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Original article

Retinal nerve fibre layer thickness in a healthy Nepalese population by spectral domain optical coherence tomography

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Abstract

Objective: To determine the normal values for peripapillary retinal nerve fibre layer thickness (RNFL) measured by spectral domain optical coherence tomography (SD-OCT) in a healthy Nepalese population and to compare the RNFL thicknesses between the genders and among the various age groups. **Material and methods**: One hundred and fifty six eyes of 156 healthy Nepalese subjects (66 males and 88 females) of various age groups were enrolled in this observational, cross-sectional, hospital-based study. The peripapillary RNFL of the randomly chosen eye of each subject was imaged with a high resolution SD-OCT (Spectralis HRA+OCT, Heidelberg Engineering). The RNFL thickness was measured around the optic nerve head using 16 automatically averaged, consecutive, circular B scans with a 3.4 mm diameter and compared between the genders and among the various age groups. **Results**: The mean age of the subjects was 38.94 ± 17.00 years (range - 14 to 76 years). The average peripapillary RNFL was found to be 102.64 ± 9.56 µm (95 % CI 97.01 - 101.93). The mean ± SD peripapillary RNFL measurements at the superior, nasal, inferior and temporal sectors in the study population were 129.51 ± 15.09 µm, 76.55 ± 12.02 µm, 134.53 ± 17.19 µm and 70.74 ± 15.53 µm respectively. The average RNFL thickness was 99.47 ± 10.18 µm in the male whereas it was 105.09 ± 8.31 µm in the female participants. The RNFL decreased by 2.26 µm per age perdecade. **Conclusion**: The Average RNFL thickness is 102.64 ± 9.56 µm in the Nepalese population. Gender and age related variation in the RNFL can serve as a useful guideline in the diagnosis of glaucoma in our population.

Keywords: retinal nerve fibre layer, optical coherence tomography, optic nerve head

Introduction

RNFL thickness can be measured using optical coherence tomography (OCT), which is an imaging technique first described by Huang et al (1991). The spectral domain optical coherence tomography (SD-OCT) has improved the depth resolution by a factor of two and has enabled a higher acquisition rate by more than 50 times, resulting in considerable improvement in the image quality. The study by Sakamoto et al, (2008) has shown that the averaging of more than four OCT B scans provided much better visualization of retinal structures and identification of thinning and defects in the retinal layer. OCT is an imaging modality that relies on low-coherence interferometry to measure the echo time delay and the intensity

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of the back-scattered and back-reflected light from the internal microstructure in biologic tissue (Marsh et al, 2010). Because of its minimal invasiveness and quick image acquisition time, combined with high resolution and cross-sectional image quality, OCT analysis of optic disc has become a very valuable potential tool in the evaluation and management of patients with primary open angle glaucoma (POAG) and glaucoma suspects.

Recent studies on RNFL thickness have shown that structural damage to the RNFL is seen preceding the functional damage detected in the automated visual field in glaucoma. A visual field defect is detected only after the loss of 30 to 50% of retinal ganglion cell axons at the location being tested (Quigley et al, 1982). So, the defects in the RNFL can be an early sign of glaucoma. According to the World Health Organization (WHO), glaucoma is a leading cause of irreversible blindness. If diagnosed and treated in the earliest phase, its progression can be stopped or delayed (Lopez-Pena et al, 2011).

Various studies have suggested that clinically-detected morphologic changes secondary to glaucoma can appear in the very early phases of the disease, before the appearance of functional defects (Sommer et al, 1991). The peripapillary RNFL is also found to be thinner in older age, with a greater axial length (AL) and a smaller optic disc area. Studies have shown that for every 1mm increase in the AL, the mean RNFL thickness is thinned by 2.2 microns, and with every decade of life, the thinning is seen by 2 microns. Caucasians have a slightly thinner RNFL than Hispanic and Asians. There is no evident relation seen between the RNFL thickness and gender (Budenz et al, 2007). Thinning of the RNFL is also seen in eyes with optic nerve diseases (Costello et al, 2008; Jeanjean et al, 2008). Normal reference values of RNFL thickness, using the stratus OCT analyser, has been established in cohort of the normal population which is incorporated in the stratus OCT RNFL analysis software package (Budenz et al, 2007). There may be a racial difference in the NFL thickness between the western and our populations. Till date, to the best of our knowledge, no such studies regarding the NFL thickness measurement have been done among our populations. There are a few SD-OCT machines being operated for the measurement of RNFL thickness in Nepal. Since the reference values fed in the machine are from western populations, it is comparing the data from two different races. So, we would like to study the normal range of the peripapillary RNFL thickness in non-glaucomatous healthy individuals without any retinal pathology in our population.

Material and methods

Study population

Patients above 10 years of age from the general out-patient department (OPD) were recruited for the study. The research adhered to the tenets of the Declaration of Helsinki for research involving human subjects. The study protocol was approved by the Institutional Review Board of the Institute of Medicine and informed consent was obtained from all the participants of the study.

Procedures

Complete ophthalmic examination was performed on every candidate for enrolment into the study. Ophthalmic evaluation included refraction, best corrected visual acuity (BCVA), detail anterior segment evaluation with a slit-lamp biomicroscope, detailed fundus evaluation after full dilatation using a slit-lamp biomicroscope with a + 90 D lens (Volk) and indirect ophthalmoscope with a + 20 D lens (Volk). Intraocular pressure measurement with a Goldmann applanation tonometer was done in all patients. White on white perimetry (Octopus 301 Haag- Streit, Interzeag International - AG, Schlieren, Switzerland) was also performed. The anterior chamber angle structure was evaluated in all cases using a Goldmann, one-mirror, indirect gonioscopy lens.

Inclusion criteria

Subjects with a normal slit-lamp biomicroscopic examination, normal funduscopic examination, normal range of tonometry (corrected IOP < 21 mm Hg), and normal automated perimetry were included in the study. The normal funduscopic evaluation was taken as a cup-disc ratio (CDR) of less than 0.4:1, asymmetry of CDR in both the eyes being less than 0.2, a healthy appearance of the neuroretinal rim (NRR) following the 'ISNT' rule and no peripapillary atrophy. When both the eyes were eligible, one randomly selected eye was included in the study.

Exclusion criteria

Patients with a history of intraocular surgery other than cataract surgery, a high refractive error of more than ± 5 D, patients with active retinopathy, maculopathy, age-related macular degeneration (ARMD) and other retinal diseases were excluded from the study.

RNFL thickness measurement using Spectral Domain Optical Coherence Tomography (SD-OCT)

All subjects were scanned using the commercially available SD-OCT (Spectralis HRA+OCT, Heidelberg Engineering). This instrument used a wavelength of 820 nm in the near infrared spectrum in the SLO mode. The light source of the SD-OCT was a super luminescent diode with a wavelength of 870 nm. Infrared images and OCT scans (40,000 A-Scans/sec) with the dual laser scanning systems were acquired simultaneously. Sixteen consecutive circular B-scans (3.4 mm diameter, 768 A-scans) centred at the optic disc were automatically averaged to reduce the speckle noise. An online tracking system compensated for the eye movements. The Spectralis software version 3.2.1 allowed separate measurements of the total retinal thickness and the RNFL thickness. The RNFL borders were clearly identified and marked automatically by the segmentation software. The retinal vessels within the RNFL were considered to be part of the RNFL. To show the distribution of the RNFL thickness around the optic disc, the thickness data of the circular scans was averaged for 4 sectors (45-degree each), and the superior and inferior segments each were further grouped into 2 sectors (22.5-degree each) as supero-temporal, supero-nasal, infero-temporal and infero-nasal. A single user performed at least two retinal scans in all the cases and the scans with the best image quality were considered for the study. Both sectoral and average RNFL thicknesses were included in the study.

Statistical methods

Statistical analysis was done with SPSS 17.0 (SPSS Inc, Chicago, IL). The level of significance was á = 0.05 in all the statistical tests. Descriptions of the results included mean and standard deviation. Regression analyses were performed with the average and sectoral RNFL thicknesses as dependent variables and age as the influencing variable. Linear models were employed with the corresponding scatter plots showing best-fit regression curves and regression coefficients. Association of the RNFL values with age was examined using Pearson correlation coefficient while that with gender was examined using Spearman rank order correlations. Unpaired t-test was used to study the gender-wise comparisons of the RNFL measurements.

Results

The study included 156 normal eyes (71 right eyes, 85 left eyes) of 156 healthy subjects fulfilling the inclusion criteria. The mean age of the subjects was 38.94 ± 17.00 years (range 14 to 76 years). The distribution of the subjects according to the age group and gender is shown in Table 1.

Table 1: The distribution of subjects by age range and gender

Age group (in years)	Gender		Number of subjects
	Male	Female	
10 - 19	6	18	24
20 - 29	18	10	28
30 - 39	8	16	24
40 - 49	7	23	30
50 - 59	14	15	29
60 yrs and above	15	6	21
Total	68	88	156

Table 2: The average and sectoral RNFL thickness in the different age groups

Age range (in years)	Retinal nerve fiber layer thickness in μm (Mean ± SD)				
	Average	Superior	Nasal	Inferior	Temporal
10-19	106.29±7.26	133.88±10.97	72.21±9.46	141.46±10.84	77.17±13.95
20-29	102.68±9.37	131.14±14.19	78.29±12.24	131.11±20.41	72.43±17.58
30-39	105.83±6.04	134.25±12.74	77.17±7.19	139.67±10.96	68.83±7.53
40-49	105.87±9.27	132.20±14.41	81.23±13.79	134.97±18.80	74.27±17.11
50-59	103.59±7.34	132.97±12.06	80.45±11.79	136.48±19.65	70.31±16.96
60 yrs and above	88.86±6.49	108.29±10.34	66.43±9.67	121.86±6.93	58.86±10.42

Table 3: The correlation between the age and the RNFL thickness (in μm) in all Participants

Parameter	Equation	P	R
Superior RNFL thickness	y = - 0.346x + 142.96	< 0.01	- 0.389
Nasal RNFL thickness	y = - 0.065x + 79.09	0.251	- 0.092
Inferior RNFL thickness	y = - 0.157x + 140.65	< 0.01	- 0.221
Temporal RNFL thickness	y = - 0.238x + 80.01	< 0.01	- 0.297
Average RFNL thickness	y = - 0.227x + 111.46	< 0.01	- 0.403

Table 4: The average and the sectoral RNFL thickness (in μm) distribution by gender and their correlation

Gender	Retinal nerve fiber layer thickness in μm (Mean ± SD)				
	Average	Superior	Nasal	Inferior	Temporal
Male	99.47±10.18	126.16±16.44	74.71±12.14	130.79±16.82	67.59±14.25
Female	105.09±8.31	132.09±13.49	77.98±11.79	137.42±17.01	73.17±16.11
ρ	0.294**	0.164*	0.141	0.221**	0.166*
P+	<0.01	<0.05	0.092	<0.01	<0.05

* Correlation is significant at 0.05 level
**Correlation is significant at 0.01 level
+ Independent t-test showing gender-wise comparisons of various parameters

Table 5: The average and sectoral RNFL thickness in emmetropia, myopia and hyperopia

Group	Average RNFL thickness (in μm)	Superior RNFL thickness	Nasal RNFL thickness	Inferior RNFL thickness	Temporal RNFL thickness
Myopia	102.50 ±4.95	136±1.41	63.50±3.53	127.50±12.02	81.00±11.31
Emmetropia	112.06 ±7.86	134.58±16.6	86.69±9.56	143.69±10.17	73.50±8.46
Hyperopia	108.0 ±8.68	142.5±15.9	81.25±9.16	141.83±12.92	74.08±12.38

The average RNFL thickness in the study population was 102.64 ± 9.56 µm (95% CI 97.01-101.93). The mean ± SD peripapillary RNFL measurements at the superior, nasal, inferior and temporal sectors in the study population were 129.51 ± 15.09 µm, 76.55 ± 12.02 µm, 134.53 ± 17.19µm and 70.74 ± 15.53 µm respectively. Figure 1 provides the summary of the RNFL thickness in each of the 4 measured sectors. The RNFL was thickest in the inferior sector followed by the superior, nasal and temporal sectors. The RNFL thicknesses (µm) in all 4 sectors differed at the $P < 0.01$ level. The thickness in the inferior sector was significantly greater than at the superior one, t (310) = - 2.745, p = 0.006. Similarly, the thickness in the nasal sector was significantly greater than at the temporal one, t (310) = - 3.699, $p < 0.001$.

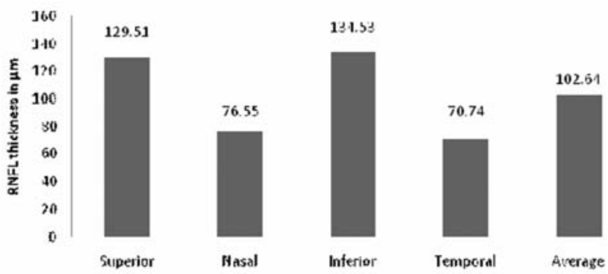

Figure 1: The average and sectoral RNFL thickness in all subjects

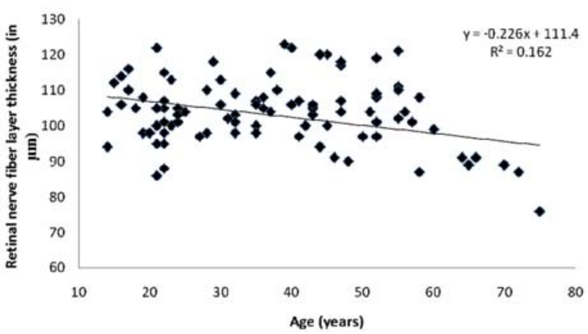

Figure 2: The relationship between the average retinal nerve fiber layer thickness and age

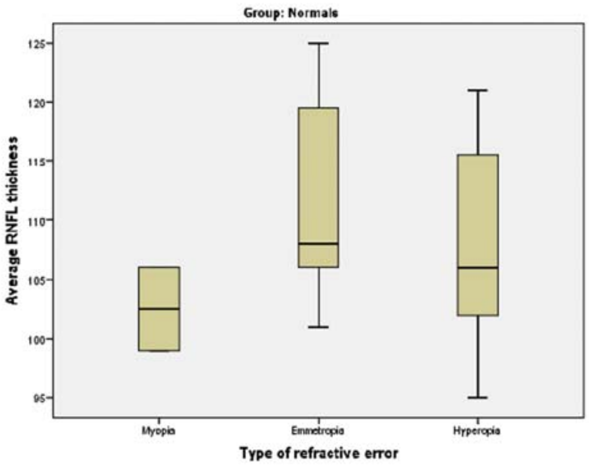

Figure 3: Average retinal nerve fiber layer thickness of emmetropics, myopics and hypermetropics

The average and sectoral RNFL thicknesses were also studied in the various age groups. The average RNFL thickness was 106.29 ± 7.26 µm in the 10 - 19 years group, 102.68 ± 9.37 µm in the 20 - 29 years group, 105.83 ± 6.04 µm in the 30 - 39 years group, 105.87 ± 9.27 µm in the 40-49 years group, 103.59 ± 7.34 µm in the 50 - 59 years group and 88.86 ± 6.49 µm in the 60 years and above age group. Table 2 shows the summary of the average as well as the sectoral RNFL thicknesses in the different age groups.

The association between the average RNFL thickness and age was statistically significantly ($r = - 0.403$, $p < 0.01$). The superior, inferior and temporal RNFL thicknesses showed a negative correlation with age, whereas the nasal sector showed no significant age dependency (Table 3). The linear regression analysis, with the average RNFL thickness as the dependent variable and age as the influencing variable, showed the average RNFL thickness being significantly influenced by age, with the RNFL decreasing by 2.26 µm per age-decade decrease (Figure 2). The coefficient of determination (R2) for the model was 0.16. The study on the effect of age on the sectoral RNFL thickness measurements revealed a decrease of 3.46 µm per decade in the superior sector, 1.57 µm per decade in the inferior sector and 2.38 µm per decade in the temporal sector respectively **(Table 3).**

The average RNFL thickness was 99.47 ± 10.18 µm in the male participants whereas it was 105.09 ± 8.31 µm in the female participants. Comparisons using unpaired t-test revealed that all the RNFL measurements were significantly thicker in females than in males except for the nasal RNFL thickness, t (154) = - 1.696, p = 0.092.

The average as well as the superior, inferior and temporal RNFL thicknesses correlated significantly with gender but the correlation between the nasal RNFL thickness and gender was not statistically significant (p = 0.141, p = 0.08) **(Table 4).**

The average RNFL thickness was lowest in myopia (102.50 ± 4.95 µm) followed by hyperopia (108.0 ± 8.68) and emmetropia (112.06 ± 7.86). However, the comparison of the average RNFL thickness among the three groups revealed no statistically significant difference. The RNFL thickness was further analyzed in different quadrants for comparison among the three groups of refractive error which revealed a statistically significant difference only in the nasal quadrant ($p < 0.05$) **(Table 5)**

Discussion

Optic nerve head evaluation as well as RNFL assessment are important diagnostic tools for the early detection and management of glaucoma. Studies have shown that RNFL damage occurs in glaucoma (Bowd et al, 2000; Sommer et al, 1977; Sony et al, 2004; Quigley et al, 1980). RNFL thinning is also seen in myopia (Budenz et al, 2007; Hougaard et al, 2006) and in old age (Lee et al, 2000; Sony et al, 2004). RNFL thickness may show a racial variation as well (Bendschneider, 2010; Poinoosawmy et al, 1997; Repka et al, 1989; Sony et al, 2004; Tsai et al, 1995). According to Zeimer, quantitative detection of glaucomatous damage at the posterior pole by retinal thickness mapping may provide a unique method for the detection and monitoring of early glaucomatous tissue loss (Zeimer et al, 1998). The mean thickness of the RNFL seen in our study was 102.64 µm which is comparable to the other studies done in other Asian countries (103.0 to 108.4 µm) (Choi et al, 2005; Hsu et al, 2008; Sony et al, 2004). However, the studies from western populations showed the mean RNFL thickness to be lower (85.8 - 99.36 µm) than in Asian populations (Bendschneider et al, 2010; Bowd et al, 2000; Lopez-Pena et al, 2011). The differences in the RNFL thickness among Asians, coloured people and Western populations may have racial causes or may be dependent upon some physical phenomena. A darker choroid may alter the B-scan thickness in comparison that by a lighter choroid due to the altered reflectance of laser light.

When the sectoral thickness was evaluated, the RNFL thickness was thickest at the inferior quadrant, followed by the superior, nasal and temporal quadrants, with two humps at the superior (129.51µm) and inferior (134.53µm) quadrants and two troughs at the nasal (76.55µm) and temporal (70.74µm) quadrants, following the 'ISNT' rule. A study by Sony et al (2004) also described the similar pattern of thickness but the difference in thickness of the superior (131.00 ± 9.0 µm) and inferior quadrant (132.34 ± 14 µm) was not statistically significant. Kanamori et al (2003), in their study of 160 normal eyes, showed slightly higher values. They found that the thickness of the RNFL was maximum at the superior quadrant (145.5 ± 19.6µm), followed by the inferior (143.1 ± 19.5 µm), temporal (98.7 ± 20.8µm) and minimum at the nasal quadrant (92.6 ± 20.4 µm). Their observation also did not follow the previously described 'ISNT' rule. Bowd et al (2000) found lower values as compared to that of our study although their study included only 30 normal cases. They noted a higher inferior quadrant RNFL thickness (107.6 µm) followed by the superior (105.7μm), whereas the temporal quadrant had a greater thickness (66.2 μm) when compared to the nasal quadrant (61.8 μm). These variations in the quadratic RNFL thickness can again be attributed to the racial variation in the population studied. In addition, the study from Korea has shown the nasal quadrant to be thinner than the temporal (Choi et al, 2005).

There was a significant difference in the RNFL among males and females. The males had a thinner (99.47 ± 10.18 μm) and a statistically significant difference in the RNFL thickness when compared to that of the females (105.09 ± 8.31 μm). Sony et al(2004) did not observe the significant difference in males and females but Schuman et al (1995) showed that the nerve fibre layer of men was usually thinner than that of women, as seen in our study, but the difference was not statistically significant.

Various reports have shown that with advancing age, the RNFL thickness decreases (Costello et al, 2008; Sommer et al, 1977). Similar findings were also seen in our study. The average RNFL thickness was significantly associated with age. The superior, inferior and temporal RNFL thickness showed a negative correlation with age but the nasal quadrant did not do so. The linear regression analysis showed that the RNFL thickness decreased by 2.26 microns per decade of age. Budenz et al (2007) showed the decay per decade to be 2.0 microns. Similarly, Bendschneider et al (2010) reported the decrease in the RNFL thickness to be 1.90 microns per decade. Closer home, Mansooriet et al (2012) has also shown a negative correlation of the RNFL with age where the average RNFL thinning was 0.116 microns per year.

When the RNFL was compared among emmetrops, myops and hypermetrops, the average RNFL thickness was lowest in myopia (102.50 ± 4.95 μm) followed by hyperopia (108.0 ± 8.68) and emmetropia (112.06 ± 7.86). But the difference was not significant statistically. This could be because cases of higher refractive error were excluded from the study. The RNFL thickness was further analyzed in the different quadrants for comparison among the three groups of refractive error which revealed a statistically significant difference in only the nasal quadrant ($p < 0.05$).

As we all know, the OCT is an expensive machine; and, with the limited resources, we may not be able to use it exclusively for the examination of RNFL, Many centres in the country are using the machine for various macular disease evaluation, and, so, the OCT can also be utilized to evaluate the RNFL in various other diseases as well.

Since this was a hospital-based study with a small sample size, the results may not reflect the actual state of the population as a whole. A population-based study with a larger sample size would be more reliable to justify our findings. A longitudinal study with serial RNFL measurements would be more relevant to prove the decay of RNFL with age. As the thin CCT is also considered to be a risk factor for glaucoma, the comparison of CCT and RNFL thickness could be of significant value in diagnosed cases of glaucoma. Since we excluded the cases of high refractive error (greater than ± 5 D), we could not compare the RNFL thickness with significantly long or short axial lengths. In this study, we just tried to compare the RNFL thickness of our population with that of western populations; but further study to compare the same among the various ethnic groups of the country may give some important information as well.

Conclusion

Our study provides a normative database for the retinal nerve fibre layer thickness in normal Nepalese eyes by SD-optical coherence tomography. The gender as well as the age-related variations in the RNFL can serve as a useful guideline in the diagnosis of glaucoma in our population.

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