Original Article

Assessment of peripapillary retinal nerve fiber layer thickness using scanning laser polarimetry (GDx VCC) in normal Indian children

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Purpose: To obtain reference values of RNFL thickness in normal Indian children and to study the association of RNFL thickness with central corneal thickness(CCT) and axial length(AL). **Materials and Methods:** 200 normal Indian children (mean age 8.6 ± 2.9 yrs) were examined on the GDxVCC. The inferior average (IA), superior average (SA), temporal-superior-nasal-inferior-temporal (TSNIT) average and nerve fiber index (NFI) values were recorded and compared between males and females as well as between the different age groups. The association of TSNIT average with AL and CCT was examined. **Results:** Values for the RNFL parameters were-SA: 64.9 ± 9.7, IA: 63.8 ± 8.8, TSNIT average: 53.5 ± 7.7 and NFI 21.5 ± 10.8. Superior, inferior and TSNIT averages did not differ significantly between males and females ($P = 0.25$, $P = 0.19$, $P = 0.06$ respectively; Mann-Whitney U test). No significant differences were found in TSNIT average across age groups. There was a statistically significant positive correlation between CCT and TSNIT average ($r = 0.25$, $r^2 = 0.06$, $P < 0.001$). The correlation TSNIT average and AL($r = -0.12$; $r^2 = 0.01$) was not significant ($P = 0.2$). **Conclusion:** Reference values for RNFL parameters reported for Indian children are similar those reported in adults. There is a small correlation between central corneal thickness and RNFL as reflected in average TSNIT.

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Glaucoma in childhood is a potentially blinding condition, accounting for 2.5% of blindness in children in India.[1,2] The prognosis is largely dependent on early diagnosis, successful treatment by lowering intraocular pressure (IOP) and preventing/treating amblyopia.[3] Difficulty in diagnosis and treatment of glaucoma in these eyes may often be responsible for the disproportionate percentage (up to 18%) of such children in blind institutions around the world.[4]

The diagnosis of glaucoma in young children can be problematic. Reliable visual field examination is often not possible in eyes of children below the age of 8 to 10 years. Evaluations of the IOP and optic nerve head and soft signs like corneal/axial length enlargement are used to assess damage and monitor the progression of glaucoma. Often, these examinations have to be carried out under anesthesia. Objective imaging technologies like GDx VCC (Variable corneal compensation), HRT (Heidelberg Retinal Tomograph) and the Optical coherence tomography (OCT) are now available to document the retinal nerve fiber layer (RNFL) thickness. These allow earlier diagnosis of glaucoma,[5,6] in adults. Scanning laser polarimeter (SLP) is a non-invasive, objective, accurate and reproducible imaging technology designed to quantitatively assess peripapillary RNFL thickness.[7] The RNFL is a birefringent structure that causes phase shift or retardation of polarized light. The GDx VCC is a confocal scanning laser ophthalmoscope with an integrated ellipsometer to measure retardation. Although it does not measure the anatomical thickness of RNFL, it indirectly infers the RNFL thickness point by point in the peripapillary region by measuring the total retardation in the light reflected from the retina.[8,9] The reproducibility of measurements taken using SLP is high in healthy children and adults, as well as in adult glaucomatous patients.[10-18] Variable (VCC) and enhanced corneal compensation (ECC) have improved the sensitivity and specificity of the GDX in the diagnosis of glaucoma.[19-23] The current GDx VCC has a built-in normative database created on the basis of RNFL thickness results obtained from subjects older than 18 years. The RNFL thickness in children differs from that in adults.[18,24-26] Being user-friendly, fast and objective, this technology has potential for use in the paediatric population.[18]

Previous SLP studies have reported a significant age-related RNFL thinning in healthy adults.[27-30] Moreover, SLP derived RNFL thickness is known to be influenced by other variables including ethnicity and refractive error. It has been reported that RNFL is thinner in myopic eyes as compared to emmetropic eyes.[31]

The central corneal thickness (CCT) is also reported to be thinner in myopic eyes and reports suggest a correlation between CCT and RNFL.[32,33] CCT is an important variable in measurement and interpretation of IOP in adult eyes and may influence the progression of glaucoma.[34] We felt it would be of interest to study the association between between CCT and RNFL as well as axial length (AL) and RNFL.

There are three reports of GDx data in children of different ethnicities, of which two had small sample sizes. To the best of our knowledge, there is no published literature on RNFL values in eyes of normal Indian children. The aim of this study was to obtain RNFL parameters using GDx VCC in this population of healthy children of Indian origin, as well as to determine whether RNFL thickness is associated with CCT or AL.

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Materials and Methods

This prospective, observational, cross-sectional study included 200 eyes of 200 consecutive children attending our pediatric clinic between January to September 2010. All children between the age of 3 to 17 years attending our pediatric clinic were included in the study. These children were either referred from their schools for an annual ophthalmic examination or came to our pediatric clinic because the parents were concerned about possible need for spectacles. None of them had any systemic diseases or ocular pathology. Exclusion criteria were: IOP >21 mm/Hg, optic nerve head vertical C/D ratio of > 0.4, axial length (AL) of >24 mm, refractive error of > ± 3D sphere, astigmatism of >2D, BCVA of <0.18LogMAR, previous ocular trauma or surgery, peripapillary atrophy, optic disc hemorrhage, pallor or medullated nerve fibers at the optic disc. Eyes with an optic disc diameter of <1.4 mm or >1.8 mm as measured on the GDx VCC, a typical scan score of <25 or quality score of <8 even after two repeat examinations were also excluded from analysis. Children who were unco-operative for either one of/all of the following examinations, vision assessment, Goldmann applanation tonometry, fundus examination, biometry, refraction and GDx VCC examination were also excluded. Those in whom any of the exclusion criteria was fulfilled in one or both eyes were excluded. When both eyes of the patient fulfilled the inclusion criteria, one eye was randomly included in the study.

The study was approved by the Institutional Ethics Committee and followed the tenets of the Declaration of Helsinki. Informed, written consent was obtained from the parents/legal guardians of the subjects.

A complete ophthalmologic evaluation was performed for each child. This included unaided visual acuity (UAVA), best corrected visual acuity (BCVA), slit lamp biomicroscopy, Goldmann applanation tonometry, axial length (AL), central corneal thickness (CCT), dilated retinoscopy, fundus and GDx VCC examinations. Visual acuity was measured on the ETDRS LogMAR chart in school going children and with the Cardiff Acuity cards for preschool children. CCT was measured using an ultrasonic pachymeter (Ocuscan, Alcon) by a single, trained observer. Axial length was measured on the IOL Master (Zeiss) in all eyes. A standardized dilatation regime was used for all eyes, which included dilatation with cyclopentolate 1% eyedrops instilled twice in both eyes at an interval of ten minutes.

A single, trained observer who did not perform the AL, CCT and refraction performed all the SLP examinations. SLP imaging was performed using the GDx VCC (Carl Zeiss Meditec Inc, Dublin, CA).

The patient's name, age, ethnicity, gender and spherical equivalent of refraction were entered prior to examination. The GDX examination was performed according to the recommendations of the manufacturer. Briefly, the procedure was explained to the child by our pediatric co-ordinator and his/her head was aligned on the headrest of the machine. The child was then asked to maintain fixation on the red internal fixating light. As the first step, an initial image was acquired to compensate for anterior segment birefringence. Next, a compensated image with a 3.2mm scan circle diameter was used which was centered on the optic nerve head; the mean of three measurements was used. In case of a poor image quality, artificial tears were instilled and the examination was performed again. All children in whom we could obtain GDX images cooperated for the other parts of the examination.

The GDx VCC parameters studied were: superior average (SA, 25° to 144°), inferior average (IA, 215° to 334°), nerve fiber indicator (NFI) and the temporal-superior-nasal-inferior-temporal (TSNIT, entire 360°) average. These parameters were compared between boys and girls. The TSNIT map is displayed at the bottom of the GDx VCC printout and stands for Temporal-Superior-Nasal-Inferior-Temporal. It displays the RNFL thickness values along the calculation circle starting temporally and moving superiorly, nasally, inferiorly and ending temporally. In a normal eye the TSNIT plot follows the typical 'double hump' pattern, with thick RNFL measures superiorly and inferiorly and thin RNFL values nasally and temporally.

Statistical analysis

Data was entered in an Excel workbook (Microsoft Excel®) and analyzed using Statistical Product and Service Solutions (SPSS software,version 12, Windows). Shaipro-Wilk's test was used to assess normality of quantitative variables. The test results showed deviation from normal distribution. (In all the cases the significance of the test was below 0.05). As the data were not adhering to normal distribution; non-parametric tests have been used for comparison of location/median between different groups. Mann Whitney test has been used as an alternative to independent samples *t* test to compare two groups, and the Kruskal Wallis test has been used as a nonparametric alternative to ANOVA (analysis of variance) test for comparing more than two groups.

Descriptive statistics were used for describing actual values of the Superior, Inferior Average, TSNIT average and NFI in terms of mean and standard deviations alongwith 95% confidence interval.

The SA, IA, TSNIT average and NFI were further compared between males and females to check for any statistically significant differences. For this purpose, the Mann Whitney U Test was used and a $P < 0.05$ was considered to be statistically significant.

These parameters were also compared between different age groups, for which the Kruskal Wallis test was used and a $P < 0.05$ was considered to be statistically significant.

Pearson's correlation test was performed to detect an association between the TSNIT average and CCT/AL.

Results

The recruited numbers were 204 eyes of 204 patients in the study. A total of 466 patients were seen in the pediatric clinic during the study period, of which 204 patients fulfilled the inclusion criteria and gave informed consent to participate in the study. Of the 262 patients excluded, 30 refused to participate in the study, 55 were diagnosed with co-existing ocular morbidity, 10 had a history of ocular trauma and the remaining 167 patients had refractive error of >±3D sphere or astigmatism of >2D. Both eyes of all 204 eligible patients were found to fulfill inclusion criteria, but only one eye was randomly selected for the study.

A typical scan score of >8 was achieved in 192 eyes on the first examination. In 8 eyes, an initial scan score of <8 was obtained, which improved on the second test. Four eyes were excluded because of inability to obtain good quality GDx VCC scan even with repeat testing. The mean age of the subjects was 8.62 ± 2.84 years (range 3 to 15 years). There were 120 males (60%) and 80 females (40%). There were 98 right eyes (49%) and 102 left eyes (51%). The mean IOP was 13.02 ± 2.3 mm/Hg (range 8-18 mm/Hg). BCVA was 0.13 ± 0.17 logMAR, with a mean spherical equivalent of refraction of 1 ± 1.06 dioptres. The mean AL was 22.88 ± 1.00 mm (range 20.73-23.92 mm).

Table 1 lists the mean, SD and 95% confidence interval for the output parameters studied, i.e. SA, IA, TSNIT average, and NFI (nerve fiber indicator). Table 2 shows the SA, IA, TSNIT average and NFI in males and females. The TSNIT graph followed the "double hump" pattern, similar to that seen in adult eyes. No significant difference was found between the RNFL parameters of males and females (P = 0.25 for SA, P = 0.19 for IA, P = 0.06 for TSNIT average, and P = 0.36 for NFI; Mann Whitney U Test).

The 200 eyes were divided into 4 groups according to age: ≥3 - ≤/6 years, >6 - ≤/9 years, >9-≤12 years, and >12-≤15 years. Although we intended to enroll children upto 17 years of age, the oldest child enrolled in the study was 15 years of age. RNFL parameters were compared between these groups using the Kruskal Wallis test [Table 3]. We also compared SA, IA, and TSNIT average in children aged below the median age (9 years) to those above the median. No significant difference was detected on this comparison for any of the parameters (P = 0.92 for SA, P = 0.28 for IA, P = 0.22 for TSNIT average), or between different age groups [Table 3]. Pearson's correlation test was performed to detect an association between the TSNIT average and CCT/AL. A positive correlation was seen between CCT and the TSNIT average (r = 0.25, R^2 =0.06, P < 0.0001). A weak negative correlation was found between TSNIT average and AL (r = −0.12; r^2 = 0.01; P = 0.2). However, this correlation was not found to be statistically significant.

Table 1: Retinal nerve fiber layer thickness parameters obtained on the GDx VCC in 200 normal children

Parameter	Mean	Standard deviation	95% confidence interval
Superior average	64.93	9.73	63.57-66.29
Inferior average	63.89	8.81	62.66-65.12
TSNIT average*	53.52	7.77	52.43-54.60
NFI	21.56	10.81	20.05-23.06

*TSNIT: Temporal, superior, nasal, inferior, temporal, NFI: Nerve fiber index, VCC: Variable corneal compensation

Table 2: Comparison of retinal nerve fiber layer thickness parameters between males and females

Parameter (mean±SD^)	Males (n=120,60%)	Females (n=80,40%)	P Value*
Superior average	64.32±8.81	66.93±11.72	0.25
Inferior average	63.48±7.84	65.93±9.83	0.19
TSNIT average	52.84±6.05	55.56±11.0	0.06
NFI	22.02±11.52	20.07±8.74	0.36

*Mann Whitney U test, ^SD: Standard deviation, TSNIT: Temporal, superior, nasal, inferior, temporal, NFI: Nerve fiber index

Discussion

To the best of our knowledge, this is the first study to report RNFL thickness values in eyes of healthy children of Indian origin on the GDx VCC. Scans could not be obtained in only four patients (2%), all of whom were in the age group of 3 to 6 years. We could reliably perform the GDx VCC examination in children as young as 3 years. Thirty nine eyes (20%) were from the patients aged 3 to 6 years; 4 patients were aged 3 years. Any test in children has to be easy to use. While this technology facilitates ease of examination, we believe that the role of technician/counselor very important and contributed to the number of successful scans. Taking time with the patient as well as parents, and explaining the procedure to them in a friendly manner, was crucial to obtaining co-operation. In many instances the mother underwent the examination prior to the child. The mother then told the child how easy and fun this exam was comparing it to a video game/television show. The entire procedure, including counseling took about 20 to 25min per child.

Three other studies[18,24,35] have reported the use of the GDx VCC in normal children of different ethinicities. Table 4 shows the TSNIT average in the current study as well as the other three reports. The SA, IA and TSNIT averages in these studies appear to be higher than the Indian population. However, statistical comparison between these studies with our study was not possible because the study designs were different and the published results are based on normally distributed data, which, in our case are not normal. We also compared the values from the current study to reported RNFL parameters on the GDx and GDx VCC in adult Indian and non-Indian eyes (Dada T *et al*., Paper presentation, abstract in the Asian J Ophthalmol 2006; 8: supplement no. 2) [Table 5] and found that in our study, the values in children were similar to those reported in adults. Unless studies with larger numbers show otherwise, based on the results of our study, it seems that normative RNFL data from adult eyes can probably be used for children as well.

Table 3: Comparison of retinal nerve fiber layer parameters according to age groups

Parameter (mean±SD^)	±3-<6 yrs (n=39)	>6-≤9 yrs (n=40)	>9-<12 yrs (n=62)	>12-≤15yrs (n=59)	P value*
Superior average	65.30±8.90	63.95±9.45	64.65±7.66	67.49±15.50	0.56
Inferior average	64.19±8.19	62.7±9.30	63.96±7.77	66.06±11.14	0.36
TSNIT average	53.73±5.21	52.07±6.82	53.27±5.08	57.28±15.28	0.10
NFI	22.21±11.11	22.93±12.27	20.76±10.0	19.77±9.02	0.61

Kruskal Wallis test, ^SD: Standard deviation, TSNIT: Temporal, superior, nasal, inferior, temporal, NFI: Nerve fiber index

Table 4: GDx VCC parameters (superior asverage, inferior average, and TSNIT average) reported in published pediatric studies

Authors	Ethnicity	No. of eyes	Age (yrs) (mean±SD)	Superior average (mean±SD)	Inferior average (mean±SD)	TSNIT average (mean±SD)
Lundvall *et al*.[18]	White (Swedish)	72	8 (4.5-15.5)*	73.5±2.3	73.5±2.0	61.1±1.5
Salvetat *et al*.[24]	White (Italian)	98	8.5±2.8	70.6±8.0	69.4±7.8	58.8±5.5
Coloma-Gonzales *et al*.[35]	Spanish	217	6-9 yrs#	71.35	70.08	59.43
Current Study	Indian	200	8.62±2.85	64.94±9.76	63.90±8.83	53.56±7.77

*Median (range), #Range, SD: Standard deviation, TSNIT: Temporal, superior, nasal, inferior, temporal, VCC: Variable corneal compensation

Table 5: GDx VCC parameters (superior average, inferior average, and TSNIT average) reported in adult normal population and the current study

Authors	No. of eyes	Age (Yrs)	Superior average (µm, mean±SD)	Inferior average (µm, mean±SD)	TSNIT average (µm, mean±SD)
Dada *et al*[!]	200	40-60 (range)	66.8±6.7	62.1±6.6	54.8±4.1
Deleon-Artega *et al*.[41]	149	40.3±11.3 (mean±SD)	68.5±1.3	65.4±1.3	56.0±0.9
Badala *et al*.[45]	46	58.9±6.8 (mean±SD)	65.9±2.3	64.8±2	55.1±1.7
Reus and Lemij[16]	73	59.0±11.0 (mean±SD)	66.7±1.7	61.5±2.1	54.8±1.4
Da Pozzo *et al*.[28]	62	64.7±6.5	64.7±1.3	60.9±1.7	53.7±1.0
Vijaya L, *et al**[36]	180	Males (36.79±15.61, mean±SD) Females (33.68±16.37, mean±SD)	Males: 75.37±15.1 Females: 73.62±10.16	Males: 82.39±13.41 Females: 77.73±10.32	-
Current study	200	3-18	64.94±9.76	63.90±8.83	53.56±7.77

[!](Dada T *et al*. Paper presentation, abstract in the Asian J Ophthalmol 2006;,8:supplement no. 2), *GDx without variable corneal compensator was used in this study, SD: Standard deviation, TSNIT: Temporal, superior, nasal, inferior, temporal, VCC: Variable corneal compensation

The effect of age on the optic nerve axonal counts in normal individuals has been the subject of various studies which showed a negative association between age and optic nerve axonal counts.[27-29,35,36] Balazsi *et al*.,[25] reported a mean loss of 5,637 axons per year, corresponding to a total loss of about 35% of optic nerve axons during the course of an individual's life span. Another study using the OCT reported a non-uniform reduction in RNFL thickness with age.[30] The numbers in our study are small but there was no significant difference between the ages (in the small age range) that was used [Table 3].

The SA, IA, TSNIT average and NFI were not significantly different between males and females [Table 2]. Similar results have been reported in children by Lundvall and colleagues.[18]

In a previously published study, Kaushik *et al*.,[33] compared peripapillary RNFL thickness parameters as measured on the OCT to CCT in eyes with ocular hypertension (OHT) as well as in normal control subjects. Their study reports that OHT patients with thinner corneas had thinner RNFL measurements compared to those with thicker corneas as well as normal subjects. However, in their study, there was no difference in RNFL thickness measurements in normal subjects with thinner or thicker corneas. In another study, Henderson and colleagues[37] found on the GDx VCC that OHT patients with thinner corneas had a higher NFI, indicating a thinner RNFL. However, no correlation is reported between CCT and RNFL thickness in healthy adult eyes.[38] We found a positive weak correlation between the CCT and RNFL ($r = 0.25$, r^2 6%). However, in these normal pediatric eyes, we were unable to explain this trend. Future studies aimed specifically at comparing CCT and RNFL thickness with a larger sample size would be required to study this possible association and its clinical significance.

We also found a weak negative correlation between AL and TSNIT average ($r = -0.12$, $P = 0.2$) Certain studies have demonstrated that RNFL thickness as measured on the Cirrus (HD) OCT has a significant correlation with AL, RNFL thickness decreasing as AL increases.[31,39,40] Our study excluded eyes with long axial lengths and was not powered to detect such an association. The other available report also did not report a correlation between AL and RNFL thickness as measured by the GDx ECC.[31]

A weakness in our data is that we included only eyes with AL of ≤24 mm and a spherical equivalent of refraction of ≤±3D. It is also known that optic disc size is variable in the general population and, axon count increases as optic disc size increases.[41] It is therefore, not possible to extrapolate this data to eyes with significant refractive error or large/small optic discs, factors known to affect RNFL thickness.[25,31,41-46] Additionally, this technology does not help in the difficult young age group which is more at risk for amblyopia. Finally, another limitation is that the study population was a selected from those attending our clinic, and therefore, may not be a true representative of the Indian children in general.

In conclusion, our study provides preliminary reference values for RNFL data for normal children of Indian origin using the GDx VCC. Based on our study results, RNFL values in children appear to be similar to those reported in normal adults. Comparison with the published literature suggests that there is no clinically significant difference between the different races either. These findings suggest a role for GDx VCC as an objective means of diagnosis of glaucoma in the pediatric population.

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