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A Study of Macular Thickness, Retinal Nerve Fiber Layer Thickness and Optic Nerve Head Parameters in Healthy Subjects Using Spectral Domain Optical Coherence Tomography (SD-OCT)

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Abstract
Purpose: *To find out macular thickness, retinal nerve fiber layer (RNFL) thickness and optic nerve head parameters using Spectral Domain optical coherence tomography (SD-OCT) in healthy subjects and to find out the effect of age on these parameters.*
Materials and Methods: *The present cross-sectional study was conducted on 446 eyes of healthy volunteers at Upgraded Department of Ophthalmology, S.M.S Medical College, Jaipur. The macular thickness, retinal nerve fiber layer thickness and optic nerve head parameters were measured using SD OCT (TOPCON 3D OCT 2000). Statistical analysis was performed using t test for comparative study and Pearson's co-efficient for correlation. Where indicated, linear regression was used to describe parametric association and to generate graphic representations of the same.*
Results: *The overall macular thickness was found to decrease significantly with age. The overall RNFL thickness was found to have negative correlation with age, which was statistically significant. There was increase of cup area and cup volume with increase in age in the study population which is statistically significant as denoted by the p-value. There was decrease in rim area and rim volume with increase in age which was statistically significant as denoted by the p-value. There was increase of Cup/Disc ratio, Linear and Vertical Cup/Disc ratio with increase in age in the study population which was statistically significant as denoted by the p-value.*
Conclusion: *The present study provides the largest normative database for SD OCT, TOPCON 3D 2000 in Indian population. In conclusion, global and regional changes due to the effects of age on macular thickness, RNFL thickness and ONH parameters on OCT should be considered while assessments of these are undertaken.*
Keywords: *Macula thickness; Retinal nerve fiber layer thickness; Optic nerve head; Optical coherence tomography*

I. Introduction

Optical coherence tomography (OCT) is an in vivo, non-invasive test that can impart accurate measurements of macular and retinal nerve fiber layer thickness.[1] It has emerged as a useful tool that provides cross- sectional imaging of the tissues that is comparable to histological analysis[2,3] and by providing detailed morphometric and quantitative information.[4,5]

The changes in macular thickness (MT), retinal nerve fiber layer (RNFL) thickness around the optic nerve head (ONH) and optic nerve head parameters like disc size, cup size, are commonly seen in eyes with pathologies, like glaucoma, coloboma, age related macular degeneration, diabetic retinopathy etc., but can also be attributed to normal aging process. Therefore normal aging can be confused to be pathologically associated with the disease. Likewise structural damage in glaucoma occurs before the detectable visual field loss. Hence, knowledge of normal macular thickness, RNFL and ONH parameters with respect to the age must be known in order to distinguish pathological changes from normal aging process.

Thus, the present study was undertaken to find out the macular thickness, retinal nerve fiber layer (RNFL) thickness and Optic nerve head parameters using Spectral Domain optical coherence tomography (SD - OCT) in healthy subjects and to find out the effect of age on these parameters.

II. Materials and Methods

This was a descriptive type of observational, cross-sectional, hospital based study conducted at Upgraded Department of Ophthalmology, SMS Hospital and Medical College, Jaipur.

446 eyes of randomly selected healthy volunteers from the outpatient department in age group 18-85 years having best corrected visual acuity of 20/40 or better, refractive error within +/- 6.0 diopters, no media opacity that interferes with fundus imaging, no evidence of retinal or ONH pathologies and with normal 24-2

standard algorithm perimetry with less than 30% fixation losses and false positive and false negative responses were included in the study.

All patients underwent complete ophthalmic examination to satisfy inclusion and exclusion criteria. Patients on any medications which were known to have any effect on RNFL thickness, i.e. anti glaucoma, ethambutol, isoniazid, cholroquine, aminoglycosides, NSAIDS etc, patients with any systemic disease that might affect retina or visual field, i.e. hypertension, diabetes, leukemia, anemia, connective tissue disorders etc. and patients with previous intraocular operations other than uneventful cataract extraction were excluded from the study.

The macular thickness, retinal nerve fiber layer thickness and optic nerve head parameters were measured using SD OCT (TOPCON 3D OCT 2000) after pharmacological pupillary dilatation with tropicamide 0.8 and phenylephrine 5% and instillation of artificial tears.

in order to avoid any errors in the thickness measurements due to difference in positioning. The module used for macular thickness measurements was 3D Radial. A false colour topographic image was displayed with numeric averages of thickness measurements for each of the nine map regions within 6×6 mm area centred on the fovea, as defined by the ETDRS.[6] According to ETDRS map, macula is divided into 9 regions with 3 concentric rings, innermost measuring 1 mm, inner ring of 3 mm and outer ring of 6 mm diameter centred at fovea. The innermost 1 mm ring is the fovea while the inner and outer rings are further divided into four equal quadrants. The macular thickness was measured as distance between the inner limiting membrane (ILM) and the inner boundary of retinal pigment epithelium (RPE) in each of the 9 regions.

The 3D - disc scans were taken with proper disc fixation to as the get the best possible disc margins identification by the machine. The images were deemed acceptable only when the scans met all the necessary criteria, and there were no artifacts caused due to blinking or eye movements. The RNFL thickness parameters were calculated as the mean of three corresponding parameters measured independently on three individual circular scans. For RNFL scans, overall mean RNFL thickness and RNFL thickness measurements averaged within the 4 quadrants and 12 clock hours were used for analysis.

$P<0.05$ was considered as statistically significant. Statistical analysis was performed using t test for comparative study and Pearson's co-efficient for correlation. Where indicated, linear regression was used to describe parametric association and to generate graphic representations of the same.

III. Results

The present study was conducted on 446 eyes of healthy individuals and data was recorded on the macular thickness, Retinal nerve fiber thickness and Optic nerve head parameters. However, 46 eyes were excluded from the study due to poor scan quality (n = 26), poor centration (n = 10), non-clinically detectable small pigment epithelial detachments (n = 6) and early epiretinal membranes (n = 4). 400 eyes of 200 subjects were evaluated.

Table 1: Observations of measurements of macular thickness

ETDRS regions		N	Mean	Std. Deviation	Minimum	Maximum
0	Overall	400	264.75	23.652	199	295
1	Superior outer	400	258.58	14.424	203	292
2	Inferior outer	400	254.12	14.587	204	297
3	Temporal outer	400	248.94	14.573	187	299
4	Nasal outer	400	274.97	16.192	224	321
5	Superior inner	400	294.36	17.168	244	342
6	Inferior inner	400	290.24	17.065	244	361
7	Temporal inner	400	282.27	19.780	223	396
8	Nasal inner	400	295.21	17.932	248	398
9	Centre	400	222.01	19.548	163	298

Macular thickness: The macular thickness was recorded in 9 ETDRS regions. The mean, standard deviation and ranges are shown in Table 1. Studying the macular scan, the fovea was the thinnest area (222.01 ± 19.55µm), the inner macular circle was thicker than the outer macula in all four regions superior, inferior, temporal and nasal ($p < 0.001$). The nasal macular thickness was found to be significantly thicker (295.21 ± 17.93 µm), $p < 0.001$ than the temporal macular thickness. Nasal quadrant was the thickest among all four 9 ETDRS regions, followed by superior, inferior and temporal quadrant (table 1)

Table 2: Correlation of macular thickness with age

Age Group (Yrs)	N	Mean	Std. Deviation
>30	90	270	20.56
30-40	82	270	23.43
40-50	86	267	25.12
50-60	78	261	25.69
>60	64	255	25.81
Total	400	264.6	24.122

Correlation of macular thickness with age: The overall macular thickness was found to decrease significantly with age (table 2). The table 2 shows the overall retinal thickness which was calculated by (Outer circle thickness x 3/16+ inner circle thickness x 1/18 + centre thickness x 1/36). All the regions show a decline in the thickness with age, however the superior outer and temporal outer, show a lower thickness between the age group of <30 years as compared to the 30 – 40 years, in the study which is not significant. The overall correlation of the thickness with increasing age had significantly decreased. The central macular thickness show a lower thickness in the 30 – 40 years age group than the 40 – 50 years age group and further decrease in the central macular thickness with the increasing age which is statistically significant. All the quadrants superior, inferior, nasal, temporal and centre showed negative correlation with age which was statistically significant, p<0.001 (table 2)

Table 3: Parameters of RNFL thickness measurement

Parameters	N	MEAN	Std. Deviation	Minimum	Maximum	P-value
Overall	400	98.91	9.866	71	136	0.0001
Superior	400	114.30	13.200	70	158	
Inferior	400	120.29	12.954	83	156	
Temporal	400	69.83	10.223	37	101	
Nasal	400	81.43	13.629	40	125	
1 o'clock	400	116.36	16.635	59	159	
2 o'clock	400	98.19	17.245	52	171	
3 o'clock	400	69.98	14.861	34	120	
4 o'clock	400	79.03	15.929	35	130	
5 o'clock	400	109.03	17.836	61	149	
6 o'clock	400	128.05	16.426	83	189	
7 o'clock	400	119.33	20.069	60	180	
8 o'clock	400	70.86	12.969	38	119	
9 o'clock	400	60.79	10.223	36	97	
10 o'clock	400	81.97	13.424	36	127	
11 o'clock	400	116.36	19.108	55	182	
12 o'clock	400	115.56	15.003	63	164	

Table 4: Correlation of RNFL Thickness with age

Age Group (Yrs)	N	Mean	Std. Deviation	P-value
<30	90	102.96	9.31	0.0000
30-40	82	102.16	8.47	
40-50	86	101.79	8.93	
50-60	78	94.77	7.56	
>60	64	90.22	8.82	
Total	400	98.91	9.87	

Retinal Nerve Fiber Layer Thickness & correlation with age: The overall RNFL thickness was found to have negative correlation with age, which was statistically significant (table 4). RNFL thickness was found to decrease with the advancing age in the study population, however some clock hours exhibited a mild increase in the RNFL thickness in the 30 – 40 age group in comparison to the <30 years age group in the 1 o'clock, 2 o'clock, 3 o'clock, 4 o'clock and 12 o'clock region. The study also showed lesser degree of RNFL thickness decrease with advancing age from 7 o'clock to 11 o'clock region in comparison to the rest of the clock hours. There was significant decrease of RNFL thickness in all the regions between the first and the last decade of study population (table 3 &4)

Table 5: Various observations of Optic Nerve Head Parameters

Age Group (Yrs)	N	Mean± Deviation Std.					
		Optic Nerve Head Disc Area	Optic Nerve Head Cup Area	Optic Nerve Head Rim Area	Optic Nerve Head Cup Volume	Optic Nerve Head Rim Volume	Optic Nerve Head Horizontal Disc Diameter
<30	90	2.47±0.41	0.67±0.38	1.77±0.46	0.12±0.11	0.27±0.39	1.88±0.19
30-40	82	2.51±0.38	0.77±0.43	1.74±0.45	0.16±0.14	0.18±0.15	1.87±0.13
40-50	86	2.54±0.30	0.83±0.41	1.71±0.37	0.18±0.17	0.16±0.19	1.88±0.12
50-60	78	2.52±0.36	0.99±0.46	1.54±0.39	0.21±0.17	0.13±0.09	1.89±0.14
>60	64	2.27±0.35	0.82±0.45	1.49±0.35	0.17±0.15	0.14±0.08	1.79±0.12
Total	400	2.47±0.37	0.81±0.43	1.66±0.42	0.17±0.15	0.18±0.23	1.87±0.15
P- Value	-	-	0.0001	0.0000	0.0028	0.0002	-

Optic Nerve Head parameters: There was increase of cup area and cup volume with increase in age in the study population which is statistically significant as denoted by the p-value (table 5). There was decrease in rim area and rim volume with increase in age which was statistically significant as denoted by the p-value (table 5)

Table 6: Various ratio of Optic Nerve Head Parameters

Age Group (Yrs)	N	Mean± Deviation Std.		
		Optic Nerve Head Cup/ Disc Ratio	Optic Nerve Head Linear Cup/Disc Ratio	Optic Nerve Head Vertical Cup/Disc Ratio
<30	90	0.27±0.13	0.49±0.15	0.51±0.16
30-40	82	0.30±0.15	0.53±0.15	0.53±0.16
40-50	86	0.32±0.15	0.55±0.14	0.56±0.15
50-60	78	0.38±0.15	0.59±0.14	0.60±0.14
>60	64	0.34±0.15	0.56±0.15	0.58±0.17
Total	400	0.32±0.15	0.54±0.15	0.55±0.16
P- Value		0.0000	0.0003	0.0018

Ratios of Optic Nerve Head Parameters : The Optic Nerve Head Cup/Disc ratio in different age groups, their mean with standard deviation. There was increase of Cup/Disc ratio, Linear and Vertical Cup/Disc ratio with increase in age in the study population which was statistically significant as denoted by the p-value.(table 6)

IV. Discussion

The changes in macular thickness, retinal nerve fiber layer thickness and optic nerve head parameters are seen in pathological conditions on routine practice. However the knowledge of normal anatomical values of these parameters is essential to differentiate the abnormal changes from what is attributed to normal due course of ageing. Also ethnicity has shown to play a role in various studies.[7-11] Therefore it is important to have a different database for each ethnic group.

The macular thickness was evaluated using the macular scan. The fovea was the thinnest area (222 ± 19.54 µm) and the inner nasal macular thickness was found to be thickest region (295.21 ± 17.93 µm). This result was in accordance with the previous studies conducted by Duan XR et al[12] and Appukuttan B et al.[1] Similarly, Adhi M et al[13] mean macular thickness of 262.80 ± 13.342 µm and foveal thickness of 229.01 ± 24 µm among subjects from Pakistan. Giani A et al[14] reported foveal thickness of 229 ± 24 µm, while Sull AC et al[15] reported a foveal thickness of 231 ± 16 µm. This shows that Indian population has a slightly thinner macular thickness as compared to what reported as above, but a slightly higher than reported by Hyang J et al[16] (foveal thickness of 221.76 ± 15.95 µm,) and thinner than reported by Bruce A et al[17] (foveal thickness of 244.83 ± 17.84 µm) in healthy subjects using Topcon OCT. This also explains the ethnic differences in macular thickness which have been described in a number of studies,[7-11,18] and the need for a different database according to the origin.

Previous studies on Indian eyes using Straus OCT by Tewari HK et al,[19] showed a thinner central foveal thickness of 149.16 ± 21.15 µm. This difference in the measurements was due to the fact that time domain (TD-OCT) measures retinal thickness as the distance between internal limiting membrane (ILM) and the third hyper- reflective band, whereas SD OCT measures the distance between ILM and the retinal pigment epithelium (RPE) resulting in higher SD OCT readings compared to those obtained by TD-OCT.[20] Nevertheless,

macular thickness in our subjects decreased from the center towards the periphery of retina, and was found to be thickest nasally and thinned out temporally. This was consistent with finding reported elsewhere.[15,16]

Correlation of macular thickness with age showed that the overall macular thickness was found to slightly decrease with age which is in consistent with findings of Tewari HK et al,[19] Duan XR et al,[12] Guedes V et al[10] and Kyung RS.[21] But in contrast to previous studies we did not found any positive correlation with the central macular thickness which did not decrease significantly with advancing age, this may be related to the thickening of the internal limiting membrane and the centripetal force of the posterior vitreous resulting in the elevation of fovea, thus preventing the thinning at fovea.[22] In another study by Kashani AH et al,[23] a significant increase in centre in centre point foveal thickness and mean foveal thickness with age. They suggested the presence of interstitial edema from foveal capillary dropout with age as a probable reason.

The overall RNFL thickness was found to have negative correlation with age, which was statistically significant in all the four quadrants This was in accordance with the study conducted by Parikh RS et al[24] and Appukuttan B et al.[1] However, Sung KR et al,[21] reported the slope change of RNFL over people of different ages was statistically significantly different from a slope of zero for overall, and in superior, inferior and nasal quadrants but not statistically significant in the temporal quadrant This is also in accordance with our study showing highly significant values in superior , inferior and nasal regions but only significant in temporal quadrant of lesser degree of RNFL thickness decrease with advancing age from 7 o'clock to 11 o'clock region in comparison to the rest of the clock hours. This difference in rate may be due to the concentration of thinner nerve fibers in the papillomacular bundle at the temporal aspect of the ONH as has been reported in histology sections.[25] Identical number of axonal loss will cause a shallower decline in locations predominantly composed by thinner fibers.

Optic nerve head analysis showed that while the disc size remained stable there was a significant decrease in rim area and increase in the cup area. The rate of change varied substantially among the various parameters and differs from the rate in other locations. This might indicate preferential loss at the optic nerve head level that is less pronounced in the retina itself.

Mean disc area was 2.47 ± 0.37 mm^2 and optic cup area, rim area, and horizontal integrated rim width increased with disc size, whereas vertical integrated rim area did not. Vertical integrated rim area, horizontal integrated rim width, and rim area decreased and cup area, cup disc ratio increased with age. Sung KR et al[26] and Marsh BC et al[27] also have similar findings i.e. cup area increased and rim area decreased with age, both of which were statistically significant.[26,27] Disc size did not show significant change with age, but significant cup and rim area changes likely reflect neural tissue loss.

Girkin CA et al[28] has shown in his previous studies that there are racial differences for optic nerve parameters, participants of European descent had significantly smaller optic disc area than other groups, and Indian participants had significantly smaller rim area than other groups which is also supported by the results of our study. However, the optic nerve parameters shown by an Indian study done in southern India by Mansoori T et al[29] were on the higher side as compared to our results indicating even a regional difference among the parameters and a different normative database for North Indian population with spectral OCT.

There exists a variability in measurements by commercially available OCT systems. SD -OCT gives a higher value of macular thickness as compared to the TD-OCT. This difference is due to the different resolutions and difference in the retinal segmentation. The present study is one of the pioneer studies to use SD-OCT (TOPCON 3D OCT 2000) to establish the largest normative database for macular thickness, retinal nerve fiber layer (RNFL) thickness and optic nerve head (ONH) parameters in Indian population and to determine the effect of age on them.

V. Conclusion

The present study provides one of the largest normative database for SD OCT, TOPCON 3D 2000 in Indian population**.** In conclusion, global and regional changes due to the effects of age on macular thickness, RNFL thickness, and ONH parameters on OCT should be considered while assessments of these are undertaken.

VI. Limitations

The main limitation of this study was that it was based on cross sectional data rather than longitudinal data. It would be ideal if we could follow the change of retinal tissue in each individual longitudinally but for obvious reasons this is not feasible at this stage of the technology. Therefore, we acknowledge that we are not measuring true thickness changes but rather looking at differences among a large, broad population. This can cause some artifacts as can be observed in age group <30 years that had thinner RNFL thickness as compared to the 30-39 year old, and some decline in the Disc area noted in the >60 years age group.

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