Original Article

Retinal nerve fiber layer thickness in normal Indian pediatric population measured with optical coherence tomography

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Purpose: To measure the peripapillary retinal nerve fiber layer (RNFL) thickness in normal Indian pediatric population. **Subjects and Methods:** 120 normal Indian children ages 5-17 years presenting to the Pediatric Clinic were included in this observational cross-sectional study. RNFL thickness was measured with stratus optical coherence tomography (OCT). Children with strabismus or amblyopia, with neurological, metabolic, vascular, or other disorders and those with abnormal optic discs were excluded. One eye of each subject was randomly selected for statistical analysis. The effect of age, refraction and gender on RNFL thickness was investigated statistically. **Result:** OCT measurements were obtained in 120 of 130 (92.3%) subjects. Mean age was 10.8 ± 3.24 years (range 5-17). Average RNFL thickness was (± SD) 106.11 ± 9.5 μm (range 82.26-146.25). The RNFL was thickest inferiorly (134.10 ± 16.16 μm) and superiorly (133.44 ± 15.50 μm), thinner nasally (84.26 ± 16.43 μm), and thinnest temporally (70.72 ± 14.80 μm). In univariate regression analysis, age had no statistical significant effect on RNFL thickness (P = 0.7249) and refraction had a significant effect on RNFL thickness (P = 0.0008). **Conclusion:** OCT can be used to measure RNFL thickness in children. Refraction had an effect on RNFL thickness. In normal children, variation in RNFL thickness is large. The normative data provided by this study may assist in identifying changes in RNFL thickness in Indian children.

Access this article online
Website:
www.ijo.in
DOI:
10.4103/0301-4738.121185
Quick Response Code:

Key words: Imaging in glaucoma, optical coherence tomography, pediatric, retinal nerve fiber layer

Optical coherence tomography (OCT) is a non-invasive, non-contact imaging technology giving a cross sectional image of the retina and its substructures in a real time mode and *in vivo*.[1,2] OCT has been shown to measure the thickness of the retinal nerve fiber layer (RNFL) with micrometer-scale sensitivity. Optical coherence tomography is been used increasingly to diagnose and manage a variety of retinal diseases and glaucoma.[2-4]

Several reference data exist regarding normative values of retinal thickness across age distributions, ethnicities, and ocular disease states.[5-11] Preliminary studies have shown that retinal thickness changes with age and ethnicities.[9-11] The normal range of RNFL thickness in adults has been measured by several investigators using OCT[6-8] but less is known about normative RNFL thickness values in children. The published reports of OCT values in the healthy eyes of children are limited by consideration of either a single age group[12,13] or a single race.[14-22]

The reproducibility and good diagnostic ability of OCT for both adults and children have been proved in various studies.[23,24] As the OCT normal database is commercially not available for pediatric Indian eyes, we aimed to study the RNFL measurement profile by OCT for the normal Indian pediatric population.

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Manuscript received: 27.08.12; Revision accepted: 24.03.13

The population distribution of RNFL thickness as measured by stratus OCT has not yet been established for young children in India. The development of reference standards may allow for better investigation and detection of various ocular disease in children.

Subjects and Methods

Subjects

All subjects were recruited from patients' ages 5 years to 17 years presenting to the Pediatric Clinic. Ethical review board permission was taken. Informed consent from parents was taken. To be enrolled, subjects had to have no ocular problems other than refractive error.

Exclusion criteria were strabismus or amblyopia, any abnormalities of the disc or the retinal nerve fiber layer, family history of glaucoma, or any other hereditary eye disease, history of intraocular surgery or any kind of laser therapy mentally challenged, children with neurological, metabolic, vascular disorders, other systemic disease possibly affecting the eye, presence of a media opacity, best-corrected visual acuity of less than 20/30, hypermetropia more than + 3D, myopia more than –5D, or astigmatism more than 2D were also excluded.

Normal-appearing disc, cup, and neuroretinal rim on careful examination of the optic nerve head with 90-D aided stereoscopic slit-lamp indirect ophthalmoscopy, with a cup-disc ratio of less than 0.7 or cup-disc ratio asymmetry of less than 0.2 being the selection criteria for further evaluation.

All subjects received a full ophthalmic examination including cycloplegic refraction, assessment of intraocular pressure (Goldmann applanation tonometry where feasible), assessment of ocular motility and alignment, slit-lamp biomicroscopic evaluation, and dilated fundus examination.

Pupils were dilated with tropicamide 1% and cyclopentolate 1% or 2% drops, depending on age of the subject.

RNFL measurements were obtained through dilated pupils using a 3rd-generation optical coherence tomographer (stratus OCT, software version 4.0.4, Carl Zeiss, Dublin, CA). This OCT uses partial coherence interferometry technology (wavelength, 820 nm) to obtain cross-sectional images (equivalent to B-scan ultrasound) and achieves an axial resolution of approximately 10 um.[1,2]

OCT also has been shown to have good intra-observer and inter-observer reproducibility,[23,24] and generally is not affected by optical aberrations or pupil diameter.[25] Peripapillary measurements were performed using the fast scan protocol (fast RNFL thickness scan [3.4 protocol]; this consists of a circular scan pattern in which the diameter is 3.46 mm for eyes with standard axial length [24.46 mm] and refraction 0 diopters [D]). Each scan consists of 256 individual Ascans along a circular scan path. Three such circular scans were performed successively, with a total acquisition time of 1.92 s. The average of the 3 scans was used in the analysis.All scans were performed by the same investigator. An internal fixation target was used in all scans, and the location of each scan on the retina was monitored on the built-in infrared-sensitive video camera. Scans were accepted only if they were free of artifacts and had signal strengths of at least 6. Mean RNFL thickness in micrometers along the whole circle circumference, four quadrants, 12'o clock hours, and at 256 A-scan lengths were obtained. 12 30° sectors were also defined in clockwise order for the right eye and in counterclockwise order for the left eye: 1-superior-nasal, 2-nasal-superior, 3-nasal, 4-nasal-inferior, 5-inferior-nasal, 6-inferior, 7-inferior-temporal, 8-temporal-inferior, 9-temporal, 10-temporal-superior, 11-superior temporal, and 12-superior). Maximum RNFL thickness in superior and inferior quadrants was also analyzed.

Statistical analysis

One eye of each subject was selected randomly for statistical analysis by selecting right and left eyes in an alternating fashion from the randomly ordered sample. For statistical comparison of variables between right and left eyes, all eyes were used. For purposes of analysis, 30°-segment measurements of left eyes were mirror imaged (i.e., 3'o clock was nasal for all eyes). Univariate and multivariate regression analyses were used to analyze the effect of age, gender, and refraction on RNFL thickness. Statistical comparisons of variables between right and left eyes were performed using the paired student's *t* test. For comparisons of variables between genders, the unpaired Student's *t* test was used. All statistics were done using the Stata: Data analysis and statistical software (Version 8.1, Texas, USA).

Results

Demographics

130 subjects consented to participate in this study. Of these 3 (3.3%) did not co-operate for the OCT imaging (3 were 5 years old).Of the remaining 127 subjects, seven were excluded because scan quality was not good. Thus, 120 subjects were eligible for statistical analysis. After random selection of one eye of each subject, 60 right and 60 left eyes entered statistical analysis. The demographic characteristics of the study subjects are listed in Table 1. There was no statistically significant difference between male and female subjects for refraction ($P = 0.26$) and age ($P = 0.959$).

RNFL thickness

Mean global RNFL thickness was 106.11 ± 9.50 μm (range, 82.26-146.25) 105.64 ± 8.87 μm (range, 84.68-129.75) in right eyes and 107.54 ± 8.96 μm (range 91.35-140.33) in left eyes; the difference was not statistically significant ($P = 0.453$). On average, the RNFL was thicker inferiorly and superiorly, thinner nasally and thinnest temporally [Table 2].The distribution of global RNFL thickness for all eyes is shown in Fig. 1. The RNFL thickness in different peripapillary locations for all eyes is shown in Fig. 2.

Table 1: Demographics of study subjects included in the analysis (*n*=120)

Characteristic	Value
Age (years)	
Mean±SD	10.8±3.24
Range	5-17
Gender (%)	
Female	54 (45)
Male	66 (55)
Refraction (SE in diopters)	
Mean±SD	−0.93±1.31
Range	−4-1.5

SD: Standard deviation of the mean, SE: Spherical equivalent

Table 2: RNFL thickness for all quadrants and sectors in study subjects

Parameter	Mean (SD) μm	Median μm	Range in μm
RNFL (average)	106.11 (9.51)	106.04	82.26-146.25
S Max	164.98 (18.05)	164	124-218
I Max	165.78 (20.99)	166	126-235
Quadrants			
Superior	133.44 (15.50)	135	94-171
Nasal	84.27 (16.43)	84	49-124
Inferior	134.1 (16.16)	136	106-193
Temporal	70.72 (14.81)	70	32-170
Sectors			
Superior	142.33 (23.79)	142.5	80-194
Superior nasal	129.52 (21.05)	127.5	76-188
Nasal superior	104.88 (24.19)	104	51-194
Nasal	70.06 (18.08)	69	36-130
Nasal inferior	80.07 (17.08)	80	39-130
Inferior nasal	118.4 (23.79)	118	68-214
Inferior	150.78 (23.62)	151	102-212
Inferior temporal	132.41 (22.91)	131	82-214
Temporal inferior	71.06 (14.89)	69.5	43-119
Temporal	56.83 (9.54)	56	36-88
Temporal superior	82.5 (17.48)	82	52-184
Superior temporal	131.72 (21.88)	131.5	77-187

RNFL: Retinal nerve fiber layer, SD: Standard deviation of the mean, S Max: Superior maximum, I Max: Inferior maximum

The mean RNFL thickness in four quadrants and for all clock hours is provided in [Table 2]. The mean RNFL thickness was highest in the inferior quadrant followed by superior, nasal, and temporal quadrants [Table 2]. The RNFL was thickest inferiorly (134.10 ± 16.16 μm) and superiorly (133.44 ± 15.50 μm), thinner nasally (84.26 ± 16.43 μm), and thinnest temporally (70.72 ± 14.80 μm). The RNFL

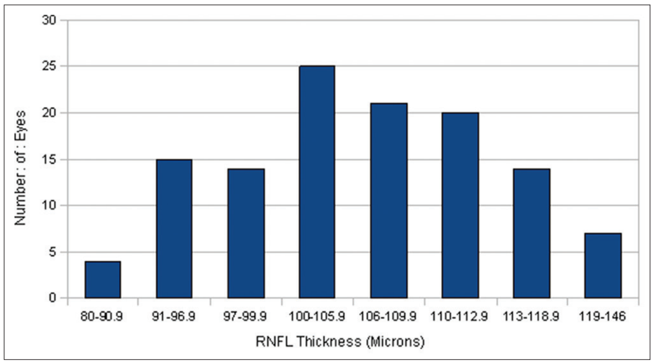

Figure 1: The distribution of global RNFL thickness for all eyes (*n* = 120)

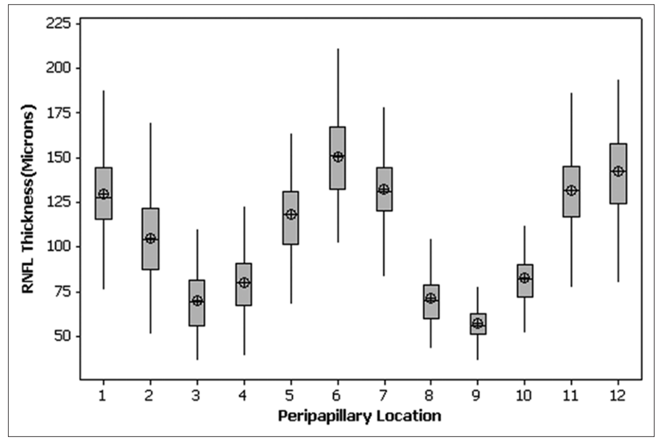

Figure 2: Retinal nerve fiber layer thickness in normal children as a function of peripapillary location (*n* = 120 eyes). Peripapillary location is given in clock hours (3 = nasal, 6 = inferior, 9 = temporal, 12 = superior). Mean, Boxplot gives five number summaries- the smallest observation (sample minimum) lower quartile (q1) median (q2) upper quartile (q3) and largest observation (sample maximum)

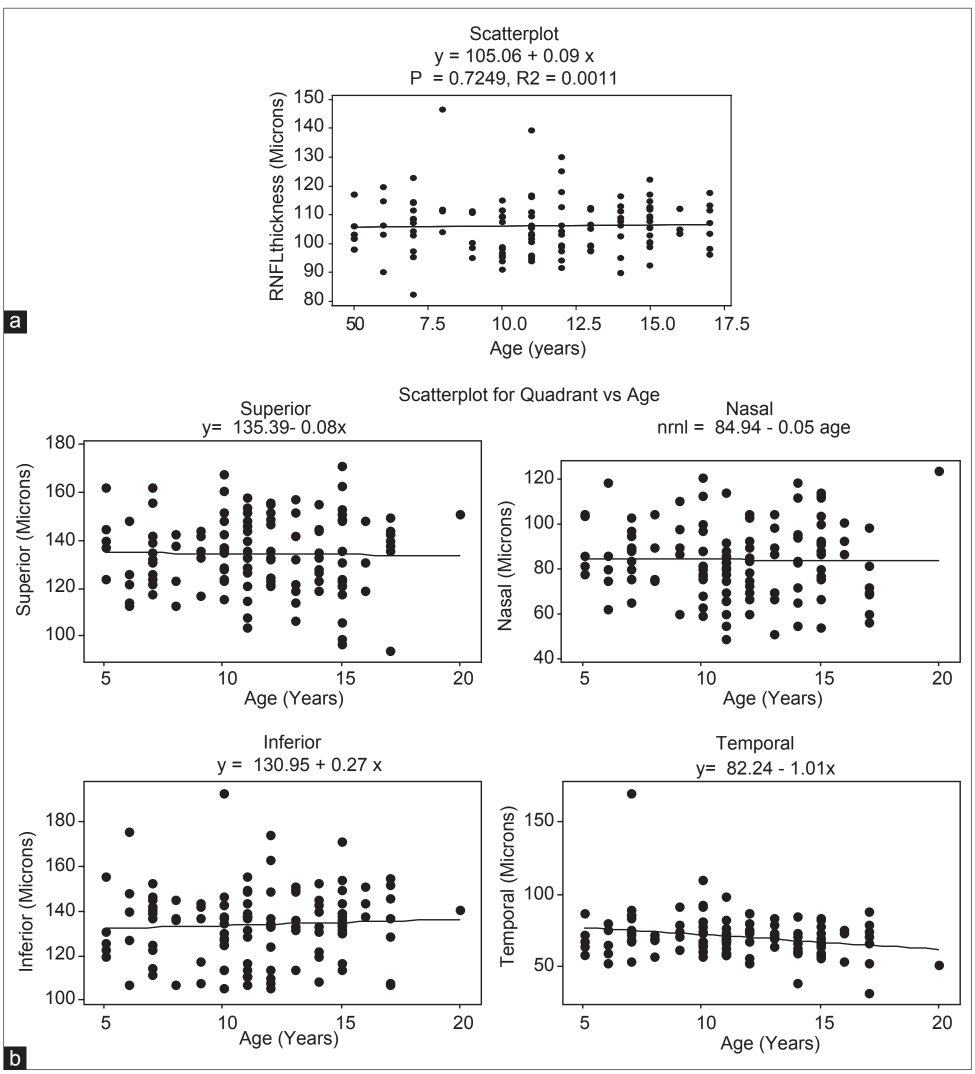

Figure 3: (a) Scatter plot showing average global retinal nerve fiber layer (RNFL) thickness in normal children as a function of age (*n* = 120 eyes). (b) Quadrant thickness as function of age shows no significant relation with age except in temporal quadrant

thickness was not statistically different in males and females ($P = 0.342$).

Regression analysis

In univariate regression analysis, age had a no statistically significant ($P = 0.7249$, $R^2 = 0.0011$) effect on RNFL thickness [Fig. 3a] while it had statistically significant effect on only in temporal quadrant when analyzed for individual quadrant RNFL thickness [Fig. 3b]. The equation for the regression line [Fig. 3a] was $y = 105.06 + 0.0915x$ (95% confidence interval [CI] for the coefficient −422 to. 6052) (y = global RNFL thickness, x = age). There was no significant linear relationship between age and global thickness.

Refraction had a statistically significant ($P = 0.0008$, $R^2 = 0.2102$) effect on RNFL thickness [Fig. 4a]. The equation for the regression line in [Fig. 4a] was $y = 107.38 + 3.205x$ (95% CI for the coefficient, 1.40 to 5.01) (y = global RNFL thickness, x = refraction). There was a linear relationship between refraction and global thickness. After removing outlier, ($n = 119$ eyes) refraction still had statistically significant effect on RNFL thickness. The equation for the regression line after removing outlier was $y = 106 + 2.35x$ (95% CI for the coefficient, 0.70 to 3.99) (y = global RNFL thickness, x = refraction). $P = 0.0060$, $R^2 = 0.1496$. Refraction had statistically significant effect only in nasal quadrant when analyzed for individual quadrant RNFL thickness [Fig. 4b].

Gender did not have a statistically significant effect on global RNFL thickness ($P = 0.342$).When age was controlled for in multivariate regression analysis, refraction still had a statistically significant effect on RNFL thickness ($P = 0.002$); however, when refraction was controlled for, age no longer did ($P = 0.820$). The inclusion of gender into the multivariate analysis did not affect these results.

Discussion

OCT has become a widely used tool in clinical and scientific ophthalmology. Normative data are provided automatically by OCT, but the database only includes individuals 18 years and older, limiting its use in children. It is informative to compare our results with findings from previous studies.

The average RNFL thickness in our study was determined 106.11 ± 9.50 μm which is consistent with findings reported in the literature. Table 3 shows OCT values of previous studies of children and our present study as well. Average RNFL thickness values for our total sample were comparable with

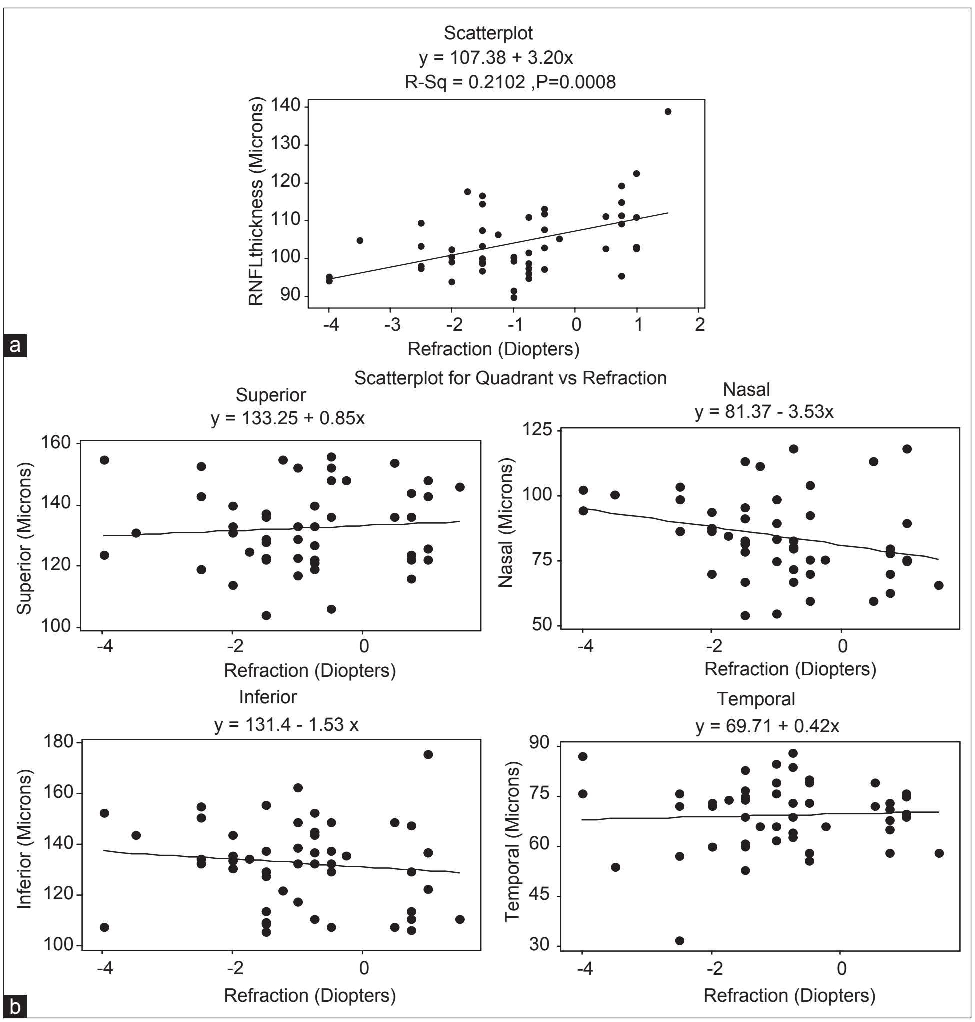

Figure 4: (a) Average global retinal nerve fiber layer (RNFL) thickness in normal children as a function of refraction (n = 120eyes). (b) Quadrant thickness as function of refraction shows no significant relation except in nasal quadrant

Table 3: Comparison of studies reporting OCT-measured global RNFL thickness (μm) in normal pediatric subjects

Author	Ethnicity	Number of children	Age range (years) (mean±SD)	Global RNFL thickness μm (mean±SD)	OCT version
Our study	Indian	120	5-17 10.8±3.24	106.11±9.5	Stratus OCT
Salchow *et al.*[14]	92% Hispanic, 8%, African American, 1%, Caucasian	92	4-17 9.7±2.7	107±11.1	Stratus OCT
Huynh *et al.* Sydney childhood eye study[12,13]	White and East Asian	1765	6	103.7±11.4	Stratus OCT
Gupta *et al.*[22]		18	6-13	100±2.64	Not mentioned
Freedman *et al.*[18]	Black White	286	3-17	108.27±9.8	Stratus OCT
Parikh *et al.*[11]	Asian Indian	59	5-20	100.15±10.8	Stratus OCT
Ahn *et al.*[15]	Korean	72	9-18	105.53±0.33	Stratus OCT
Mrugacz and Bakunowicz-Lazarczyk[17]	Polish	26	10-18	132±24.5	OCT 2000
Larsson *et al.*[26]	Swedish	56	5-16 10.1±3.0	98.4±7.88	Stratus OCT
Leung, *et al.*[19]	Hong kong Chinese	97	9.75 (6.08 to 17.58)	113±9.8 RE 113.1 LE	Stratus OCT
Zhang *et al.*[21]	Chinese (63%) White, (24%) Hispanic, (8%) black, (3%) Asian subjects	199	5-18 10.4±2.7	112.36±9.21	Stratus OCT
Repka *et al.*[29]	White, Black, Hispanic, Latino	37	7–12 9.2±1.5	109.6	Stratus OCT
Samarawickrama *et al.* Sydney childhood eye study (incorporating the Sydney myopia study)[20]	European Caucasians	3382	6 12	101.95 104.57	Stratus OCT
	East Asian		6 12	105.45 107.921	
Turk *et al.*[27]	Turkish	107	6-16 10.46±0 94	106.45±9.41	Spectralis OCT

OCT: Optical coherence tomography, RNFL: Retinal nerve fiber layer, SD: Standard deviation of the mean

those of Salchow *et al*. and Ahn *et al*. (population largely Hispanic and all Korean, respectively) but higher than those of the younger subjects reported by Parikh *et al*. (population mostly Asian Indian), Gupta *et al*. and Larsson *et al*.[11,14,15,22,26]

Compared with the large study of Australian 6-year-olds by Huynh *et al*.[12,13] average RNFL thickness were higher in our study population. Average RNFL thickness of our study was lower with those of Mrugacz and Bakunowicz-Lazarczyk, Leung *et al.*, and Qian *et al*.[17,19,21] By contrast, pediatric OCT values reported by Gupta *et al.* differed from all other reports, possibly owing to technical differences in the protocols used,[22] the authors in this small study did not specify which generation of OCT machine they used.In a recent study by Turk *et al*.[27] the average RNFL thickness by spectral-domain OCT (SD-OCT) for Turkish pediatric patients with ages between 6 years and 16 years was determined as 106.45 ± 9.41 (83.33–141.17) similar to our study though our study measured RNFL on time domain stratus OCT. Possible explanations of difference from various studies include our stricter optic nerve and refractive criteria, and the existence of variation between distinct geographic areas and ethnic groups. The differences between different versions of the device have also been found in adults and may be due to the use of different algorithms between spectral and time domain OCT devices.[28]

The distribution of RNFL thickness (thickest inferiorly and superiorly, thinner nasally, and thinnest temporally) in our study is in agreement with the normal distribution of RNFL around the optic nerve as has been previously reported for OCT data from the normal children[14,15] and adults.[3,5-8] The OCT measurements of RNFL thickness have been previously described as dependent on race, age, axial length, and disc area in adult eyes,[8] with a reported average RNFL thickness decrease of 2.2 μm for every 1-mm increase in axial length, and an average RNFL thickness decrease of 2 μm for every decade of aging.[10] These findings are consistent with pathology studies by Dolman *et al*. suggesting that change due to age-related decay of the optic nerves is not significant until after age 60 years.[28] Compared with adult studies, average RNFL thickness in our population was comparable with but slightly higher than that reported in the younger adults (age, <40 years) by Budenz *et al*. and Leung *et al*.[8,19]

Measurements of RNFL thickness were not dependent on age in our population of children when controlling for refraction. Similarly, Leung *et al*. determined that the RNFL thickness among pediatric patients shows no significant correlation with age.[19] Salchow *et al*. concluded that age did not show a significant effect on RNFL thickness after controlling for the effect of refractive error.[14]

In our study, mean RNFL thicknesses were similar in boys and girls, in accordance with the studies by Salchow *et al*.[14] The effects of refraction on RNFL thickness have been debated; some authors have found no difference

(Mrugacz and Bakunowicz-Lazarczyk 2005) and others have described thinning of the RNFL with greater negative refraction (Huynh *et al*. 2006).[12,17] In the study by Turk *et al*., there was no significant correlation between age, refraction defect, axial length values, and RNFL thickness.[27] In the present study when age was controlled for in multivariate regression analysis, refraction still had a statistically significant effect on RNFL thickness ($P = 0.002$); however, when refraction was controlled for, age no longer did ($P = 0.820$).

The measurements obtained from the different OCT models can vary.[30] Furthermore, this difference may also occur among different models of certain SD-OCT devices.[27,30] Taken together, these findings suggest that differences in OCT technology may be responsible for the differences between our results and previously published data on RNFL thickness.The reason why different versions of OCT yield different values for RNFL thickness is not clear. It is possible that different software algorithms used to delineate the RNFL may be among the factors responsible for this observation.

Our statistical analysis identified factors with a significant effect on RNFL thickness.The current study has several limitation. RNFL thickness is significantly correlated with age and axial length. However, in this study we did not consider potential influential factors for RNFL measurements, including axial length, optic disc size. Secondly axial length and refraction are usually correlated which is not addressed by the present study. Limited numbers and the exclusion of eyes with high refractive errors may have limited our ability to identify additional relationships between RNFL thickness and axial length.

In conclusion, this study is the first to present normative stratus OCT data for Indian pediatric patients. This study demonstrates normative values of retinal thickness and RNFL thickness in the Indian pediatric age group. This information should facilitate evaluation of OCT assessments performed in children with diagnosed or suspected glaucoma as well as those with other optic neuropathies.

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Cite this article as: Pawar N, Maheshwari D, Ravindran M, Ramakrishnan R. Retinal nerve fiber layer thickness in normal Indian pediatric population measured with optical coherence tomography. Indian J Ophthalmol 2014;62:412-8.

Source of Support: Nil. **Conflict of Interest:** None declared.