Original article:

To assess the influence of disc area on retinal nerve fibre layer thickness measured and to find significant association between disc area and retinal nerve fiber layer thickness by spectral domain optical coherence tomography Method Single observer

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ABSTRACT

Purpose: To assess Retinal nerve fiber layer (RNFL) thickness measurement using optical coherence tomography (OCT)

Subjects: 200 normal patients

Methods: Single observer, hospital based, cross sectional study of 200 normal subjects. Subjects underwent RNFL scanning using OCT/SLO, all images were acquired by single observer.For ONH analysis, optic nerve topography scan mode was used. The topography stack covers an area of 6mm × 6mm with a depth of 2 mm. A three dimensional tomographic image of the optic nerve region was generated from a stack of sequential OCT and SLO images. The operator makes sure that the center of the Optic disc is the center of the SLO image. Results Our study comprised of 400 eyes of 200 normal subjects, between the age group of 18 to 45 years.

Results: The mean disc area was 2.88±0.70mm2. Based on disc area subjects were divided in to 3 groups Group 1 disc area less 3 mm2 (Mean±SD 2.40 mm2±0.30 mm2) Group 2 disc 3-4 mm2(Mean±SD 3.37 mm2±0.27 mm2) Group 3 disc area more than 4mm2(mean ± SD 4.25 mm2±0.17 mm2).

Conclusion: In view of positive correlation between disc area and RNFL thickness from our study and previous studies, there definitely appears some association between disc area and RNFL thickness. However, to come on definite conclusion about association between disc area and RNFL thickness various factors such as age, axial length, race, gender, refractive error etc, should be considered with inclusion of more subjects.

INTRODUCTION

Retinal nerve fiber layer (RNFL) thickness measurement using optical coherence tomography (OCT) is useful adjunct in detection and monitoring of glaucoma1.OCT is non contact , non invasive imaging technique which produces high resolution, cross sectional images of optic nerve head (ONH) and RNFL2-4. It provides objective and quantitative estimation of RNFL thickness5-7. Assessment of the optic disc is of utmost importance for the diagnosis of optic nerve anomalies, glaucoma and neuro ophthalmologic diseases. Size of the neuro retinal rim and the optic cup vary with disc size,8 there is a large variation in disc size within a population and also among populations.9-10 Large discs are often associated with large cups, in order to exclude glaucomatous cupping, normative values of optic disc and RNFL measured using SD–OCT help to delineate normal variation from pathological changes.11 RNFL damage is known to precede optic disc change and visual field (VF) damage.12,13 RNFL is known as a sensitive indicator of structural

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damage.14,15 knowledge of normal limits of RNFL with respect to disc area is of clinical importance in early glaucoma diagnosis.

The fast acquisition rate of SD-OCT allows for much faster scanning time, reducing motion artifacts, and enabling denser patterns across the ONH. Axial resolution of SD-OCT <6 μm, scan velocity of 27,000 axial scans per second.16 Reproducible, three dimensional representation of the human eye is possible using OCT during a routine undilated clinical examination.17-20

SD-OCT automatically outlines ONH, optic cup and disc borders similar to mental estimations by clinicians, and also calculates more objective measurements such as optic disc area and neuro retinal rim area in addition to the classic clinician subjective average and vertical cup to disc ratios.21

In the measurement of peripapillary RNFL thickness, a circle diameter of 3.4 mm is used around ONH to avoid intersecting tissue within the ONH margin in large disc and to avoid areas with peripapillary atrophy and is close enough to disc to allow dense sampling covering the entire distribution of RNFL measurements. This location has shown to be the most reproducible compared with other scan circles of different diameters.22 Few limitations like pupil dilation, cataract, corneal and retinal pathology affect results, small normative database, no progression software, potential artifacts, and interpretation errors.23,24 Previous studies have demonstrated positive correlation between disc size and RNFL thickness in normal eyes. However, others studies did not find such correlation using OCT. As optic disc and RNFL thickness show large inter individual variation within and among population. Purpose of this study was to assess the influence of disc area on RNFL thickness in normal eyes measured by SD-OCT.

MATERIALS:

Study titled "Influence of disc area on retinal nerve fiber layer thickness measurement by spectral domain Optical Coherence Tomography" was carried out in 400 eyes of 200 normal subjects in Department of Ophthalmology, National Institute of Medical Science (NIMS) Medical College and Hospital, Shobha Nagar, Jaipur, Rajasthan.

Inclusion Criteria:

• BCVA of ≥6/9, refractive error within ±3.5 D of sphere and ±2 D of
cylinder.
• IOP <21 mm Hg.
• Open angles on gonioscopy.
• Normal appearing optic disc with healthy neuro retinal rim.
• No asymmetry in cup disc ratio between 2 eyes.
• No evidence of peri papillary atrophy, tilted disc, disc haemorrhageetc

Exclusion Criteria

• Subjects with family history of glaucoma. • Ocular trauma.
• Neuro ophthalmic disease
• Intra ocular surgeries.
• ONH abnormalities.
• Media opacities like cataract, corneal opacities.

METHODOLOGY

a. Institutional ethical and scientific committee approval was taken.
b. 200 healthy subjects attending Ophthalmology OPD in National Institute of
Medical science, Shobha Nagar, Jaipur were selected.
c. Informed consent of the patient taken after explaining the purpose of the study, in

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an appropriate local language.

d. The consent form is appended.

e. Patient who did not give consent to participate were excluded from the study.

f. Demographic data, history, ocular examination findings were noted in case record form.

PROCEDURE:

Ocular examination

• Visual acuity was assessed using Snellens Chart.

• Refraction was done in all the subjects.

• Pupillary reaction was noted.

• Slit lamp examination was done to rule out ocular pathology.

• IOP measurement was done by applanation tonometer.

• Anterior chamber angle was evaluated by Gonioscopy using volk single mirror goniolense graded according to Schaffer's grading.

• Optic disc evaluation was done by direct ophthalmoscopy and slit lamp biomicrocsopy using +90D.

OCT Examination

• Subjects coming to department of ophthalmology after ocular examination were subjected for OCT examination.

Subjects underwent RNFL scanning using OCT/SLO, all images were acquired by single observer.

• Subjects were asked to look at internal fixation target and a circular scan of diameter of 3.4mm centered around optic disc and location was observed on the SLO image to ensure proper positioning of scan in relation to ONH. RNFL analysis uses an automated OCT software algorithm to identify anterior and posterior margin of RNFL.

Following RNFL parameters were evaluated

• Average peripapillary RNFLT (360°) and four quadrants RNFL thickness (superior, nasal, inferior and temporal) were measured. The sectors were defined in clockwise order for right eye and anti clockwise order for the left eye.

• For ONH analysis, optic nerve topography scan mode was used. The topography stack covers an area of 6mm × 6mm with a depth of 2 mm. A three dimensional tomographic image of the optic nerve region was generated from a stack of sequential OCT and SLO images. The operator makes sure that the center of the Optic disc is the center of the SLO image.

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Criteria for determining scan quality

a. Signal strength >6.

b. Clear fundus image allowing optic disc and scan circle visibility prior to and during image acquisition.

c. Even and dense color saturation throughout all retinal layers with red color visible in the retinal pigment epithelium and RNFL.

d. Continuous scan pattern without missing or blank areas. e. Automated detection of disc margin in ONH analysis.

Figure 1 OCT view

Figure 2 OCT view

Statistical analysis:

• Continous variable were summarized as mean and SD, were analyzed by using one way Anova test, followed by Turkey HSD test.

• Correlation was assessed by Pearson Correlation coefficient.

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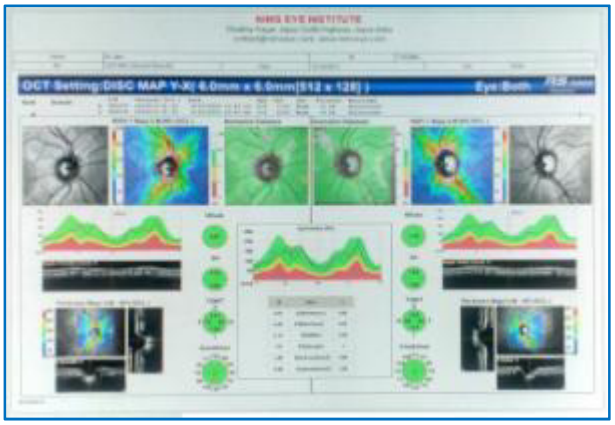
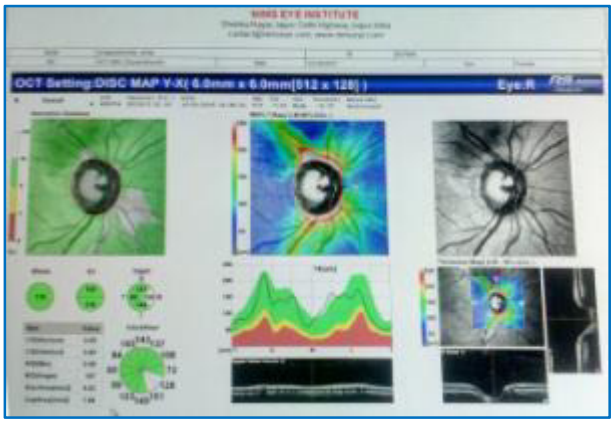
• Normal / oblique categorical variables were summarized as percentages and were analyzed by using Chi –square test.

• MedCalc 12.2.1.0 version software was used for Statistical calculation and Microsoft Excel was used to generate graphs and tables.

• P<0.05 was considered as significant value.

• P≤0.001 was considered as highly statistically significant value. • P≥0.05 was not considered as statistically significant value.





Discussion

Assessment of the optic disc is of utmost importance for the diagnosis of optic nerve anomalies, glaucoma and neuro ophthalmologic diseases. Size of the neuro retinal rim and the optic cup vary with disc size, there is a large variation in disc size within a population and also among populations.8 with disc area ranging from 0.8 mm2 to 6mm2.

Large discs are often associated with large cups, in order to exclude glaucomatous cupping, normative values of optic disc and RNFL measured using SD-OCT help to delineate normal variation from pathological changes.11

Since RNFL damage is known to precede optic disc changes and visual field damage,12,13 RNFL is known as sensitive indicator of structural and functional damage,14,15knowledge of normal limits

of RNFL with respect to disc area is of clinical importance in early glaucoma diagnosis.

The aim of the study was to assess the influence of disc area on RNFL thickness measured by SD-OCT. After systematic and meticulous collection of data, the results were analyzed and interpreted.

In our study total number of males (118) outnumbered females (82), mean age of males (28.46± 6.90), mean age of females (27.83± 7.02), mean RNFL thickness in males (101.9±3.83), mean RNFL thickness in females(103±5.75), no statistically significant difference in RNFL thickness was observed among males and females, which is in line with previous study by Bundez et al,25Pakravan M et al,26 Bendsehneider D et al,27 Qus et al,28 Savini et al,29 Mansoori T et al.30 In our study mean disc area in males 2.79D, mean disc area in female 2.97 D and were not statistically significant.Which is in line with previous studies by Bowd C et al,31 Dacosta S et al,32Onmez F E et al,33Mansoori T et al.30

In our study no statistically significant difference was observed in mean RNFL thickness in right (102.82 μm) and left (101.90 μm) eyes of male subjects and right (101.78 μm) left(103.04 μm) RNFL thickness of female subjects, which was in line with previous study by Budenz et al,25 Pakravan M et al.26 RNFL thickness was thickest in the inferior quadrant, followed by superior, nasal and temporal quadrants and similar results were found in our study.

Our study shows RNFL thickness measured by SD-OCT positively correlates with ONH size, firstly our results are simply confirmatory of previous histological studies showing that the optic nerve fibre count increases with enlarged ONH size.

Varma et al,34 reported in histological study convergence of ganglion cell axons from retinal periphery towards the optic disc gives rise to increase RNFL thickness as ONH is approached. Funaki et al35 found significant positive correlation (R=0.497, P<0.001) between optic disc size and integral peri papillary RNFL thickness using SLP. The results of study support the concept that the cross sectional area occupied by RNFL, increased significantly with the increase in optic disc size. Clinical significance of evaluating thicker RNFL measured by OCT for large disc area and vice versa lies in greater susceptibility to develop glaucoma in eyes with small disc with congenitally low axonal count or greater anatomic reserve in eyes with large disc.

In 2006 Mederios et al 36 reported with regard to optic disc area, larger optic discs were associated with decreased sensitivity for the Stratus OCT parameter average thickness and GDx VCC parameter for nerve fiber indicator, whereas small optic discs were associated with increased sensitivity. In our study positive correlation was found between disc area 3mm2 and RNFL thickness average (R= 0.75, P<0.001), inferior (R=0.90, P<0.001), superior (R= 0.82, P<0.001), nasal (R= 0.42, P<0.001), temporal (R= 0.73, P<0.001).

Positive correlation was found between disc area 3-4mm2 and RNFL thickness average (R= 0.84, P<0.001), inferior (R=0.60, P<0.001), superior (R= 0.75, P<0.001), nasal (R= 0.67, P<0.001).

Positive correlation was found between disc area more 4mm2 and RNFL thickness, inferior (R=0.94, P<0.001), superior (R=0.68, P<0.001), nasal (R= 0.93, P<0.001), temporal (R= 0.95, P<0.001), but not with average RNFL thickness.

If they are coursing more obliquely (either heaping up into the disc rim or already diving downward into it), the measurement by the instrument might give a thicker value than in eyes in which the axons are further from the disc rim and are completely perpendicular to the measuring light. When fixed diameter circular scan is used the distance between the scan and the ONH margin is reduced in the presence of a large ONH that will lead to thicker RNFL measurement in patients with large ONH as the measurement will be made closer to the optic disc edge.

Primary aim of the study was to establish a correlation between disc area and RNFL thickness was studied, analyzed and achieved.

However, RNFL thickness is influenced by many factors viz. Age, axial length, refractive error, race etc this study is not fulproof from its results and findings.

LIMITATIONS OF THE STUDY

- Lack of analysis of confounding factors such as axial length, race, age refractive error, corneal diameter etc.
- Less subject in disc area group more than 4 mm.2
- Not a population based study and is prone to selection bias.
- Hence, to overcome above limitations, the next step should be to take into account the confounding factors such as axial length, race, age and refractive error etc.

RESULTS:

Based on disc are subjects were divided in to three groups. Group 1 disc area less 3 mm^2 Group 2 disc 3-4 mm^2Group 3 disc area more than 4mm^2.

Baseline characteristics of sample population

	Disc Area <3 mm^2	**Disc Area 3-4 mm^2**	**Disc Area >4 mm^2**	**Total**
Number of Subjects	**122**	**56**	**22**	**200**
Age (mean±SD) in years	25.91±5.83	32.07±7.06	31.05±7.19	26.20±6.94
Males/Females	77/45	29/27	12/10	118/82
Disc area (mean±SD) in mm2	2.40±0.30	3.37±0.27	4.25±0.17	2.88±0.70

*ANOVA - Analysis of Variance

Mean value of age were not alike in disc area group and is statistically significant P<0.001.

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Distribution of study participants according to age & Disc Area

Age	Disc Area <3 mm^2		Disc Area 3-4 mm^2		Disc Area >4 mm^2		Total	
	No.	%	No.	%	No.	%	No.	%
≤25 Year	69	56.56	12	21.43	4	18.18	85	42.50
26-35 Year	42	34.43	22	39.29	12	54.55	76	38.00
>35 Year	11	9.02	22	39.29	6	27.27	39	19.50
Total	122	100.00	56	100.00	22	100.00	200	100.00

Chi-square = 35.404 with 4 degrees of freedom; P<0.001

Association of disc area with age and is statistically significant

Distribution of study participants according to sex & Disc Area

Sex	Disc Area <3 mm^2		Disc Area 3-4 mm^2		Disc Area >4 mm^2		Total	
	No.	%	No.	%	No.	%	No.	%
Male	77	63.11	29	51.79	12	54.55	118	59.00
Female	45	36.89	27	48.21	10	45.45	82	41.00
Total	122	100.00	56	100.00	22	100.00	200	100.00

Chi-square = 2.239 with 2 degrees of freedom; P = 0.326

Number of males outnumbered females in all three disc area groups

Average RNFL Thickness

Disc area (mm^2)	N	Mean± SD	95% CI for Mean	Range	'p' Value*	'p' Value# (1-2)	'p' Value# (1-3)	'p' Value# (2-3)
<3 mm^2	240	100.56±4.79	99.95-101.17	94-140	<0.001	<0.001	<0.001	0.088
3-4 mm^2	116	103.93±5.56	102.24-105.62	99-138				
>4 mm^2	44	105.6±3.07	105.04-106.17	97-109				

*ANOVA # Tukey HSD

RNFL thickness of total studied eyes (N=400)

Disc area (mm^2)	RNFLT	N	Mean	SD	95% Confidence Interval for Mean		Range	
					Lower Bound	Upper Bound	Minimum	Maximum
Total	Average	400	102.39	5.01	101.90	102.88	94	140
	Inferior quadrant	400	134.35	7.33	133.63	135.07	13	161
	Superior quadrant	400	128.45	4.03	128.06	128.85	90	145
	Nasal quadrant	400	85.04	6.99	84.35	85.73	60	183
	Temporal quadrant	400	71.13	4.79	70.66	71.60	50	89

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