ORIGINAL ARTICLE

Normative retinal nerve fber layer thickness in a healthy pediatric South Asian cohort: a spectral‑domain optical coherence tomography study

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Received: 6 February 2023 / Accepted: 31 December 2023 © The Author(s), under exclusive licence to Springer-Verlag London Ltd., part of Springer Nature 2024

Abstract

Currently, the normative values for retinal nerve fber layer (RNFL) thickness in our population have not been widely studied. Our study aimed to assess the peripapillary RNFL thickness (RNFLT) with Optopol Copernicus REVO80® spectral-domain optical coherence tomography (SD-OCT) amongst healthy children and its associations. One hundred eighty-two eyes of 91 consecutive healthy children 3 to 16 years of age with a refractive error $\leq \pm 5$ D were included after a thorough eye exam including visual acuity, refraction, tonometry, pachymetry, axial length estimation, and slit lamp exam including fundus assessment. RNFLT was measured via Optopol Copernicus REVO80® high resolution SD-OCT by a single experienced observer with 3D disc mode within a circular area of diameter 3.45 mm and the ring further divided into four quadrants: inferior, superior, nasal, and temporal. The mean age was 11.12 ± 3.12 years (range, 3–16). The average RNFLT was *120.13 ± 12.6* μm. The mean *superior* RNFL was the thickest at *138.21 ± 16.6* μm, next was the mean *inferior* RNFLT at *137.62 ± 17.2* μm, followed by the *nasal 91.61 ± 18.5* μm and then the *temporal at 74.58 ± 11.7* μm. No signifcant diferences in RNFLT were noted between the two eyes. The mean RNFLT was signifcantly higher in *males* as compared to *females*, in vertical quadrants and at an average ($p < 0.05$). No significant relationship was found between the average RNFLT and factors such as age, axial length, corneal thickness, cup-to-disc ratio, intraocular pressure, or refractive error. This study establishes normative values of RNFLT for this subgroup of Pakistani children for the Optopol Copernicus REVO80® SD-OCT device.

Keywords Retinal nerve fber layer thickness · Optical coherence tomography · Pediatric, Children, Imaging

Introduction

Hereditary or acquired optic neuropathies, including glaucomatous, ischemic, infammatory, metabolic, nutritional, or traumatic optic nerve disorders including papilledema, optic disc malformations, optic neuritis, and CNS tumors, comprise a signifcant cause of visual impairment and morbidity both in children and adults. The disabling visual loss can be permanent or may recover to some or the full extent. Even if vision recovers, color vision and contrast sensitivity

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may not recover, and permanent visual feld defects may remain $[1-5]$ $[1-5]$ $[1-5]$.

Since its first description in 1991, optical coherence tomography (OCT) has gained high and wide utility for rapid, easy, three-dimensional, high resolution, and in vivo quantitative anatomic imaging of the retina and choroid with exquisite detail. The retinal nerve fiber layer (RNFL) is composed of the nonmyelinated axons of the retinal ganglion cells (RGC) and is the structure of choice to examine and interpret in a variety of processes: neurodegenerative, neuro-reparative, and neuroprotective $[6, 7]$ $[6, 7]$ $[6, 7]$ $[6, 7]$ $[6, 7]$.

The normative values for RNFLT in the pediatric population are not well researched upon in Pakistan. Hence, this study was carried out to determine normative data of RNFLT for the SOCT Copernicus REVO80® device in our healthy children and its correlations, in order to better diagnose and monitor pediatric optic nerve disease.

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Materials and methods

Study design and participants

This prospective, observational study was carried out in the Eye outpatient department of Fauji Foundation Hospital, Rawalpindi which is affiliated with Foundation University Islamabad, Pakistan, from September 1, 2020, to February 15, 2022. Ethical approval from the 'Foundation University Ethical Review Committee' was taken which is according to the Declaration of Helsinki. After a comprehensive ocular examination, visual acuity testing with a Snellen chart and cycloplegic autorefraction with 1% cyclopentolate drops (RK-F1 Full Auto Ref-Keratometer®, Canon, Japan) taking a mean of three readings, intraocular pressure (IOP) measurement with Goldmann applanation tonometry, and slit lamp examination for detailed anterior segment and fundus exam with a Volk® Superfield lens, healthy children between the ages of 3 and 16 years were recruited. Children having a best corrected visual acuity of $\leq 20/20$ measured with the age appropriate Snellen chart, a spherical equivalent of $\leq \pm 5.00$ D, an IOP of ≤ 21 mm Hg, normal-appearing discs (healthy pale, pink in color with a healthy neuroretinal rim, and symmetrical cups), and absence of any ocular pathology (amblyopia, corneal scarring, uveitis, cataract, glaucoma, retinal disorders, trauma history), or systemic disorders, were included in the study. Axis-II A-scan (Quantel Medical®, France) was used to calculate axial length via the contact method by the author after anesthetizing the cornea with proparacaine hydrochloride 0.5% eye drops and taking a mean of 10 readings in primary gaze with adequate centration. SOCT Copernicus REVO80® (Optopol Technology, Poland) high resolution spectral domain (SD-OCT) was used to perform pachymetry to evaluate central corneal thickness (CCT). Our research included both eyes which were examined in detail for the absence of pathology. Informed consent was taken prior to examination.

Spectral‑domain optical coherence tomography

The RNFLT analysis in the peripapillary region was done by a single experienced observer using the SOCT Copernicus REVO80® high resolution SD-OCT (Optopol Technology, Poland Software Version 10.0.1) (840-nm superluminescent diode source, transverse resolution of 12 μm, axial resolution of 5 μm, and 80,000-A-scan/s scanning speed). Each child and guardian was explained that the procedure was noncontact and painless, and the test was performed with the child sitting either independently or in the parent's lap with adequate chin and forehead placement. Clear instructions were given to look into the lens and blink freely while looking at the center of the internal fxation target (green cross) and to follow it for capturing the optic disc. The examination procedure has been described by us previously as well [\[8,](#page-7-3) [9](#page-7-4)].

 $A 6 \times 6$ mm disc 3D scan was performed manually for each eye with quality index (QI) \geq 7 without any errors or artifacts. The optic nerve head has to be in the center of dashed horizontal lines in the scanned area which is observed live on the screen. The scan angle is set at 0° . A pseudo SLO live image which shows the en face view of fundus is also visible, and the circling ring was centered at the optic disc, and images were captured manually. If any kind of blink or motion artifact was noted, the imaging was repeated to avoid any errors. Segmentation errors were checked during the exam, and the scan was repeated if they occurred.

The RNFLT is the distance of the internal limiting membrane and the external edge of the RNFL ⁹ . This is assessed within a circular ring centered around the disc with a diameter of 3.45 mm, which is automatically computed by the device. This is subdivided into four quadrants for RNFLT analysis: *superior, inferior, nasa*l, and *temporal.* The average RNFLT as well as the quadrantic RNFLT was noted.

Statistical analyses

IBM SPSS statistics version 20 was used to analyze the data. Both eyes of the children were included in the data analysis. Since both eyes are likely to be correlated, the total data was considered a single unit. *Age*, *gender*, *eye laterality*, *intraocular pressure*, *spherical equivalent*, *CCT*, *CDR*, and *axial length* were the demographic and clinical variables studied, and their relationship with RNFLT was observed. Frequencies, means, and standard deviations for the descriptive data were calculated for all these variables.

The *Friedman test* was used to compare the means amongst the quadrant and average RNFL thickness values. The *independent t-test* was used to compare the means of the variables amongst gender between the two eyes and also amongst the stratifed age groups of the children. After determining correlation amongst the demographic variables, a linear regression analysis and *Pearson's correlation* was used to investigate the impact of age, gender, intraocular pressure, CCT, CDR, spherical equivalent, and axial length on the RNFL thickness in these individuals. A p -value of \lt 0.05 was considered to be signifcant.

Fig. 1 Bar graph depicting the RNFL thickness in four

quadrants

Table 1 Patient characteristics and RNFL parameters observed with SOCT Copernicus REVO80® SD-OCT

Variables ($n = 182$ eyes of 91 children)	Mean \pm SD	Minimum	Maximum
Age in years	11.12 ± 3.12	3	16
IOP (mmHg)	13.98 ± 2.84	5	21
CCT (μ m)	532.2 ± 41.6	401	721
CDR.	0.32 ± 0.18	0.00	0.80
Spherical equivalent (D)	-0.27 ± 1.28	-4.00	5.00
Axial length (mm)	22.69 ± 0.92	20.12	25.15
RNFLT inferior (μm)	137.62 ± 17.2	91	189
RNFLT superior (μm)	138.21 ± 16.6	81	189
RNFLT nasal (μm)	91.61 ± 18.5	54	211
RNFLT temporal (μm)	74.58 ± 11.7	54	115
RNFL average (μm)	120.13 ± 12.6	88	168

RNFL retinal nerve fber layer, *SD-OCT* spectral-domain optical coherence tomography

Results

Study population

There were 52 (57.1 %) females and 39 (42.9 %) male participants in our study with a total of 91 children (182 eyes). The average age, IOP, CCT, cup-to-disc ratio (CDR), axial length, and spherical equivalent (SE) are tabulated in Table [1](#page-2-0).

Average and sectoral RNFL thickness values

The *average* RNFLT was $120.13 \pm 12.6 \,\mu$ m. The RNFLT is given in Table [2](#page-2-1).

The mean RNFL in this study has been found to be the thickest in the *superior* quadrant at 138.21 ± 16.6 µm, followed closely by the *inferior* quadrant at $137.62 \pm 17.2 \,\mu$ m,

Table 2 Quadrantic RNFL parameters by age group and on average (μm) measured with SOCT Copernicus REVO80® SD-OCT

Age group		RNFL inferior	RNFL superior	RNFL nasal	RNFL temporal	RNFL average
3–5 years, $n = 10$	Mean \pm SD	135 ± 10.18	132.8 ± 12.89	81.8 ± 10.65	75.8 ± 10.39	115.8 ± 6.11
6–8 years, $n = 28$	Mean \pm SD	140.07 ± 17.44	143.6 ± 15.48	93.03 ± 13.43	$74.89 + 7.25$	123 ± 10.97
9–11 years, $n = 58$	Mean \pm SD	139.72 ± 13.84	135.5 ± 15.62	90.12 ± 18.32	74.6 ± 10.98	119.62 ± 10.76
12–14 years, $n = 54$	Mean \pm SD	135.5 ± 19.86	139.61 ± 19.56	94.44 ± 23.9	73.54 ± 13.62	120.31 ± 16.3
15–16 years, $n = 32$	Mean \pm SD	136.06 ± 19.57	137.72 ± 13.81	91.37 ± 12.83	75.69 ± 13.45	119.56 ± 11.62

RNFL retinal nerve fiber layer, *SD-OCT* spectral-domain optical coherence tomography

then *nasally* at 91.61 \pm 18.5 µm, and lastly *temporally* at 74.58 \pm 11.7 µm. The quadrantic and average RNFLT is also given in Table [1.](#page-2-0) Figure [1](#page-2-2) depicts a bar chart showing the RNFL distribution in the four quadrants.

The children were also segregated into five groups according to age, and the RNFLT is detailed in Table [2.](#page-2-1) There were signifcant diferences observed amongst the RNFLT in each quadrant and at an average $(p < 0.05)$ with the Friedman test.

Inter‑eye variation

Sectoral variability was observed between the two eyes with the superior and inferior RNFLT means revealed greater values in the left eye, and the nasal and temporal sectors had greater RNFLT values in the right eye. The *left* eye showed a slightly greater average RNFLT as compared to the *right* eye (120.93 μm vs 119.32 μm); however, there was no statistically signifcant inter-eye diference according to the *independent t*-*test*, and this is elaborated in Table [3](#page-3-0).

Relationship of RNFL thickness with demographic and clinical variables

This study did not fnd a statistically signifcant relationship of the average RNFL with any of the variables: age, IOP, CCT, CDR, axial length, or refractive error/spherical equivalent (*p* > 0.05). However, *Pearson's correlation* showed a signifcant negative correlation of the inferior RNFLT with *axial length* (*r* −0.193; *p* 0.009); the nasal RNFLT had a signifcant positive correlation with *spherical equivalent* (*r* 0.167; *p* 0.025) (direct relationship with hypermetropia), and the temporal RNFLT was positively correlated with *CCT* (*r* 0.236; *p* 0.001) (Table [4\)](#page-4-0).

Gender diferences in RNFL thickness

The average RNFLT in *females* was 117.4 ± 13.2 μm and in *males* was 123.7 ± 10.8 µm. The mean superior RNFLT in females was 134.6 ± 16.8 μm versus 142.9 ± 15.1 μm in males. The mean inferior RNFLT in females was $134.2 \pm$ 17.6 μm versus 142.1 ± 15.6 μm in males. The mean nasal RNFLT in females was 90.8 ± 19.9 μm versus 92.7 ± 16.4 μm in males. The mean temporal RNFT in females was 73.5 \pm 12.5 μm versus 75.9 \pm 10.5 μm in males. A significant diference between the two genders is noted between the *average*, *superior*, and *inferior* RNFLT (*p* < 0.05) and the RNFLT being thicker in all quadrants in males and also at an average. Table [5](#page-5-0) depicts the gender diferences amongst the RNFLT.

Discussion

Pediatric OCT imaging is rapidly transforming the way we diagnose and track pediatric optic nerve and retinal diseases. Assessing a pediatric examination is intricate and demanding. The absence of standardized reference data further complicates the diagnostic and treatment decision-making process. The provision of an in vivo histological structural quantifcation of the retinal nerve fber layer provides a non-invasive modality to identify and monitor retinal and optic nerve pathologies in children. Since OCT machines do not have a normative database for children, there is a need for studying pediatric normative database for the different OCT devices that are available for each setting. This study presents normative values for the average and sectoral RNFLT in a healthy pediatric cohort between 3 and 16 years of age in a single tertiary care setting, for the SOCT Copernicus REVO80® device. The '*ISNT rule*' is characterized by decreasing values for peripapillary RNFL thickness sectors, following this sequence: *inferior* > *superior* > *nasal* > *temporal*. The average RNFLT was 120.13 μm, but the 'ISNT rule' was not followed in this study with the superior RNFL sector demonstrating greatest thickness on average, followed by the inferior, then nasal, and lastly temporal (*SINT*). Amongst the demographic and clinical variables, none demonstrated a signifcant relationship with the mean RNFLT; however, sectoral RNFLT varied with the variables. *A signifcant negative relationship of the inferior RNFLT with axial length was observed in our cohort; the nasal RNFLT had a signifcant positive association with hypermetropia or positive spherical equivalent, and the temporal RNFLT was positively correlated with CCT*.

Gender differences in RNFLT were observed in our cohort, showing thicker values in all sectors amongst male

RNFL retinal nerve fber layer, *SD-OCT* spectral domain optical coherence tomography

	Female, $n = 104$ $mean \pm SD$	Male, $n = 78$ $(\text{mean} \pm \text{SD})$	p -value
RNFL inferior (μm)	$134.2 + 17.6$	$142.1 + 15.6$	$0.002*$
RNFL superior (μm)	$134.6 + 16.8$	$142.9 + 15.1$	$0.001*$
RNFL nasal (μm)	90.8 ± 19.9	$92.7 + 16.4$	0.488
RNFL temporal (μm)	$73.5 + 12.5$	$75.9 + 10.5$	0.164
RNFL average (μm)	$117.4 + 13.2$	$123.7 + 10.8$	$0.001*$

Table 5 Gender diferences between RNFL thicknesses measured with SOCT Copernicus REVO80® SD-OCT

*Signifcant *p*-value

RNFL retinal nerve fber layer, *SD-OCT* spectral-domain optical coherence tomography

children as compared to females and significant differences in the superior and inferior quadrants, as well as at an average.

The RNFLT has been observed to vary amongst diferent *races*, *regions*, and *populations*. It also varies according to the *OCT machine* used to measure it. Therefore, it is important to have a normative database for each population in order to better diagnose and monitor optic nerve and retinal diseases. The *retinal nerve fber layer* is an important structure that is afected early in glaucoma wherein structural damage surpasses functional damage heralded by visual feld defects, thus highlighting the importance of OCT in glaucoma diagnosis and management [\[7](#page-7-2), [10](#page-7-5)].

Comparison with Pakistani OCT studies

In Pakistan, Irfan Ullah et al. [[11\]](#page-7-6) in the only other study on pediatric RNFLT have reported *Spectralis OCT* normative pediatric RNFLT values in their cohort of 56 children, with mean RNFLT being 101.25 μm, which is thinner than our study and followed the ISNT rule, contrary to our study. They reported thicker RNFL in males similar to our study. Mubashir et al. [[12](#page-7-7)] in their study on Pakistani high myopics have reported mean RNFLT in the age range of 12–20 years to be 92.17 μm and 92.20 μm in the right and left eyes, respectively, and a thinner RNFLT in males, in contradiction to our results. Pediatric OCT RNFL data is not studied widely yet in Pakistan. There is a need for more studies, especially at a larger scale and to report data.

Comparison with OCT studies from other populations and ethnicities

Rao et al. in their *Cirrus* OCT study on their Indian pediatric cohort of 74 children reported mean RNFLT to be 94 μm and 93 μm in the right and left eyes, respectively, and displayed the RNFLT normative values to be thickest in the superior quadrant, and also found a signifcant association with refractive error, and axial length, but no effect with age [\[13](#page-7-8)]. Huynh et al. [[14\]](#page-7-9) have reported mean RNFLT 103.7 μm, the thickest RNFL values in the superior quadrant similar to us in their large Australian *Stratus* OCT study. They have also noted thicker values in males, similar to us, an association with increased axial length and negative refractive error. They also noted signifcant diferences between white and East Asian children in their study. Salchow et al. [[15\]](#page-7-10) in their *Stratus* OCT study on 92 Caucasian children presented mean RNFLT to be 107 μm, with the inferior quadrant to be the thickest, followed by superior, then nasal and temporal. They also reported a signifcant efect of refraction on RNFLT. Pawar et al. presented RNFLT values for their pediatric Indian cohort of 120 children in their *Stratus* OCT study with mean RNFLT 106.11 μm and showed the inferior quadrant to be the thickest and a signifcant association with refractive error [[16](#page-7-11)]. The Anyang Childhood Eye Study utilizing the *iVue*-*100* SD-OCT showed the mean RNFLT values in 12-year-olds in China to be 103.08 μm and reported the inferior quadrant to be thickest and a negative association with axial length and hyperopic refractive error, thicker RNFL values in female children, and no association with age or body mass index (BMI) [[17](#page-7-12)]. Yao et al. have reported *Topcon* 3D OCT normative RNFLT data in Tibetan children and reported mean RNFLT to be 112.33 μm and found a signifcant diference amongst genders, negative correlation with IOP, and positively correlation with spherical equivalent and BMI [\[18](#page-7-13)].

The Gobi Desert Children Eye Study [\[19](#page-7-14)] analyzed the RNFLT with *Spectralis* SD-OCT and found it to be the thickest inferotemporally, followed by superotemporally, a decrease in RNFLT with increased myopia, male gender, IOP rise, and low birth weight. An association with age could not be established in this study as well. Another study utilizing the *Topcon* 3D OCT 2000 by Ayala et al. in their Swedish pediatric cohort showed thickest RNFL values inferiorly and a signifcant association with refractive error but not age [[20\]](#page-7-15). The Hong Kong Children Eye Study on school children showed a positive association with age and a signifcant association of temporal RNFL values with axial length with *Spectralis* SD-OCT [[21\]](#page-7-16). On the contrary, our study showed a negative association of inferior RNFLT values with axial length.

Lee et al. in their study with *Spectralis* SD-OCT compared RNFLT of emmetropes with myopes and hypermetropes and found an association of thinner RNFL with increasing age, a myopic refractive error, and axial length [\[22](#page-7-17)]. Ethnic diferences were highlighted amongst European Caucasian and East Asian children, with the latter having thicker RNFL and larger CDR [\[23\]](#page-7-18). This is in agreement with our study on our South Asian children, whose RNFLT values are thicker as compared to European Caucasians as observed in previous studies. Runge et al. [[24\]](#page-7-19) have elaborated segmented retinal layer normative pediatric values in

with *Spectralis* SD-OCT on German children. It has been suggested that nerve fber layer loss happens later in life after the age of 50 years; hence, the absence of RNFL correlation with age in children was noted in some of the studies. Although, the "*ISNT rule*" is generally said to be followed in normal eyes and is indeed followed in on the average RNFLT in male and female children in this study, but *SINT*, *ISTN*, and *SITN* have also been observed in diferent studies, and this represents a wide variation amongst diferent ethnicities [\[13–](#page-7-8)[24\]](#page-7-19).

Currently, our OCT machines incorporate adult data for normative values and diferences generated in the OCT scan. A pediatric normative database for every cohort is the need of the moment to efectively evaluate pediatric glaucoma. It is also required to study diseases causing optic nerve edema or atrophy, and even in a variety of CNS diseases especially multiple sclerosis [\[25](#page-7-20), [26\]](#page-7-21), a multitude of degenerative, autoimmune, and infammatory diseases, and systemic diseases with ocular manifestations as well [\[27](#page-7-22)].

Comparison with REVO SOCT device

Krumova et al. [[28](#page-7-23)] have reported the normative average RNFL in Caucasian children as 117.11 μm and no correlation with age using Copernicus REVO SOCT. The only other Copernicus REVO SOCT study on pediatric RNFLT has been done by Nemeș-Drăgan et al. [\[29\]](#page-7-24) who reported thicker mean RNFLT values of 127.05 μm with this device, in Romanian children, while presenting a comparison between two tomographs, the other being Spectralis OCT, which showed thinner RNFL values. For the REVO SOCT, signifcant inter-eye diferences were not observed. This is similar to our study where the average RNFLT is found to be greater than most other OCT studies discussed above, and these are the only other studies to report thicker mean RNFLT.

The potential of OCT in diagnosis and management in future is tremendous and should be utilized wherever necessary to reach an accurate and early diagnosis to prevent visual morbidity and mortality. While the outcomes from both early and late technologies show similarities, the variations in scanning speed, axial and transverse resolution, and scanning protocols amongst SD-OCT instruments prevent the direct transposition of measurement results [\[27\]](#page-7-22).

Strengths and limitations

There is a paucity of pediatric data with the Copernicus REVO80® SD-OCT device, and this study is one that establishes a normative RNFLT database for this South Asian cohort. Strengths of our study are that it is one of the scant studies done on our population. Limitations are that it is a small single-center, hospital-based study rather than a population study. The efect of race and ethnicity cannot be assessed in our cohort of South Asian children.

Future implications

This normative pediatric database for RNFLT will guide us in future in the diagnosis and therapy of pediatric ocular disorders. Additional OCT research, encompassing diverse ethnic populations, is essential in the future to facilitate glaucoma monitoring through imaging in the pediatric age group, in order to facilitate neuropediatric diagnosis amongst all ethnic backgrounds.

Conclusions

This study provides normative data of retinal nerve fber layer thickness values in a Pakistani pediatric cohort for the Optopol Copernicus REVO80® SD-OCT device. This will serve as a starting point to compare with similar studies in the Pakistani pediatric population and facilitate diagnosis of retinal, optic nerve, and neurological disorders of children in our clinical practice.

Author contributions SN: conception and design of the study, acquisition and analysis of data, and drafting of the manuscript and fgures.

Data availability The data for this study is available via the Open Science Framework with the DOI 10.17605/OSF.IO/EKQMB, and the link is [https://osf.io/ekqmb/?view_only=e2b19666a5564e428830](https://osf.io/ekqmb/?view_only=e2b19666a5564e428830a2079ec010de) [a2079ec010de.](https://osf.io/ekqmb/?view_only=e2b19666a5564e428830a2079ec010de)

Declarations

Ethical approval Permission from the "Foundation University Ethical review committee" [FF/FUMC/215-43 Phy/20] was taken previously, which is in concordance with the Declaration of Helsinki. Parental informed consent and patient consent to participate in the study were taken prior to evaluation.

Competing interests The author declares no competing interests.

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