

Mapping the thickness of retinal layers using Spectralis spectral domain optical coherence tomography in Indian eyes

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Purpose: This study aimed at establishing the normative data for the thickness of macular layers on Spectralis Spectral-domain optical coherence tomography (SD-OCT) in healthy Indian eyes and testing the effects of age, gender, central corneal thickness (CCT), and intraocular pressure (IOP) on such values. **Methods:** This cross-sectional study was done on 308 eyes of 159 healthy subjects. OCT scans were obtained using the posterior pole asymmetry scan protocol. From the thickness map, data were grouped into nine Early Treatment Diabetic Retinopathy Study (ETDRS) macular sectors. Correlation between retinal thickness and age/IOP/CCT was done using Pearson correlation. Correcting for age as a covariate, multivariate regression analysis was done to know which retinal layers showed significant differences in thickness between males and females. **Results:** The mean age was 46.06 ± 13.06 years (range: 20–75 years). Significant central subfield (CSF) thickening with age was noted in retinal nerve fiber layer (RNFL), inner nuclear layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), and outer nuclear layer (ONL) ($P < 0.04$). The average thickness of the outer ring reduced with age in the ganglion cell layer (GCL)/IPL/INL ($P = 0.001$). Women had thinner inner and outer retinal thickness than men in all ETDRS rings ($P < 0.001$). There was no interocular asymmetry ($P > 0.05$) and no correlation between IOP/CCT and retinal layer thickness. **Conclusion:** In CSF, age-related thickening was noted in RNFL, IPL, INL, OPL, and ONL. The average inner ring thickness decreased with age in GCL and IPL and increased in the RPE layer. The average outer ring thickness decreased with age in GCL, IPL, and INL layers and increased in OPL. The average IR and OR thickness was significantly less in women compared to men in all sub-fields. There was no correlation between IOP/CCT and retinal layer thickness.

Key words: Age, gender, macular layers, normative, optical coherence tomography, thickness

Spectral-domain (SD) optical coherence tomography (OCT) is a powerful imaging technique to visualize the ultra-structural changes in the human retina.^[1] This technique has been shown to have a good histological correlation.^[2] The development of auto-segmentation software has helped us study the multi-layered structure of the retina in greater detail. However, the segmentation algorithms vary among different manufacturers of SD-OCT.^[3] The built-in auto-segmentation software of the Spectralis SD-OCT device (Heidelberg Engineering, Inc., Heidelberg, Germany) has been previously used in studies^[4,5] and has shown excellent repeatability and reproducibility.^[6] The active eye-tracking technology in Spectralis SD-OCT allows measuring even small structural changes in the retina over time and hence can be used for tracking disease progression.^[7] The output is in the form of pseudo-colored macular thickness maps divided into zones as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS).^[8]

Different retinopathies affect different layers of the retina. Of late, research is being done to find a correlation between the thickness of different retinal layers and different retinopathies

such as glaucoma, diabetic retinopathy, and multiple sclerosis.^[9-11] Studies have shown that changes can occur in the thicknesses of the retinal layers on OCT even before the features of retinopathy appear clinically.^[5] This can help in the early detection of retinal diseases and also monitor the subtle progression of the disease.

However, the normal thickness of the retina varies among different ethnic populations.^[12] Also, within a population, age, gender, and refractive errors determine the normal values of retinal thickness.^[13-15] Hence, establishing a normative database for one's population is necessary to help physicians compare the thickness profiles of patients with such normative data. Currently, to the best of our knowledge, a normative database for the thickness of the individual retinal layers within the macula for Spectralis SD-OCT using the posterior pole asymmetry protocol does not exist in the Indian population. Hence, this study was undertaken. The aims of this study were 1) to establish normative data in the ETDRS thickness map for the following macular layers—retinal nerve fiber layer (RNFL),

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ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), retinal pigment epithelium (RPE), inner retina (IR), outer retina (OR), and total retina (TR) on Spectralis SD-OCT in healthy Indian eyes, and 2) to test the effects of age, gender, central corneal thickness (CCT), and intraocular pressure (IOP) on such values.

Methods

Study design

This was an observational cross-sectional study. The study was approved by the institutional ethics committee. The study followed the Declaration of Helsinki. Subjects were recruited between February 2021 and July 2021.

Sample size

Based on the previous literature, the variance in the ganglion cell layer thickness change from young to older populations was 32%.^[16] Hence, keeping the type 1 error at 5% and the power of the study at 80%, the sample size was calculated for the study using the G*power ver. 3.1.9.4 software. The calculated sample size was 198 eyes, which was rounded off to 200 eyes.

Study population

Subjects were chosen from people attending the ophthalmology OPD for a routine eye check-up, staff, and students of the hospital. We included subjects between 18 and 70 years, with best-corrected visual acuity (BCVA) \geq 20/20, a refractive error between -2 and $+2$ dioptres, and normal retinal status on examination. We excluded subjects with systemic illnesses, current or past ocular diseases (such as retinal diseases, amblyopia, IOP more than 21 mmHg, glaucoma, and previous ocular surgery), and OCT scans with a signal strength of <25 .

Assessments

All subjects underwent a complete ophthalmological examination including BCVA, refraction, IOP, and CCT measurements by non-contact tonometry (NCT), anterior segment examination using a slit lamp, and posterior segment examination using a plus 90D lens, and indirect ophthalmoscope.

OCT imaging

Heidelberg OCT-Spectralis HRA imaging system (Spectralis HRA + OCT; Heidelberg Engineering, Heidelberg, Germany) was used to acquire high-quality OCT images. Posterior pole asymmetry scan protocol was used to acquire the macular thickness measurements. This protocol involves 61 horizontal single lines with 15 frames on an average ($30^\circ \times 25^\circ$ volume scan centered at the fovea). [Fig. 1a] Scans with a signal strength of ≥ 25 were taken for analysis. Automated retinal segmentation was applied to obtain the thickness values of different retinal layers. Scans were manually checked for proper auto segmentation. From the retinal layer thickness map, data were grouped into nine macular sectors as defined by ETDRS.^[8] The extent of the different layers is shown in Fig. 1b.

Statistical analysis

The retinal thickness values for each layer were presented as mean and standard deviation. Average thickness values from the outer ring of the grid (composed of four subfields

between 3 and 6 mm distant from the center) and the inner ring (composed of four subfields with a distance between 1 and 3 mm from the center) were calculated for each layer. Correlation between retinal thickness values and age/IOP/CCT measurements was done using the Pearson correlation test. The correlation between age and thickness of different retinal layers was presented as a linear regression together with the 95% confidence bands of the regression slope [Fig. 2].

Correcting for age as a covariate, we analyzed which retinal layers showed significant differences in thickness between males and females using multivariate regression analysis. All *P* values were adjusted by the Bonferroni factor. A *P* value <0.05 was considered significant. Statistical analyses were performed using the SPSS package version 28 (SPSS Inc., Chicago, IL, US).

Results

Demography

A total of 308 eyes of 159 patients were analyzed. A total of 148 eyes of 76 men and 160 eyes of 83 women were analyzed. The mean age of the subjects was 46.06 ± 13.06 years (range: 20–75 years). There was no significant difference in the mean age between men and women ($P = 0.13$) [Table 1].

Retinal layer thickness measurements in normal subjects and their relationship with age

The thickness of the different retinal layers (mean \pm SD) is shown in Table 2.

Total retinal thickness increased in the CSF ($r = 0.28$, $P = 0.001$) and decreased in the outer ring ($r = -0.208$, $P = 0.001$) with age. RNFL thickness also increased in the CSF ($r = 0.117$, $P = 0.04$) with age. Mean GCL and IPL thickness decreased with age in the inner (GCL: $r = -0.204$, $P = 0.001$; IPL: $r = -0.19$, $P = 0.001$) and outer rings (GCL: $r = -0.336$, $P = 0.001$; IPL: $r = -0.27$, $P = 0.001$). Also noted was an increase in CSF thickness with age in the IPL layer ($r = 0.137$, $P = 0.016$). Mean INL thickness reduced in the outer ring ($r = -0.246$, $P = 0.001$) and increased in the CSF ($r = 0.29$, $P < 0.001$) with age. Mean OPL thickness increased with age in the CSF ($r = 0.256$, $P = 0.001$)

Table 1: Demographic profile of subjects

Demographic characteristic	Frequency
Gender distribution (<i>n</i>)	
Males	76
Females	83
Age (mean \pm SD) (years)	
Overall	46.06 \pm 13.06
Males	47.82 \pm 15
Females	44.63 \pm 11
Number of eyes (<i>n</i>)	
<30 years	50
30-50 years	142
>50 years	116
Right eyes (<i>n</i>)	154
Left eyes (<i>n</i>)	154
Central corneal thickness (mean \pm SD) (μ m)	533 \pm 27
Corrected intraocular pressure (mean \pm SD) (mmHg)	15.9 \pm 2.8

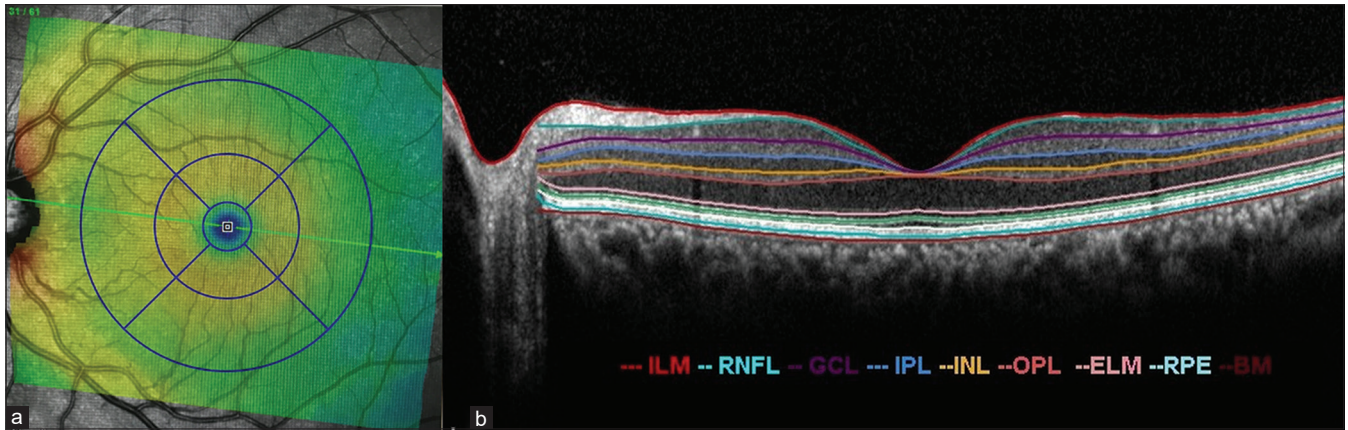


Figure 1: (a) HRA image showing the extent of the macular area scanned in the posterior pole asymmetry protocol and the ETDRS grid (Central subfield: 1 mm, Inner ring [composed of four subfields between 1 and 3 mm distant from the center], and Outer ring [composed of four subfields between 3 and 6 mm distant from the center]). (b) OCT image showing layer-wise extents: RNFL (from the internal limiting membrane [ILM] to RNFL), GCL (from RNFL to GCL), IPL (from GCL to IPL), INL (from IPL to INL), OPL (from ONL to OPL), ONL (from OPL to the external limiting membrane), RPE (from upper RPE to Bruch’s membrane), IR (from RNFL to ONL), OR (photoreceptors and RPE) and TR (all retinal layers)

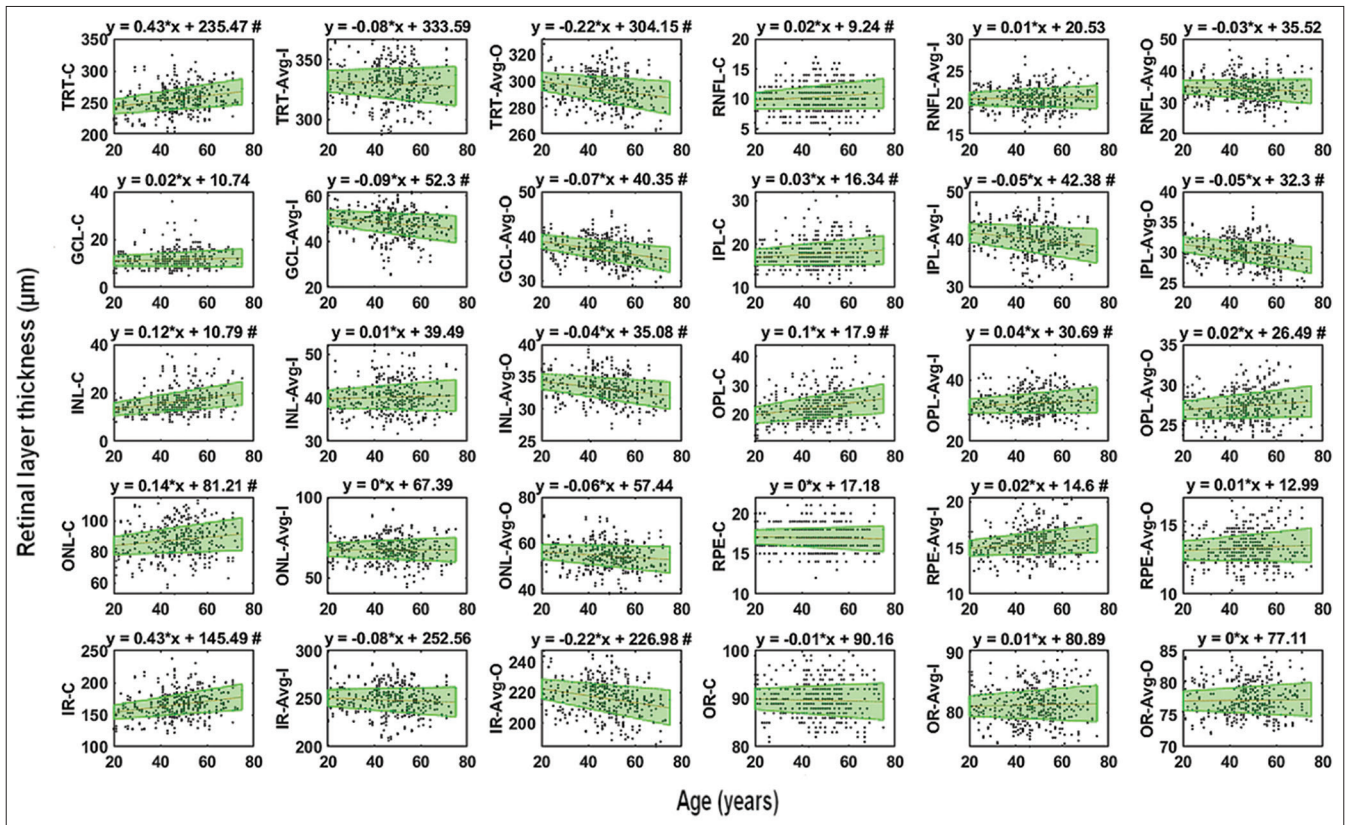


Figure 2: Scatterplots of simple linear regression between age (x-axis) and the thickness of different retinal layers (y-axis). (C: Central sub-field, Avg-O: Average thickness values from the outer ring of the ETDRS grid [composed of four subfields between 3 and 6 mm distant from the center], Avg-I: Average thickness values from the inner ring [composed of four subfields with a distance between 1 and 3 mm from the center], Total retinal thickness (TRT), Retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), Inner nuclear layer (INL), Outer plexiform layer (OPL), Outer nuclear layer (ONL), retina pigment epithelium (RPE), Inner retina (IR), Outer retina (OR), micrometre (µm), # indicates $P < 0.05$)

and outer rings ($r = 0.116, P = 0.04$). ONL thickened with age in the CSF ($r = 0.197, P = 0.001$). The mean RPE layer thickness of the inner ring increased with age ($r = 0.164, P = 0.004$) [Fig. 2].

Overall, CSF thickening with age was noted in all retinal layers, which reached statistically significant levels in RNFL, IPL, INL, OPL, and ONL layers. The average thickness of the

Table 2: Age-wise distribution of retinal thickness values (mean±SD) (µm) using Spectralis SD-OCT in normal subjects

Age (years)	CSF	IS	II	IN	IT	OS	OI	ON	OT
Total Retinal thickness (µm)									
<30	246.55±18.73	338.47±13.77	335.10±14.29	337.14±14.21	320.47±14.57	298.14±12.45	289.55±13.29	317.80±13.10	281.29±13.69
30-50	252.41±21.68	334.89±17.79	330.41±19.35	332.84±19.94	318.20±18.24	297.08±13.19	288.13±14.25	314.95±15.15	281.59±14.45
>50	263.01±20.55	333.53±15.57	330.43±15.43	334.77±17.09	319.28±14.83	292.38±12.77	281.29±12.98	308.89±16.58	277.82±11.53
RNFL thickness (µm)									
<30	9.94±1.98	22.63±2.23	24.43±2.54	20.14±1.65	16.31±0.99	35.92±4.45	39.51±5.16	46.82±5.25	17.49±1.07
30-50	9.90±2.55	22.82±3.14	23.31±3.67	19.76±2.14	16.85±1.01	36.82±5.02	38.63±5.38	44.85±6.62	18.20±1.23
>50	10.62±2.50	22.31±2.59	23.54±2.90	19.92±2.16	17.29±1.17	34.83±4.68	36.19±5.81	43.62±6.50	18.70±1.32
GCL thickness (µm)									
<30	11.22±2.82	53.22±3.71	52.37±4.26	50.80±4.30	47.14±4.46	36.41±2.63	34.94±3.02	41.04±2.88	38.12±3.63
30-50	11.67±4.84	49.95±6.41	49.01±7.20	47.36±8.00	44.30±7.25	36.24±2.70	35.03±2.92	41.13±2.84	37.34±3.69
>50	12.03±3.68	49.09±5.42	48.23±5.58	46.82±6.30	43.11±5.50	34.86±3.20	33.20±2.80	38.90±3.73	34.96±3.71
IPL thickness (µm)									
<30	17.10±2.44	41.90±2.55	41.65±3.02	41.88±2.85	40.49±3.00	29.20±2.16	28.12±2.38	31.45±2.42	32.80±2.29
30-50	17.45±3.59	40.11±3.80	39.57±4.31	40.10±4.62	39.14±4.33	29.21±2.27	28.30±2.35	31.60±2.20	32.97±2.65
>50	18.34±3.31	39.50±3.25	38.93±3.41	39.73±3.48	39.34±3.62	28.06±2.40	26.92±2.23	29.96±2.88	31.94±2.38
INL thickness (µm)									
<30	13.84±2.64	39.55±3.08	40.65±4.04	39.39±3.29	36.8±3.4	32.51±1.99	32.35±2.64	35.35±2.13	33.90±2.22
30-50	15.64±5.41	40.82±3.99	41.24±4.69	40.09±4.22	38.29±4.03	32.52±2.46	32.31±2.53	35.29±2.53	33.64±2.68
>50	18.13±5.54	40.43±4.57	40.96±4.31	40.70±4.48	38.09±4.20	31.98±2.32	31.25±2.22	34.36±2.60	32.50±2.14
OPL thickness (µm)									
<30	20.76±4.61	32.24±6.99	34.69±9.75	29.49±3.38	32.00±4.73	25.61±1.93	27.33±3.74	27.94±2.22	27.45±2.10
30-50	21.69±4.38	31.80±7.64	33.99±10.49	29.47±3.95	31.28±4.76	25.89±2.70	27.00±3.26	27.85±2.13	27.66±2.44
>50	24.29±5.94	33.75±8.77	35.32±9.56	31.19±5.06	32.65±4.81	26.63±3.20	27.28±2.87	28.90±2.72	27.98±2.43
ONL thickness (µm)									
<30	84.51±9.84	67.61±10.01	60.86±10.45	72.57±7.28	66.12±6.86	59.86±6.35	50.69±5.77	57.37±5.36	54.98±6.04
30-50	87.16±10.71	67.94±12.25	63.05±12.07	73.97±8.54	67.36±8.87	58.44±7.31	50.45±6.95	56.73±7.43	54.01±6.54
>50	90.10±10.53	66.87±10.35	63.00±13.15	73.57±9.45	67.23±8.25	57.69±7.19	50.04±6.11	55.67±6.11	54.01±5.36
RPE thickness (µm)									
<30	16.96±1.51	15.35±1.56	14.45±1.36	15.63±1.30	14.27±1.29	13.67±1.48	12.92±1.32	13.55±1.42	12.41±1.19
30-50	16.92±1.66	15.85±1.96	15.24±1.75	15.85±1.73	14.81±1.59	13.64±1.24	12.94±1.20	13.69±1.66	12.95±1.24
>50	16.81±1.55	16.28±1.88	15.40±1.73	16.04±1.74	15.22±2.10	13.63±1.46	13.05±1.46	13.40±1.76	13.34±1.89
Inner retinal thickness (µm)									
<30	156.69±19.34	257.20±12.52	254.98±14.05	254.24±13.93	239.00±13.83	219.57±12.00	212.90±12.71	239.69±12.51	204.71±11.77
30-50	162.71±21.50	253.48±17.05	250.17±18.89	250.35±19.24	237.18±17.57	219.09±12.26	211.74±12.97	237.43±17.57	203.86±12.47
>50	172.80±20.45	251.92±14.30	249.95±14.42	252.75±17.43	237.69±13.73	214.51±11.65	205.18±12.50	231.30±16.14	200.10±10.60
Outer retinal thickness (µm)									
<30	90.04±3.94	81.33±3.38	80.24±3.25	83.10±3.08	81.47±2.89	78.63±3.40	76.59±2.66	78.10±3.01	77.24±2.73
30-50	89.69±3.85	81.14±3.51	80.17±3.26	82.38±3.42	81.06±3.03	77.99±2.96	76.21±2.62	77.73±2.81	77.21±2.68
>50	90.12±3.91	81.68±3.53	80.50±3.25	82.30±6.87	81.62±3.46	78.25±3.26	76.15±2.74	77.59±3.08	77.69±3.25

Central sub-field (CSF), Inner superior (IS), Inner inferior (II), Inner nasal (IN), Inner temporal (IT), Outer superior (OS), Outer inferior (OI), Outer nasal (ON), Outer temporal (OT), Retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), Inner nuclear layer (INL), Outer plexiform layer (OPL), Outer nuclear layer (ONL), retina pigment epithelium (RPE), micrometre (µm)

outer ring reduced with age predominantly in the GCL/IPL/INL of the inner retina.

Difference in retinal thicknesses between men and women

Table 3 shows the retinal layers with significant differences in the thickness between males and females after correcting for age as a covariate in a multivariate model.

Table 4 shows the detailed gender-wise distribution of retinal layer thickness.

Overall, the average IR and OR thickness was significantly less in women compared to men in the CSF, inner and outer rings ($P < 0.001$).

There was no difference in the thickness of retinal layers between the right and left eyes ($P > 0.05$).

There was no correlation between IOP/CCT and the thickness of different retinal layers.

Discussion

SD-OCT has emerged as a powerful tool to study ultrastructural changes in the retina and detect micrometer level changes in retinal thickness. This can be potentially useful to diagnose retinal and optic nerve diseases even before clinical signs appear and also follow up on such cases more closely.^[9-13] However, to interpret the changes in retinal thickness values, we need a normative database for the population under study. There have been a few studies on the normal thickness of individual retinal layers in the Caucasian population using Spectralis OCT.^[14,17,18] However, to the best of our knowledge, studies on the normal thickness of individual retinal layers in the Indian population using Spectralis SD-OCT are lacking.

Overall, we found CSF thickening with age in all retinal layers, which reached statistically significant levels in RNFL, IPL, INL, OPL, and ONL layers. The average thickness of the outer ring reduced with age predominantly in the GCL/IPL/INL of the inner retina. There was no significant difference in the thickness of retinal layers between the left and right eyes. The thickness of the retinal layers was less in women than in men.

Our finding of thickening of the CSF with age is consistent with the findings in several previous studies^[19-23] but inconsistent with others.^[14,24,25] CSF is composed primarily of OR layers. The possible reasons mentioned for the thickening of CSF with age include an excessive metabolic strain that accumulates over the years in this part of the retina (increase in the density of residual bodies and accumulation of lipofuscin, accumulation of basal deposits); optical "pseudothickening"^[13]; foveal cones remaining stable with aging; and subclinical vitreous traction on the fovea.^[26] Similarly, thinning of the IR layers with age in the peri and parafoveal macula as we observed is consistent with the observations in most other studies.^[13,14,22,24,26] IR layers thin with age because the GCL and their axons are vulnerable to loss during aging,^[16,27] and aging is associated with loss of other neurons or glial cells in the INL.^[26]

We found that the TR, IR, and OR were thinner in women compared to those in men. This is consistent with the findings of most studies.^[14,21,22,24-26,28,29] However, Tewari *et al.*^[30] (in Indian eyes) and Grover *et al.* (in Caucasian eyes)^[18] found no significant difference in the average foveal thickness and minimum foveal thickness between men and women. Sull

Table 3: Difference in retinal layer thickness between men and women

Retinal layer-zone	Absolute mean difference (males-females)	P	95% confidence interval for difference	
			Lower bound	Upper bound
TRT-C	13.932*	<.001	10.059	17.806
TRT-Avg-I	12.428*	<.001	9.339	15.518
TRT-Avg-O	5.349*	<.001	2.918	7.78
RNFL-C	1.563*	<.001	1.088	2.037
RNFL-Avg-I	0.791*	<.001	0.432	1.15
RNFL-Avg-O	0.422	0.287	-0.355	1.199
GCL-C	1.685*	<.001	0.931	2.439
GCL-Avg-I	3.113*	<.001	1.955	4.27
GCL-Avg-O	0.542	0.063	-0.03	1.114
IPL-C	1.817*	<.001	1.173	2.46
IPL-Avg-I	2.175*	<.001	1.482	2.869
IPL-Avg-O	0.403	0.076	-0.042	0.847
INL-C	2.119*	<.001	1.136	3.103
INL-Avg-I	1.443*	<.001	0.711	2.175
INL-Avg-O	-0.2	0.362	-0.631	0.231
OPL-C	1.288*	0.013	0.276	2.299
OPL-Avg-I	1.180*	0.006	0.336	2.025
OPL-Avg-O	0.653*	<.001	0.267	1.039
ONL-C	3.822*	<.001	1.717	5.927
ONL-Avg-I	2.171*	0.006	0.625	3.718
ONL-Avg-O	2.040*	<.001	0.891	3.19
RPE-C	0.025	0.881	-0.299	0.348
RPE-Avg-I	0.195	0.22	-0.117	0.507
RPE-Avg-O	0.363*	0.004	0.115	0.611
IR-C	12.534*	<.001	8.652	16.416
IR-Avg-I	11.063*	<.001	8.112	14.014
IR-Avg-O	3.958*	<.001	1.655	6.261
OR-C	1.516*	<.001	0.746	2.286
OR-Avg-I	1.374*	<.001	0.771	1.978
OR-Avg-O	1.433*	<.001	0.917	1.948

C: Central sub-field, Avg-O: Average thickness values from the outer ring of the ETDRS grid (composed of 4 subfields between 3 and 6 mm distant from the center), Avg-I: Average thickness values from the inner ring (composed of 4 subfields with a distance between 1 and 3 mm from the center), Total retinal thickness (TRT), Retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), Inner nuclear layer (INL), Outer plexiform layer (OPL), Outer nuclear layer (ONL), retina pigment epithelium (RPE), Inner retina (IR), Outer retina (OR), micrometre (μm); * $P < 0.05$

et al.^[31] also reported no gender differences in the CSF or the other eight sectors of the ETDRS thickness map for TR thickness using Stratus-OCT although men had greater mean thicknesses than women. Our findings also contradict the findings of Appukuttan *et al.*^[32] who found no gender differences in the perifoveal and parafoveal retinal thickness and the RNFL thickness in Indian eyes. Kim *et al.*^[33] found no relationship between IR thickness (between ILM and INL as defined in their study) and gender in Korean eyes. In our study, the OR was thinner in the outer sectors in women compared to men, which was also reported by Palazon-Cabanés *et al.*^[28]

Table 4: Gender-wise description of the thickness of retinal layers

	RNFL thickness (µm)	GCL thickness (µm)	IPL thickness (µm)	INL thickness (µm)	OPL thickness (µm)	ONL thickness (µm)	RPE thickness (µm)	Inner retinal thickness (µm)	Outer retinal thickness (µm)	Total retinal thickness (µm)
CSF										
Males	10.93±2.195	12.54±3.669	18.67±3.002	17.57±5.366	23.30±5.460	89.84±11.647	16.88±1.570	172.20±20.330	90.64±3.653	262.82±20.060
Females	9.45±2.535	10.96±4.121	16.83±3.463	15.06±5.093	21.73±5.002	85.94±9.399	16.91±1.634	159.13±21.137	89.24±3.998	248.43±21.091
OS										
Males	35.43±4.629	35.70±3.065	28.70±2.514	32.01±2.293	26.16±2.621	59.18±8.374	13.87±1.336	217.52±11.787	79.11±3.093	296.32±13.170
Females	36.40±5.096	35.81±2.744	28.86±2.226	32.63±2.404	26.07±3.026	57.61±5.747	13.44±1.363	217.38±12.624	77.33±2.984	294.70±13.111
OI										
Males	37.62±5.549	34.34±3.261	27.71±2.527	31.77±2.455	27.59±3.192	51.26±7.416	13.22±1.377	210.55±13.794	77.05±2.739	287.78±15.141
Females	38.07±5.783	34.33±2.799	27.81±2.286	32.08±2.529	26.73±3.197	49.48±5.339	12.78±1.218	208.46±12.560	75.50±2.407	283.94±12.801
ON										
Males	44.76±6.175	40.16±3.546	30.85±2.817	34.70±2.613	28.93±2.610	57.20±7.450	13.78±1.564	236.51±15.331	78.52±2.947	314.86±16.148
Females	44.65±6.746	40.39±3.221	31.06±2.442	35.19±2.460	27.61±2.102	55.68±5.837	13.36±1.743	234.50±14.724	77.00±2.786	311.45±15.336
OT										
Males	18.45±1.372	36.79±4.007	32.89±2.565	33.05±2.474	28.22±2.421	55.36±6.795	13.20±1.744	204.76±12.108	78.30±2.896	283.07±13.478
Females	18.11±1.234	36.39±3.817	32.25±2.488	33.47±2.513	27.29±2.292	53.03±5.052	12.84±1.311	200.58±11.274	76.54±2.711	277.39±12.828
IS										
Males	22.97±3.020	50.99±5.547	40.73±3.388	40.70±4.135	32.78±7.708	69.00±11.350	16.13±1.910	257.17±14.823	82.34±3.202	339.41±15.706
Females	22.26±2.581	49.35±6.045	39.64±3.563	40.26±4.103	32.39±8.369	66.03±11.004	15.76±1.869	249.97±15.421	80.45±3.541	330.39±16.091
II										
Males	23.84±2.944	50.16±5.740	40.41±3.396	41.82±4.684	35.61±10.069	63.59±13.451	15.37±1.764	255.49±15.506	81.10±2.971	336.55±15.747
Females	23.33±3.516	48.41±6.828	38.96±3.978	40.29±4.113	33.63±9.984	61.83±11.060	15.01±1.643	246.46±16.695	79.58±3.359	326.08±17.240
IN										
Males	20.24±2.078	48.94±6.578	41.07±3.976	41.08±4.456	30.80±4.504	74.50±9.727	16.02±1.788	257.14±17.402	83.45±2.986	339.97±16.643
Females	19.55±2.034	46.55±7.271	39.47±3.954	39.38±3.816	29.46±4.237	72.71±7.655	15.78±1.561	246.87±16.991	81.92±3.610	328.88±17.942
IT										
Males	17.08±1.221	45.68±6.071	40.55±3.748	38.69±4.222	32.51±4.551	68.30±9.122	14.99±2.051	242.89±14.691	82.16±2.852	324.94±15.330
Females	16.78±1.009	43.01±6.482	38.36±3.796	37.34±3.779	31.33±5.000	65.95±7.474	14.79±1.505	232.64±14.883	80.60±3.319	313.28±15.680

Central sub-field (CSF), Inner superior (IS), Inner inferior (II), Inner nasal (IN), Inner temporal (IT), Outer superior (OS), Outer inferior (OI), Outer nasal (ON), Outer temporal (OT), Retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), Inner nuclear layer (INL), Outer plexiform layer (OPL), Outer nuclear layer (ONL), retina pigment epithelium (RPE), micrometers (µm). The cells highlighted depict the sectors where the retinal thickness values were significantly (p<0.05) different between males and females

Consistent with the previous studies,^[17,32,34] we found that there was a high degree of interocular symmetry in all subfields and all layers.

The wide variation in the results being reported in different studies could be due to the different ethnicity of the population being studied, different OCT machines used (each having different segmentation algorithms and hence the measured thicknesses), varying sample sizes, and the varying proportion of the age groups included in the study. Hence, having a normative database for one's population and the specific model of OCT machine being used is necessary to make meaningful conclusions from patient data.

Limitations of the present study are conclusions on age-related changes in retinal thicknesses were based on cross-sectional data rather than longitudinal data; the subjects were not objectively checked for systemic diseases, rather self-reported health information was used; subjects of Indian ethnicity alone were included; data were collected from subjects with a refractive error between -2 and +2 dioptre only (hence, results cannot be extrapolated to eyes with greater refractive errors).

Conclusion

In the central sub-field, age-related thickening was noted in the RNFL, IPL, INL, OPL, and ONL layers. The average inner ring thickness decreased with age in the GCL and IPL and increased in the RPE layer. The average outer ring thickness decreased with age in the GCL, IPL, and INL layers and increased in the OPL. The average IR and OR thickness was significantly less in women than in men. There was no correlation between IOP/CCT and the thickness of retinal layers. Future studies should aim at prospectively following up with healthy subjects to understand the actual effect of age on retinal layer thickness. Also, studies should include subjects with a wider range of refractive errors and a larger sample size.

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Conflicts of interest

There are no conflicts of interest.

References

- Fujimoto JG, Pitris C, Boppart SA, Brezinski ME. Optical coherence tomography: An emerging technology for biomedical imaging and optical biopsy. *Neoplasia* N Y N 2000;2:9–25.
- Vajzovic L, Hendrickson AE, O'Connell RV, Clark LA, Tran-Viet D, Possin D, *et al.* Maturation of the human fovea: Correlation of spectral-domain optical coherence tomography findings with histology. *Am J Ophthalmol* 2012;154:779–89.e2.
- Tian J, Varga B, Tatrai E, Fanni P, Somfai GM, Smiddy WE, *et al.* Performance evaluation of automated segmentation software on optical coherence tomography volume data. *J Biophotonics* 2016;9:478–89.
- Seigo MA, Sotirchos ES, Newsome S, Babiarz A, Eckstein C, Ford E, *et al.* *In vivo* assessment of retinal neuronal layers in multiple sclerosis with manual and automated optical coherence tomography segmentation techniques. *J Neurol* 2012;259:2119–30.
- Li ST, Wang XN, Du XH, Wu Q. Comparison of spectral-domain optical coherence tomography for intra-retinal layers thickness measurements between healthy and diabetic eyes among Chinese adults. *PLoS One* 2017;12:e0177515.
- Ctori I, Huntjens B. Repeatability of foveal measurements using spectralis optical coherence tomography segmentation software. *PLoS One* 2015;10:e0129005.
- Barteselli G, Bartsch DU, Viola F, Mojana F, Pellegrini M, Hartmann KI, *et al.* Accuracy of the Heidelberg Spectralis in the alignment between near-infrared image and tomographic scan in a model eye: A multicenter study. *Am J Ophthalmol* 2013;156:588–92.
- Early Treatment Diabetic Retinopathy Study design and baseline patient characteristics. ETDRS report number 7. *Ophthalmology* 1991;98(Suppl 5):741–56.
- Hood DC, Raza AS, de Moraes CGV, Liebmann JM, Ritch R. Glaucomatous damage of the macula. *Prog Retin Eye Res* 2013;32C: 1–21.
- Eliwa TF, Hussein MA, Zaki MA, Raslan OA. Outer retinal layer thickness as good visual predictor in patients with diabetic macular edema. *Retina Phila Pa* 2018;38:805–11.
- Esen E, Sizmaz S, Balal M, Yar K, Demirkiran M, Unal I, *et al.* Evaluation of the innermost retinal layers and visual evoked potentials in patients with multiple sclerosis. *Curr Eye Res* 2016;41:1353–8.
- Wagner-Schuman M, Dubis AM, Nordgren RN, Lei Y, Odell D, Chiao H, *et al.* Race- and sex-related differences in retinal thickness and foveal pit morphology. *Invest Ophthalmol Vis Sci* 2011;52:625–34.
- Demirkaya N, van Dijk HW, van Schuppen SM, Abramoff MD, Garvin MK, Sonka M, *et al.* Effect of age on individual retinal layer thickness in normal eyes as measured with spectral-domain optical coherence tomography. *Invest Ophthalmol Vis Sci* 2013;54:4934–40.
- Nieves-Moreno M, Martínez-de-la-Casa JM, Cifuentes-Canorea P, Sastre-Ibáñez M, Santos-Bueso E, Sáenz-Francés F, *et al.* Normative database for separate inner retinal layers thickness using spectral domain optical coherence tomography in Caucasian population. *PLoS One* 2017;12:e0180450.
- Sowmya V, Venkataramanan VR, Prasad V. Effect of refractive status and axial length on peripapillary retinal nerve fibre layer thickness: An Analysis Using 3D OCT. *J Clin Diagn Res* 2015;9:NC01-4.
- Huo YJ, Guo Y, Li L, Wang HZ, Wang YX, Thomas R, *et al.* Age-related changes in and determinants of macular ganglion cell-inner plexiform layer thickness in normal Chinese adults. *Clin Experiment Ophthalmol* 2018;46:400–6.
- Çubuk M, Kasım B, Koçluk Y, Sükgen EA. Effects of age and gender on macular thickness in healthy subjects using spectral optical coherence tomography/scanning laser ophthalmoscopy. *Int Ophthalmol* 2018;38:127–31.
- Grover S, Murthy RK, Brar VS, Chalam KV. Normative data for macular thickness by high-definition spectral-domain optical coherence tomography (spectralis). *Am J Ophthalmol* 2009;148:266–71.
- Sung KR, Wollstein G, Bilonick RA, Townsend KA, Ishikawa H, Kagemann L, *et al.* Effects of age on optical coherence tomography measurements of healthy retinal nerve fiber layer, macula, and optic nerve head. *Ophthalmology* 2009;116:1119–24.
- Xu Q, Li Y, Cheng Y, Qu Y. Assessment of the effect of age on macular layer thickness in a healthy Chinese cohort using spectral-domain optical coherence tomography. *BMC Ophthalmol* 2018;18:169.
- Duan XR, Liang YB, Friedman DS, Sun LP, Wong TY, Tao QS, *et al.* Normal macular thickness measurements using optical coherence tomography in healthy eyes of adult Chinese persons: The Handan

- eye study. *Ophthalmology* 2010;117:1585–94.
22. Won JY, Kim SE, Park YH. Effect of age and sex on retinal layer thickness and volume in normal eyes. *Medicine (Baltimore)* 2016;95:e5441.
 23. Invernizzi A, Pellegrini M, Acquistapace A, Benatti E, Erba S, Cozzi M, *et al.* Normative data for retinal-layer thickness maps generated by spectral-domain OCT in a white population. *Ophthalmol Retina* 2018;2:808-15.e1.
 24. Song WK, Lee SC, Lee ES, Kim CY, Kim SS. Macular thickness variations with sex, age, and axial length in healthy subjects: A spectral domain-optical coherence tomography study. *Invest Ophthalmol Vis Sci* 2010;51:3913–8.
 25. Adhi M, Aziz S, Muhammad K, Adhi MI. Macular thickness by age and gender in healthy eyes using spectral domain optical coherence tomography. *PLoS One* 2012;7:e37638.
 26. Ooto S, Hangai M, Tomidokoro A, Saito H, Araie M, Otani T, *et al.* Effects of age, sex, and axial length on the three-dimensional profile of normal macular layer structures. *Invest Ophthalmol Vis Sci* 2011;52:8769–79.
 27. Gao H, Hollyfield JG. Aging of the human retina. Differential loss of neurons and retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 1992;33:1–17.
 28. Palazon-Cabanes A, Palazon-Cabanes B, Rubio-Velazquez E, Lopez-Bernal MD, Garcia-Medina JJ, Villegas-Perez MP. Normative database for all retinal layer thicknesses using SD-OCT posterior pole algorithm and the effects of age, gender and axial length. *J Clin Med* 2020;9:E3317.
 29. Motamedi S, Gawlik K, Ayadi N, Zimmermann HG, Asseyer S, Bereuter C, *et al.* Normative data and minimally detectable change for inner retinal layer thicknesses using a semi-automated OCT image segmentation pipeline. *Front Neurol* 2019;10:1117.
 30. Tewari HK, Wagh VB, Sony P, Venkatesh P, Singh R. Macular thickness evaluation using the optical coherence tomography in normal Indian eyes. *Indian J Ophthalmol* 2004;52:199–204.
 31. Sull AC, Vuong LN, Price LL, Srinivasan VJ, Gorczyńska I, Fujimoto JG, *et al.* Comparison of spectral/Fourier domain optical coherence tomography instruments for assessment of normal macular thickness. *Retina Phila Pa* 2010;30:235–45.
 32. Appukuttan B, Giridhar A, Gopalakrishnan M, Sivaprasad S. Normative spectral domain optical coherence tomography data on macular and retinal nerve fiber layer thickness in Indians. *Indian J Ophthalmol* 2014;62:316–21.
 33. Kim NR, Kim JH, Lee J, Lee ES, Seong GJ, Kim CY. Determinants of perimacular inner retinal layer thickness in normal eyes measured by Fourier-domain optical coherence tomography. *Invest Ophthalmol Vis Sci* 2011;52:3413–8.
 34. El-Ashry M, Hegde V, James P, Pagliarini S. Analysis of macular thickness in British population using optical coherence tomography (OCT): An emphasis on interocular symmetry. *Curr Eye Res* 2008;33:693–9.