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Original Article

Ophthalmology Section

Retinal Vessel Parameters and Choroidal Thickness using Optical Coherence Tomography and OCT Angiogram in Normal Children: A Cross-sectional Study

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ABSTRACT

Introduction: Optical Coherence Tomography (OCT) and OCT Angiogram (OCT-A) are non invasive techniques for imaging the microvasculature of retina and choroid. OCT is a method of analysing the in-vivo retinal architecture. OCT uses low coherence interferometry to create a cross-sectional map of the retina with a resolution of 10-15 µm. Swept Source OCT utilises light source of 1050 nm wavelength and can reproducibly measure choroidal thickness. OCT-A uses the principle of motion contrast, where the moving red blood cells are traced to depict vessels through different segments of the retina. The same tissue area is imaged repeatedly and differences are analysed between scans to detect area with high flow rates, low flow and no flow at all. Only a few studies have been reported on baseline choroidal thickness and retinal vessel parameters in children. This study was conducted to measure these parameters using Swept source OCT in normal children.

Aim: To measure retinal vasculature parameters and choroidal thickness using OCT and OCT-A in normal children.

Materials and Methods: A cross-sectional study was conducted on 68 children between 5-15 years of age in Department of Ophthalmology, Christian Medical College and Hospital, Vellore, Tamil Nadu, India between February to July 2018, after obtaining the ethical clearance from the Institutional Review Board. The children were divided into two groups, 5-10 years and >10-15 years. Children with Best Corrected Visual Acuity (BCVA) <6/6, axial length <20/>25 mm, any systemic disease or ocular pathology were excluded. All underwent comprehensive ophthalmic examination along with fundus photography, OCT and OCTA imaging. Retinal artery and vein calibre at the disc margin and Subfoveal Choroidal Thickness (SFCT) were measured. Measurement of Foveal Avascular Zone (FAZ) both superficial and deep, Vascular Density (VD) of perifoveal, papillary and peripapillary area were obtained from OCT-A. T-test was used to compare the parameters with p-value <0.05 considered as significant.

Results: A total of 136 eyes were included. Mean retinal artery measured at superior and inferior disc margin was 176.84±43.88 µm and 177.78±41.69 µm respectively. Mean retinal vein measured at superior and inferior disc margin was 218.90±46.36 µm and 235.37±48.07 µm respectively. Perifoveal images were clear and vessel densities could be anlaysed but papillary and peripapillary vessel densities were not analysed due to poor image quality in many subjects. The mean retinal artery caliber in the age group 5-10 years was 179.85±45.62 µm and 174.00±42.30 µm in the age group >10-15 years at the superior disc margin with p-value of (0.4403). There was no statistical difference retinal vessel caliber between the two groups. The mean SFCT in the 5-10 years group was 344.42±86.77 µm and 343.40± 93.56 µm in the other with a p value 0.9474. The mean superficial FAZ (SFAZ) was 189.79±117.11 µm^2 in the first group and 264.88±169.91 µm^2 in the second group, (p=0.0034). There was no statistical difference among these parameters between the two groups except SFAZ.

Conclusion: Present study describes the normative data on retinal vasculature parameters and choroidal thickness in Indian paediatric population aged 5-15 years using OCT and OCT-A. The study findings will further help in screening and monitoring the subjects in this age group.

Keywords: Choroidal imaging, Paediatric retina, Retinal vasculature

INTRODUCTION

The OCT and OCT-A are non invasive techniques used for imaging retina and choroid [1,2]. Microvascular changes in retina can be identified by these techniques well ahead of it being identified clinically. Retinal vessel caliber changes, microvascular changes in the deeper retinal layers and choroidal vessel changes are potent biomarkers for various systemic and ocular conditions, especially diabetes and hypertension [3-5]. Prevalence of various vascular diseases in children are on the rise [6]. Hypertension has the strongest association with retinal vascular calibers. In diabetes, the microvascular changes plays an important role in its pathogenesis and has shown a relation with retinal vessel calibers. Cardiovascular risk factors are also associated with retinal vessel dimensions. Stroke, obesity, dyslipidaemia, cognitive impairment and kidney diseases are other few conditions where retinal vessel caliber show changes and can act as a marker. Detecting these retinal and choroidal changes early can help diagnose and modify treatments to avoid the long-term vascular complications in these diseases [7,8]. Studies on retinal and choroidal vessels in normal adults have been studied and reported [9-11]. However, the information on these vascular parameters in normal children are limited in the literature [12-17]. The data is even more scarce in the Indian paediatric population [14]. Children with hypertension with retinal vascular changes are seen and there was no normative data to compare. The objective of present study was to document retinal and choroidal vascular parameters in children from Indian population. Thus, the participants were divided into two groups, 5-10 years and >10-15 years. The null hypothesis made was that there was no difference in parameters among the two age groups.

MATERIALS AND METHODS

A cross-sectional study was conducted in children visiting outpatient clinic in the Department of Paediatric Ophthalmology, Christian

Medical College and Hospital, Vellore a tertiary care centre in Tamil Nadu, India, from February to July 2018. Institutional Review Board (IRB) clearance was obtained, IRB No:11100 dated 10/01/18. An informed consent was obtained from the parents or guardians of each participant and a written assent was obtained from children above 8 years and an oral assent for children below 8 years. Visual acuity of all the participants was 6/6.

Sample size calculation: Based on the study taken for sample size, the mean SFCT reported was 312.1±45.5 [14]. The calculated sample size of 64 eyes with 95% CI was done using the following formula, n=4*SD*SD/(d*d) and got, for 40 SD and precision of 10 units. The sample size calculated was 64 eyes in 5-10 years and 64 eyes in >10-15 years accounting for 128 eyes. Considering poor image quality of 5% eight eyes additionally were recruited.

Inclusion criteria: Children between 5-15 years were included in the study.

Exclusion criteria: Children younger than 5 years were excluded expecting poor cooperation and assuming poor quality images. A detailed history was taken from the parent on child's systemic medications or illness like hypertension, diabetes, renal disease or parental history of hypertension and birth history like low birth weight (<2.5 kg) or prematurity (<37 weeks), if any present, were excluded. Body Mass Index (BMI) was calculated and children with BMI >25 were excluded. Children with syndromic disorder were also excluded.

Study Procedure

Retinal artery and vein calibre at the disc margin were measured from the fundus photograph. SFCT and choroid thickness 1 mm horizontally on either side of the fovea was measured from the OCT image. Measurement of FAZ both superficial and deep, Vascular Density (VD) of perifoveal, papillary and peripapillary area were measured from OCT-A. Ocular factors which alters the retinal vascular parameters and choroidal thickness like Axial Length (AXL) >25 mm or <20 mm, highmyopia (>–6 Diopter (D) or hyperopia (>+4D) were excluded. After routine comprehensive ophthalmic examination, charts were screened for any ocular pathology that might affect the outcome and were excluded. Children who met the inclusion/exclusion criteria underwent fundus photography, OCT and OCT-A imaging. Images were screened and any retinal or choroidal abnormality detectable on OCT scan, poor image quality because of unstable fixation and who were unable to cooperate for SS-OCT examination were also excluded. Fundus photo was taken at one time to reduce contact time as the study was conducted in normal children. There is a probability of arteriolar diameter varying in different phase of cardiac cycle in children also. Both eyes of each patient were included in the study.

Measurements obtained:

- The retinal vessel caliber at superior and inferior optic disc margin, from fundus photography.
- Choroidal thickness at the foveal region, 1 mm nasal and 1 mm temporal to fovea, from OCT images.
- Measurement of FAZ both superficial and deep, from OCT-A images.
- Vascular density of parafoveal, papillary and peripapillary area from OCT-A images.

This study was done using Swept Source OCT (SS OCT – DRI OCT TRITON Plus, TOPCON Inc, Tokyo, Japan) which uses light reflectance off the surface of retina. Light emitted from SS-OCT utilises a longer wavelength, close to 1050 nm which captures retinal and choroid images within 30 seconds. This advanced vascular imaging technique allows detailed non invasive assessment of retinal microvasculature. OCT-A captures images of moving red blood cells thus accurately depicting vessels through different segmented areas of the retina.

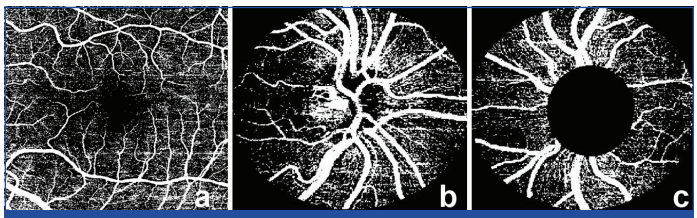
Vessel diameter: The fundus image was exported from OCT macula and the vessel calibers were measured using the inbuilt caliper, both arteriolar and venular diameter while exiting at superior and inferior optic disc margin and where the artery and vein were overlapping the measurements were not taken for analysis. The values were obtained in pixels and converted to µm. An average of three readings were taken and multiplied by 10 based on machines conversion for pixels in fundus image.

Subfoveal and parafoveal choroidal thickness: The choroidal thickness was measured from OCT macula horizontal 6 mm line scan. The thickness of choroid was measured from retinal pigment epithelial layer superiorly to the lower choroidal vascular extent at the fovea manually using the inbuilt caliper, 1 mm nasal and 1 mm temporal to fovea. Three readings were taken and average was taken in µm.

Foveal avascular zone: The foveal avascular zone superficial and deep images were exported from OCT-A macula scan and the borderline of FAZ were traced manually using the inbuilt tool and the area was derived. The values were obtained in pixels and were converted to μm^2.

Vascular density: Over optic disc, 3 mm around the disc and 3 mm around the centre of fovea was analysed using the Image J software [Table/Fig-1], an open source, which is a Java-based image processing program developed at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation (LOCI, University of Wisconsin) [18]. The vessel analysis protocol calculated the vessel density metrics using the following formula:

$$\text{Vascular density} = \frac{\text{Vessel area}}{\text{Total area of image}} \times 100 \text{ percentage area}$$



[Table/Fig-1]: Vascular density. a) Perifoveal; b) Papillary; c) Peripapillary.

STATISTICAL ANALYSIS

Data was entered in Microsoft excel spread sheet and was analysed using STATA version 15.0. The continuous variables like retinal vessel calibre, choroidal thickness, FAZ area and VD were reported by using mean and Standard Deviation (SD). The categorical variables like gender and age group were analysed and were represented as number and percentages. Age groups 5-10 years and >10-15 years were analysed separately and compared using unpaired t-test with p-values to look for any physiological variations between these age groups. Paired t-test was used to compare the differences in right and left eye. A p-value of <0.05 was taken as significant. Subgroup analysis of superior and inferior vessel calibre, temporal and nasal CT, deep and superficial FAZ, Superficial and deep perifoveal VD were also compared using paired t-test.

RESULTS

A total of 435 children were recruited for the study of which 298 were excluded due to ocular pathology and 42 were excluded due to systemi cillness/medications, 27 were excluded due to poor co-operation for imaging or poor image quality. A total of 136 eyes of 68 children were included. Mean age was 11.05±2.61 years. A total 33 children belonged to 5-10 years and 35 were from >10-15 years. Males were 38 (56%).

The retinal and choroidal parameters among the study population are shown in [Table/Fig-2]. The retinal artery caliber at superior and inferior disc margins were almost the same but the retinal vein did show some difference of 16.47 µm with inferior vein caliber being larger. Mean retinal artery measured at superior and inferiror disc margin was

176.84 micrometer (µm) (+/-43.88) and 177.78±41.69 µm respectively. Mean retinal vein measured at superior and inferiror disc margin was 218.90±46.36 µm and 235.37 ±48.07 µm respectively. The subfoveal choroidal thickness was 343.89±89.99 µm which was more than temporal and nasal thickness. The deep foveal avascular zone area was 249.37±151.55 µm^2 which was larger than the superficial foveal avascular zone. The perifoveal superficial vessel density was 59.71±3.33 % which was less than the deep by 7.04%.

Retinal vessel calibre and choroidal thickness (n=136)	Mean±SD
Retinal artery calibre at superior disc margin (µm)	176.84±43.88
Retinal artery calibre at inferior disc margin (µm)	177.78±41.69
Retinal vein calibre at superior disc margin (µm)	218.90±46.36
Retinal vein calibre at inferior disc margin (µm)	235.37±48.07
Choroidal thickness at the fovea (µm)	343.89±89.99
Choroidal thickness 1 mm nasal to fovea (µm)	314.57±86.52
Choroidal thickness 1 mm temporal to fovea (µm)	342.63±88.40
Superficial foveal avascular zone area (µm^2)	240.83±144.96
Deep foveal avascular zone area (µm^2)	249.37±151.55
Perifoveal superficial vessel density (%)	59.71±3.33
Perifoveal deep vessel density (%)	66.75±3.26

[Table/Fig-2]: Retinal and choroidal parameters in the study population.

Papillary and peripapillary vascular densities could not be analysed as the sample was low due to poor image quality in many subjects.

The [Table/Fig-3] shows the comparison between superficial and deep foveal avascular zone, perifoveal vessel density and choroidal thickness. The deep foveal avascular zone area was larger than the superficial and was statistically significant with a p-value=0.009. There was a statistical significant difference between the deep perifoveal vessel density and the superficial with a p-value <0.001, the deep being larger. There was no difference between the nasal and temporal choroidal thickness.

Parameters (n=136)	Mean±SD	Parameters (n=136)	Mean±SD	p-value
SFAZ (µm^2)	240.83±144.96	DFAZ (µm^2)	249.37±151.55	**0.009**
CTN (µm)	314.57±86.52	CTT (µm)	342.63±88.40	0.89
Perifoveal VDS (%)	59.71±3.33	Perifoveal VDD (%)	66.75±3.26	**<0.001**

[Table/Fig-3]: Comparison of parameters between superficial and deep FAZ and perifoveal vascular density, temporal and nasal CT.
CTN: Choroidal thickness 1 mm nasal to fovea; CTT: Choroidal thickness 1 mm temporal to fovea; SFAZ: Superficial foveal avascular zone; DFAZ: Deep foveal avascular zone; VDS: Vascular density superficial; VDD: Vascular density deep

The comparison between the two groups 5-10 years and >10-15 years is given in [Table/Fig-4]. It compares the various retinal vascular parameters and choroidal thickness among them. The retinal artery and vein caliber at superior and inferior disc margins were same between the groups. There was no difference in the choroidal thickness and perifoveal vessel density in both groups, but SFAZ did show a significant difference.

Vascular parameters	RE	LE	p-value	5-10 yrs (n=66)	>10-15 yrs (n=70)	p-value
RACODMS (µm)	170.32± 44.90	183.52± 42.11	0.0752	179.85± 45.62	174± 42.30	0.4403
RACODMI (µm)	181.23± 40.80	174.49± 42.98	0.3464	180.45± 42.98	175.22± 40.57	0.4683
RVCODMS (µm)	216.61± 47.68	221.42± 45.51	0.5436	223.03± 47.52	215± 45.23	0.3152
RVCODMI (µm)	237.22± 49.20	233.72± 46.44	0.6832	238.94± 44.10	232± 51.63	0.4000
SFCT (µm)	345.47± 89.82	342.32± 90.81	0.8393	344.42± 86.77	343.40± 93.56	0.9474
CTN (µm)	313.93± 87.96	315.22± 85.70	0.9309	314.73± 81.15	314.43± 91.89	0.9840
CTT (µm)	344.57± 90.22	340.69± 87.18	0.7990	343.27± 88.58	342.03± 88.87	0.9350
SFAZ (µm^2)	228.44± 148.13	228.44± 154.77	0.9999	189.79± 117.11	264.88± 169.91	**0.0034**
DFAZ (µm^2)	243.97± 167.07	218.12± 152.31	0.3475	204.37± 147.91	256.19± 167.42	0.0585
Perifoveal VDS (%)	59.72± 3.35	59.69± 3.34	0.9565	59.68± 3.12	59.73± 3.55	0.9409
Perifoveal VDD (%)	66.56± 3.37	66.94± 3.16	0.5010	66.69± 3.03	66.81± 3.49	0.8211

[Table/Fig-4]: Vascular parameters comparing subject below and above 10 years. There was no statistically significant difference in values between right and left eye or in children below and above 10 years of age except for SFAZ.
RACODMS: Retinal arteriolar caliber optic disc margin superior; RACODMI: Retinal arteriolar caliber optic disc margin inferior; RVCODMA: Retinal venular caliber optic disc margin superior; RVCODMI: Retinal venular caliber optic disc margin inferior; SFCT: Subfoveal choroidal thickness; CTN: Choroidal thickness 1 mm nasal to fovea; CTT: Choroidal thickness 1 mm temporal to fovea; SFAZ: Superficial foveal avascular zone; DFAZ: Deep foveal avascular zone; VDS: Vascular density superficial; VDD: Vascular density deep

Papillary and peri papillary vascular densities could not be analysed as the sample was low due to poor image quality in many subjects. Only DFAZ showed statistical difference with a p-value 0.006 between the right and left eye in the age group 5-10 years. Though, individual values of right and left eye were taken, the comparison p-values were adjusted for cluster effect to tackle the association between the eyes from same individual [Table/Fig-5].

Parameters	5-10 yrs		p-value	>10-15 yrs		p-value
	RE	LE		RE	LE	
RACODMS (µm)	176.36± 50.36	183.33± 40.82	0.735	164.29± 39.43	183.71± 43.39	0.997
RACODMI (µm)	183.64± 43.72	177.27± 42.67	0.212	178.82± 37.88	171.71± 43.28	0.24
RVCODMS (µm)	221.52± 50.94	224.55± 44.59	0.625	211.71± 44.42	218.29± 46.43	0.756
RVCODMI (µm)	242.73± 40.02	235.15± 48.16	0.248	231.71± 58.38	232.29± 44.73	0.522
SFCT (µm)	344.12± 84.18	344.73± 90.58	0.522	346.74± 96.05	340.06± 92.29	0.211
SFCTNF (µm)	312.06± 80.89	317.39± 82.57	0.745	315.69± 95.30	313.17± 89.71	0.37
SFCTTF (µm)	342.39± 88.15	344.15± 90.37	0.573	346.63± 93.37	337.43± 85.25	0.114
SFAZ (µm^2)	192.11± 117.32	187.48± 118.66	0.359	262.69± 166.72	267.07± 175.45	0.73
DFAZ (µm^2)	227.53± 164.03	181.21± 128.16	**0.006**	259.46± 170.80	252.91± 166.40	0.242
Perifoveal VDS (%)	59.70± 3.35	59.67± 2.92	0.951	59.74± 3.40	59.71± 3.74	0.961
Perifoveal VDD (%)	66.46± 3.40	66.91± 2.63	0.345	66.66± 3.39	66.96± 3.62	0.64

[Table/Fig-5]: Retinal and choroidal parameters of both eyes in two age groups.
SFCTNF: Subfoveal choroidal thickness nasal to fovea; SFCTTF: Subfoveal choroidal thickness temporal to fovea

DISCUSSION

This study describes the retinal vasculature parameter and the choroidal thickness in normal Indian paediatric population between 5-15 years. In the present study, significant difference between subject below and above 10 years was found only for SFAZ, thus probing the null hypothesis. To the best of our knowledge this was the first Indian study to describe the retinal vasculature calibre in this population. The only Indian study that has looked at the choroidal thickness only. Retinal imaging can document finer changes in retinal vessel diameters which can aid in early diagnosis. Arterial thinning due to chronicity is the initial change documented in hypertension [19]. Though, retinal vessel diameter variations are evident clinically, imaging can document these for future follow-up to monitor progression. Cheung N et al., have reported the retinal vessel diameter in children. It is reported as 156.4 µm and 225.4 µm [12].

The values are comparable to present study [Table/Fig-1]. Any ocular or systemic factors which could alter the retinal vascular parameters or choroidal thickness like AXL >25 mm or <20 mm, high myopia (>–6 D), hyperopia (>+4 D), systemic hypertension, diabetes, renal disease, low birth weight, BMI >25 kg/m^2 and syndrome disorders were excluded.

The choroid is a highly vascular structure, the thickness of which gets affected in various ocular and systemic conditions. These changes in thickness are seen in ocular conditions such as central serous chorioretinopathy, polypoidal choroidal vasculopathy, Vogt-Koyanagi-Harada disease, high myopia, uveitis, age related macular degeneration and systemic conditions such as diabetes mellitus and hypertension [11]. With increasing prevalence of children with systemic vascular conditions like hypertension and diabetes, a regular follow-up and screening of choroidal parameters can be of benefit to compare with the baseline values.

Park KA and Oh SY reported on choroidal thickness in normal children and the values are 348.4±82.5 µm which was highly comparable with present study results 343.89±89.99 µm. Significant choroidal thinning have been reported in children with beta thalassaemia major and type 1 diabetes mellitus without retinopathy [13,20,21].

Alteration in shape and size of FAZ has been reported in adults in conditions like diabetes, and glaucoma [22]. A baseline FAZ and VD around it are vascular parameters that can be used to monitor children with these systemic conditions. Yilmaz I et al., reported on FAZ in norma lchildren and the values of superficial FAZ area was 280 µm^2 as compared to present study result 240.83±144.96 [17]. They also reported the area of deep FAZ in normal children as 329 µm^2 while present study result showed that deep FAZ was 249.37±151.55 µm^2. The deep FAZ was wider compared to superficial FAZ and the difference was statistically significant in the present study. Studies on perifoveal and peripapillary vascular density are lacking in normal population among children.

Limitation(s)

The main limitation of this study was that children below 5 years of age were not included. Hence, further studies with larger sample size including children of all age groups are needed to establish the normogram for caliber of retinal vessels and choroidal thickness.

CONCLUSION(S)

Knowing normal retinal vasculature and choroidal parameters will help in establishing normograms in the Indian paediatric age group between 5-15 years. This will help in screening and monitoring children with systemic vascular diseases. OCT and OCT-A are non invasive techniques for evaluation of retina and choroid which will help in early diagnosis of a disease and follow-up on the effect of treatment resulting in better outcomes and quality of life.

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