Original Article

Corneal and corneal epithelial thickness distribution characteristics in healthy North Indian eyes using spectral domain optical coherence tomography

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Purpose: To determine the pattern of corneal thickness and epithelial thickness distribution in healthy North Indian eyes by using spectral domain optical coherence tomography (SD-OCT). **Methods:** The observational study measured total corneal and epithelial thickness in the central 2 mm zone and eight sectors each in paracentral 2–5 mm (ring 1) and midperipheral 5–7 mm (ring 2) zones on SD-OCT. **Results:** The study included 67 eyes of 67 subjects with a male:female ratio of 32:35 and mean age of 25.04 ± 4.54 years. The mean central corneal and epithelial thicknesses were 505.97 ± 30.12 µm and 60.48 ± 8.37 µm, respectively. The epithelium of inferior and infero-nasal sectors in ring 1 and inferior sector in ring 2 was significantly thicker than the radially opposite sectors of the respective rings ($P = 0.001$; $P = 0.01$ and $P = 0.02$, respectively). Sector-wise analysis did not reveal any significant correlation between the total corneal thickness and epithelial thickness (all $P > 0.05$) except in the outer superior sector where there was a weak positive correlation (r = 0.28, $P = 0.02$). Central epithelial thickness in males (60.59 ± 9.28 µm) and females (60.37 ± 7.58 µm) was comparable ($P = 0.91$). Pachymetry was thinnest in the inferior, inferonasal, and inferotemporal sectors in 44.79% of eyes (n = 30), while thinnest epithelium was seen in the superior, superonasal, and superotemporal quadrants in 50.75% of eyes (n = 34). **Conclusion:** The epithelial thickness distribution in this sample of topographically normal healthy North Indian eyes was nonuniform and independent of the underlying corneal thickness. Epithelium was thinner in the superior cornea, whereas total corneal thickness was minimum in the inferior part.

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The corneal epithelium plays an important role in the maintenance of corneal regularity due to its excellent regenerative capacity and rapid turnover rate, which allows it to remodel in response to underlying stromal irregularities.[1-3] The epithelium also contributes to the refractive power of the cornea, ranging from an average of 1.03 D over the central 2-mm zone to 0.85 D at the 3.6-mm zone.[4] Thus, changes in the thickness and distribution of corneal epithelium may, on one hand, be the earliest indicators of various corneal disorders, including ectasia, dystrophy, and contact lens-associated keratopathy, while on the other hand, they may be responsible for refractive surprises after keratorefractive surgery.[3,5-9] As a consequence, epithelial thickness profiles and their relationship with the underlying stroma are being increasingly utilized in algorithms designed to detect "pre topographic" keratectasia and for customization of refractive procedures to refine postoperative outcomes.[5,10]

The mean central corneal thickness in the Indian population has been demonstrated to be thinner than that in Caucasian eyes.[11] However, information regarding the epithelial and stromal distribution characteristics is sparse, with only Hoshing *et al*.[12] having recently reported the corneal epithelial thickness distribution in Indian eyes. The current observational study was conducted to determine the characteristics of corneal epithelial thickness and corneal thickness (stroma plus epithelium) distribution and their correlation across 7-mm central cornea in healthy North Indian eyes by using an ultrafast spectral-domain optical coherence tomography (SD-OCT).

Methods

Patients

The study was conducted at a tertiary care ophthalmology institute in North India. The principles of good clinical practice were adhered to in accordance with the Declaration of Helsinki. Approval from the institutional ethics committee was obtained, and informed consent was taken from all subjects.

The study population consisted of healthy volunteers and patients seeking consultation for refractive errors or laser refractive surgery. All patients underwent a comprehensive ophthalmic examination including visual acuity testing, slit-lamp bio-microscopy, dilated fundus examination, and intraocular pressure measurement with noncontact tonometry. Patients with any corneal pathologies, such as corneal scarring, dystrophies, and clinical signs of corneal ectasia on slit-lamp

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biomicroscopy, were excluded as were those with a topographic pattern suggestive of corneal ectasia on the axial curvature maps obtained by Schiempflug imaging with the Pentacam. Other exclusion criteria were history of contact lens use within the previous 3 months, long-term topical medications, dry eye disease, and pregnancy/lactation. Only one eye per patient was used for data analysis, the study eye being selected by using a computer-generated random number table.

Corneal and epithelial thickness mapping

The corneal and epithelial thickness was measured using an ultrafast SD-OCT device (Revo Nx, OPTOPOL Technology, Poland) having an 830-nm light source with a scanning speed of 110,000 measurements/s and axial and transverse resolution of 5 and 12 µm, respectively, in tissue. The cornea was mapped using the "anterior, with adaptor, topography OCT (T-OCT) module" by using the "automatic capture" option with an average duration of image acquisition of 0.3 s. The "T-OCT" module provides both corneal thickness (stroma plus epithelium) and corneal epithelial thickness maps. Only measurements with the best acquisition quality (flagged with a green checkmark by the machine) were included for statistical analysis.

The corneal and epithelial thickness was profiled over the central 7 mm with data output including the option of displaying average, minimum, and maximum thickness in 17 sectors [Fig. 1a] divided into three zones: (i) a central zone within the 0–2-mm diameter, (ii) a paracentral zone from 2 to 5 mm (inner ring/ring 1) divided into eight sectors (superior [S], inferior[I], nasal [N], temporal [T], superonasal [SN], inferonasal [IN], superotemporal [ST] and inferotemporal [IT]), and (iii) a midperipheral zone from 5 to 7 mm (outer ring/ring 2) comprising eight sectors similar to ring 1.

The inbuilt software of the OCT device automatically gives eight parameters each for the cornea and epithelium (µm): (i) central thickness, (ii) minimum thickness at 7 mm, (iii) maximum thickness at 7 mm, (iv) minimum-maximum thickness at 7 mm, (v) SN-IT thickness at 5 mm, (vi) S-I thickness at 5 mm, (vii) ST-IN thickness at 5 mm, and (viii) T-N thickness at 5 mm. Values for each of these parameters was entered in an Excel sheet for every patient and the mean was calculated [Table 1]. As the difference in the radially opposite sectors of the midperiphery, that is, 5–7-mm zone, is not given by the automated software, as an additional step, using the "average thickness" display option, the corneal and epithelial thickness was noted for all patients in each of the 17 segments. The mean corneal and epithelial thickness differences of the radially opposite sectors were then calculated by independently averaging the values in the corresponding sectors for all patients and subtracting the resultant means [Table 2].

Statistical analysis

The statistical analysis was done using the commercially available SPSS Statistics software (version 22.0, IBM Corp.). Descriptive statistics for continuous data were reported as mean ± standard deviation (SD). The independent samples Student's *t* test was used to compare the corneal and epithelial thickness in radially opposite sectors of the cornea and corneal and epithelial thickness in males versus females. One way analysis of variance (ANOVA) test was applied to compare the epithelial thickness: corneal thickness (ET/CT) ratio in the central zone with eight sectors in each of the paracentral and midperipheral zones. Pearson's correlation coefficient was used to look for any association of epithelial thickness with corneal thickness. $P < 0.05$ was considered significant.

Results

The study included 67 eyes of 67 subjects, of whom 32 (47.8%) were males and 35 (52.2%) females. There were 32 right eyes (47.8%) and 35 (52.2%) left eyes. The mean age and manifest refractive spherical equivalent (MRSE) were 25.04 ± 4.54 years (range: 13–36 years) and −2.56 ± 2.43 D, respectively. The mean simulated keratometry (K_m) and maximum keratometry (K_{max}) as recorded on the Pentacam were 43.85 ± 1.57 D (range: 40–47.6 D) and 44.84 ± 1.74 D (range: 40.2–48.7 D), respectively.

Sectoral corneal and epithelial thickness distribution

Fig. 1b and c depict the mean corneal and epithelial thickness, respectively, in the central 2-mm zone and eight sectors each in ring 1 (2–5-mm zone) and ring 2 (5–70mm zone). A "V"-shaped distribution was noticed for the corneal thickness, with the apex of the "V" coinciding with the inferotemporal sectors where the pachymetry was the least for both rings 1 and 2 [Fig. 1d]. The epithelial distribution showed an inverse "V" pattern, the thickest epithelium being seen at the apex of the inverted "V," which corresponded to the inferior sectors for both rings 1 and 2 [Fig. 1e]. A sector-wise analysis did not reveal any significant correlation between the total corneal thickness and epithelial thickness (all $P > 0.05$) except in the outer superior sector where there was a weak positive correlation (r = 0.28, $P = 0.02$). The

Table 1: Corneal and epithelial thickness profile based on data displayed by the inbuilt software of the Revo Nx spectral-domain OCT

	Corneal thickness (µm)	Epithelial thickness (µm)
Central thickness (µm)	505.97±30.12	60.48±8.37
Minimum thickness (µm) $_{[7\ mm]}$	493.18±29.12	37.43±7.39
Maximum thickness (µm) $_{[7\ mm]}$	585.22±41.07	106.66±26.94
Minimum- maximum thickness (µm) $_{[7\ mm]}$	−81.58±47.28	−66.01±36.1
SN- IT cornea (µm) $_{[5\ mm]}$	28.94±19.37	−1.78±10.06
S-I cornea (µm) $_{[5\ mm]}$	23.15±23.91	−5.39±10.50
ST- IN cornea (µm) $_{[5\ mm]}$	2.79±16.92	−2.67±12.16
T- N cornea (µm) $_{[5\ mm]}$	−23.67±21.80	−1.21±6.78

Data depicted are the mean of values provided by the inbuilt software of the machine (RevoNx, Optopol, Poland) for each parameter. I: Inferior; IN: inferonasal; IT: inferotemporal; N: nasal; S: Superior; SN: superonasal; ST: superotemporal; T: temporal

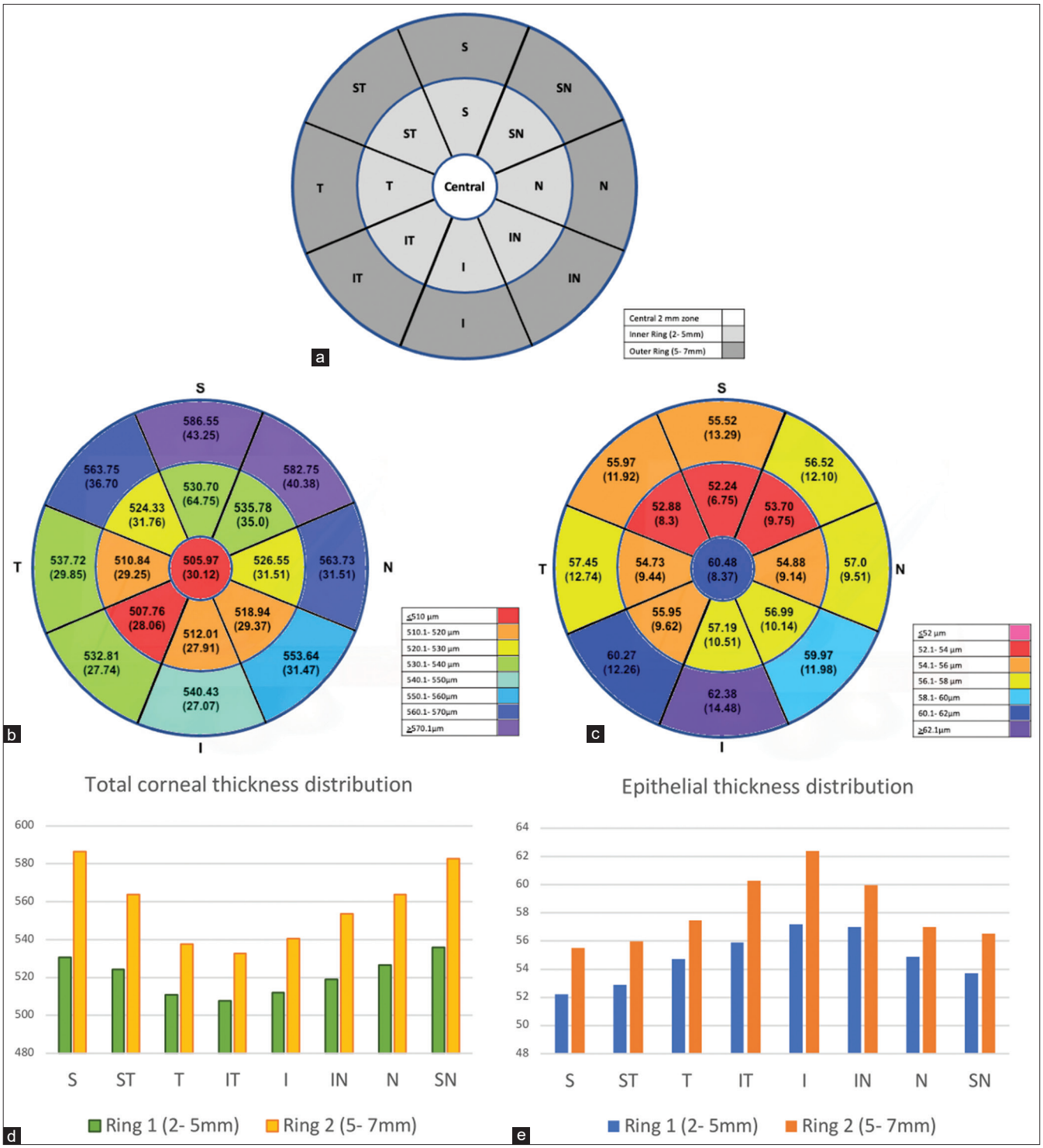

Figure 1: (a) Reference image showing distribution of the 17 sectors within the 7-mm zone of the cornea [*Central-central, (S)-superior, (ST)-superotemporal, (T) - temporal, (IT)- inferotemporal, (I)- inferior, (IN)- infero nasal, (N)- nasal, (SN)- superonasal].* (b and d) Sectoral distribution of corneal thickness (mean ± SD in µm); (c and e) Sectoral distribution of epithelial thickness (mean ± SD in µm) in the study sample. Corneal thickness (d) and epithelial thickness (e) show opposite pattern of distribution in diferent sectors. ["V" shape in d and "inverted V" in e]

mean epithelial thickness to corneal thickness (ET/CT) ratio at the center was 0.12 ± 0.02, implying a nearly 12.0 ± 2% contribution of the epithelium to the total corneal thickness. This ratio was comparable in all the sectors, with ET/CT ratio ranging from a minimum of 0.1 ± 0.02 to a maximum of 0.11 ± 0.08 ($P > 0.05$).

Corneal thickness in radially opposite sectors

Table 1 shows the mean values of the parameters provided by the inbuilt software of the machine, while Table 2 shows the mean differences in radially opposite sectors of ring 1 (2–5 mm) and ring 2 (5–7 mm), which were calculated by independently noting the values in each of the 17 sectors by using the

Table 2: Mean corneal and epithelial thickness differences in radially opposite sectors based on data noted individually in each of the 17 sectors for every patient

Zone	Sector difference	Corneal thickness difference (μm)		Epithelial thickness difference (μm)	
		Mean ± SD	*P*	Mean ± SD	*P*
Inner Ring (2-5 mm)	SN- IT cornea (μm)	28.01±19.97	<0.001*	−1.03±5.38	0.19
	S-I cornea (μm)	24.66±22.73	<0.001*	−4.95±9.96	0.001*
	ST- IN cornea (μm)	5.39±16.41	0.16	−4.1±6.88	0.01*
	T- N cornea (μm)	−15.72±15.37	0.005*	−0.15±4.15	0.93
Outer Ring (5-7 mm)	SN- IT cornea (μm)	49.94±4.1	<0.001*	−4.08±1.49	0.22
	S-I cornea (μm)	46.12±4.32	<0.001*	−7.52±2.07	0.02*
	ST- IN cornea (μm)	10.1±3.2	0.07	−4.12±1.41	0.13
	T- N cornea (μm)	−26.02±3.12	<0.001*	0.6±1.13	1

Mean difference for radially opposite sectors was calculated by noting the corneal thickness and epithelial thickness in each of the 17 sectors (using the "average thickness" display option) for all patients in an excel sheet and calculating the differences. I: Inferior; IN: inferonasal; IT: inferotemporal; N: nasal; S: Superior; SN: superonasal; ST: superotemporal; T: temporal; (* Independent Student's *t* test, *P*<0.05 significant)

"average thickness" display option for each patient and then subtracting the means. The supero nasal [SN], superior [S], and nasal [N] corneal sectors were significantly thicker than the radially opposite inferotemporal [IT], inferior [I], and temporal [T] sectors, respectively, for both the paracentral 2–5-mm zone (ring 1) and the midperipheral 5–7-mm (ring 2) zone [Table 2].

Epithelial thickness in radially opposite sectors

The epithelium was significantly thinner in the superior [S] and supero temporal [ST] sectors of ring 1 as compared to the radially opposite inferior [I] ($P < 0.001$) and inferonasal [IN] ($P < 0.01$) sectors [Table 2]. In the mid peripheral zone, that is, ring 2, a significant difference in epithelial thickness was seen only in the vertical meridian, with the superior [S] epithelium being thinner than the inferior ($P = 0.02$).

Distribution of thinnest sectors for corneal thickness and epithelial thickness

Fig. 2a and b demonstrate the percentage distribution of the sectors with the least corneal and epithelial thickness in the study population. Dividing the cornea into five regions, that is, central 2 mm, nasal (comprising nasal sectors of both inner and outer rings), temporal (comprising temporal sectors of both inner and outer rings), inferior (comprising the inferior, inferotemporal, and inferonasal sectors of both the inner and outer rings), and superior (comprising the superior, superotemporal, and superonasal sectors of both the inner and outer rings), the inferior region had the thinnest pachymetry in the maximum number of eyes (n = 30; 44.79%) [Fig. 2c]. In contrast, the minimal epithelial thickness in a majority of the eyes (n = 34; 50.75%) was noted in the superior region [Fig. 2d].

Corneal and epithelial thickness distribution by gender

Fig. 3 a-d show the sector-wise corneal and epithelial thickness distribution by gender. The corneal thickness was comparable in all the sectors for males and females (all $P > 0.05$; independent student's *t* test).

The mean central and average epithelial thickness over the 7-mm area for males was 60.59 ± 9.28 μm and 58.78 ± 10.17 μm, respectively. The central and average epithelial thickness in the central 7-mm zone in females was 60.37 ± 7.58 μm and 56.91 ± 7.17 μm, respectively. The central and average epithelial thickness was statistically comparable between genders ($P = 0.91$ and $P = 0.2$, respectively). The epithelium was thicker in males in all the sectors as compared to females, though the difference did not achieve statistical significance in any of the sectors [Fig. 3e-f].

Discussion

Central corneal epithelial thickness values for normal eyes reported in the published literature range from 48.0 ± 5 to 59.9 ± 5.9 μm.[13-21] The mean central epithelial thickness of 60.48 ± 8.37 μm in our patient cohort was at the higher end of this range and comparable to the values of 58.4 ± 2.5, 59.9 ± 5.9, and 57.4 ± 7.7 μm reported in other AS-OCT based studies by Feng *et al.*,[19] Wang *et al.*,[14] and Wirbelauer *et al.*[15] The relatively wide range of central epithelial thickness documented in different publications may be attributable in part to differences in the technology used to measure the epithelial thickness (e.g., the use of very high-frequency ultrasound (VHFUS) viz a viz AS-OCT) or different modes of data capture using the same technology (e.g., manual versus automatic measurements by using AS-OCT).[2,18,20,21]

However, even when such confounders are removed, variability in reported central epithelial thickness values can be noted. For instance, the central epithelial thickness in our predominantly North Indian population was approximately 6-μm thicker than the mean central epithelial thickness of 54 and 53.9 μm reported by Hoshing *et al.*[12] and Hashmani *et al.*[22] from western India and the western part of the Indian subcontinent, respectively. Similar to our study, both Hoshing[12] and Hashmani[22] used SD-OCT with an axial resolution of 5 μm in tissue, and the data were captured automatically using inbuilt software. Though this 10%–12% difference in epithelial thickness measurements may be a result of the relatively smaller sample size of the current study viz a viz the other two studies, the authors propose that geographic, climatic, and perhaps racial influences may also be responsible and should be investigated in future studies with larger sample sizes. These factors may be especially important in a country like India with a vast geographic expanse and a racially diverse population. Ringvold *et al.*[23] demonstrated some influence of varying latitude and ambient radiation in influencing human corneal epithelial thickness in *ex vivo* conditions.

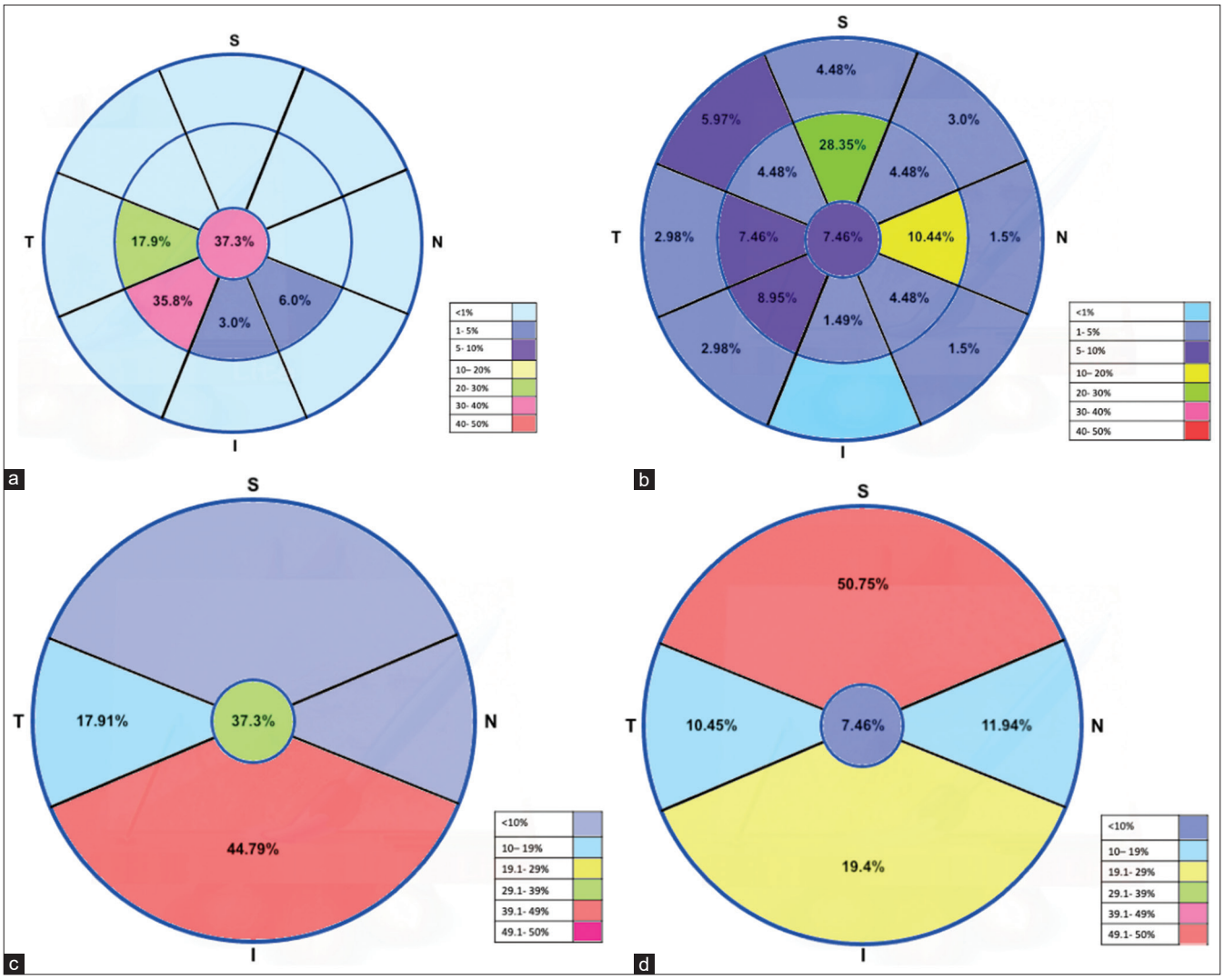

Figure 2: Percentage distribution of thinnest sectors in the study population: (a and c) Total corneal thickness; (b and d) Corneal epithelial thickness

While the central and paracentral epithelium must maintain a relatively constant profile to maintain a stable corneal power and ocular refraction in an individual over time, within-eye topographic variation has been reported for normal eyes, the epithelium usually being thinner superiorly and temporally than inferiorly and nasally.[21,24-26] A similar pattern was observed in our patients with the superior and superotemporal sectors of the 2–5-mm zone (ring 1) being 4.95 ± 9.96 and 4.1 ± 6.88 μm thinner than the radially opposite inferior and inferonasal sectors, respectively [Table 2]. This difference was greater than the 2.36 and 2.77-μm thinner superior epithelium reported in Indian subjects by Hoshing *et al*.[12] for right and left eyes, respectively, at the 2–5mm zone, but comparable in magnitude to the 5.7-μm thinner superior epithelial thickness reported by Reinstein *et al*.[2] at a 3-mm zone. Superior and superotemporal temporal epithelial thinning has been attributed to multiple factors, including frictional forces applied by the upper lid during blinking, the higher location of the outer canthus as compared to the inner canthus, the effect of gravity, and a shorter contact time of the tear film in the superior meridian leading to reduced lubrication and/or nourishing effects with subsequent thinning over time.[2,22,27,28]

The authors would like to highlight the difference between the values mentioned in Tables 1 and 2 for the radially opposite sectors in ring 1, for example, the T-N and ST-IN differences for epithelial thickness are mentioned as −1.21 ± 6.78 and −2.67 ± 12.16 μm, respectively, in Table 1 and −0.15 ± 4.15 and −4.1 ± 6.88 μm, respectively, in Table 2. These differences are a result of the way the data were acquired and handled; Table 1 presents the means obtained by averaging the values displayed by the inbuilt software, whereas Table 2 presents the data derived by independent calculation of the means in each sector followed by subtraction of the resultant means of radially opposite sectors. We propose that the method used in Table 2 is a more accurate reflection of the distribution characteristics of the epithelium than the method used in Table 1 as the latter, that is, averaging the data provided automatically by the SD-OCT software introduces a systematic error in the form of "averaging the averages." In such a situation, the result obtained is only algebraic and not distributive. This factor needs to be accounted for when interpreting data from previous publications.

No sectoral or zonal correlation of epithelium thickness and pachymetry was noted in our study, with the majority

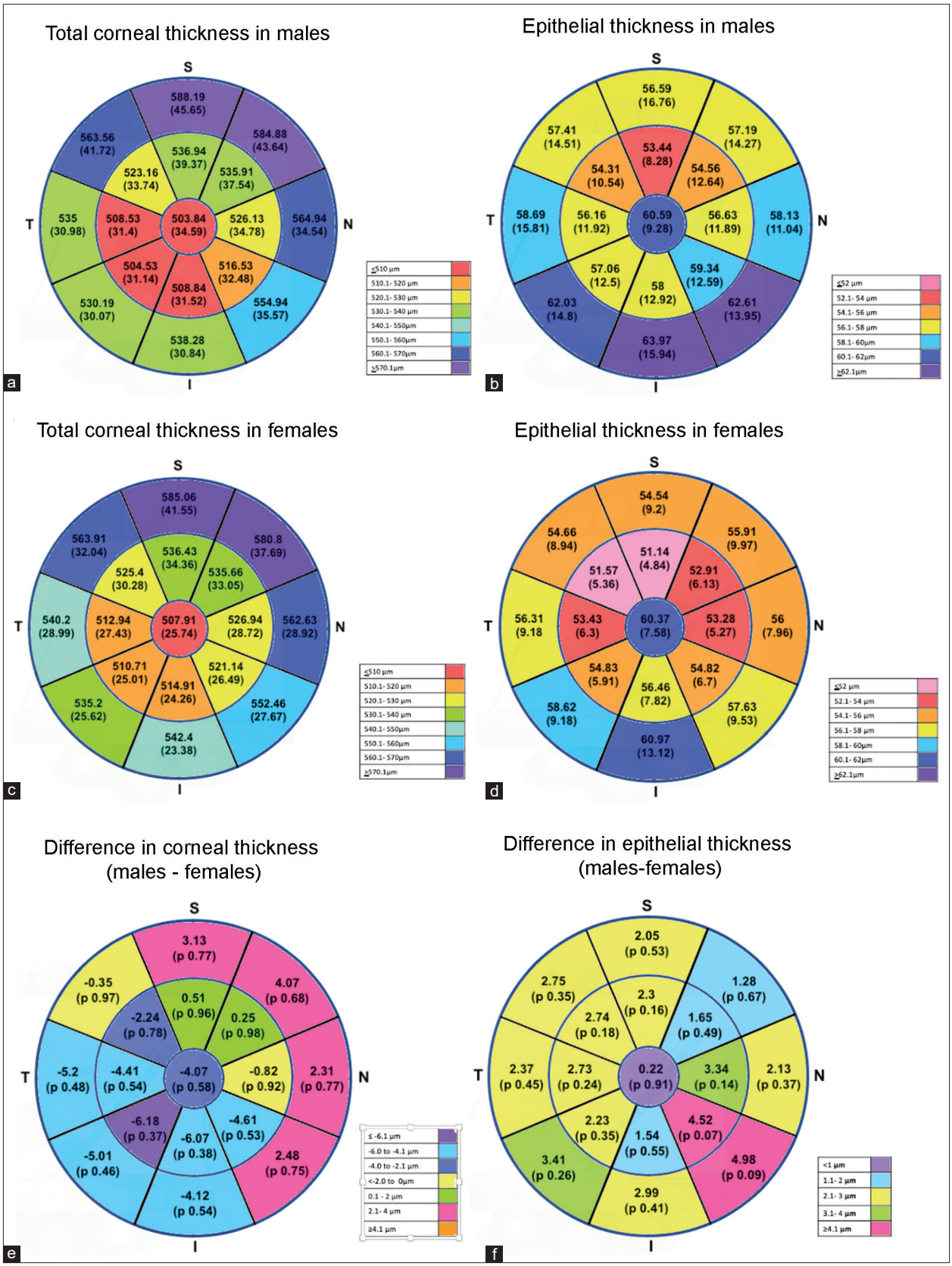

Figure 3: Sectoral distribution of total corneal thickness in µm (mean ± SD): (a) males and (c) females Sectoral distribution of epithelial thickness: (b) males and (d) females in µm (mean ± SD). Mean difference (males – females): (e) total corneal thickness (f) epithelial thickness. ($P < 0.05$ significant; student's independent *t* test)

of cases having the thinnest pachymetry inferiorly and the thinnest epithelium superiorly. These observations are consistent with the concept that the change in the epithelial thickness is independent of the underlying stromal thickness but depends on the change in the surface curvature and the rate of change of curvature.[1] This hypothesis is further strengthened by the observations of Wang *et al*.[20] who reported a positive correlation between epithelial thickness and stromal thickness only in keratoconic eyes and not in normal eyes. The topographic variability of the epithelium in normal eyes is thus more likely driven by the anatomic and physiological factors related to blink and tear film dynamics than the underlying stromal thickness.

In the current study, the average epithelial thickness in males was 1.87-µm thicker as compared to females, with the difference being > 2 µm in 13 of the 17 sectors evaluated. These observations were comparable to those of Hashmani *et al*.[22] (n = 220 eyes) and Kanellopoulos *et al*.[25] (n = 373 eyes) who also documented a thicker epithelium in males in all locations, with the difference per sector ranging from 0.7 to 2.9 µm for Hashmani *et al*.[22] and between 1.31 and 2.21 µm for Kanellopoulos *et al*.[25] Wu *et al*.[24] (n = 215 eyes) also reported the average epithelial thickness to be 1.31 µm greater in males. The gender-based thickness differences, despite being comparable in magnitude, were statistically significant for the abovementioned studies[22,24,25] but not for our cohort probably due to the smaller sample size of our study.

The authors would like to acknowledge certain limitations of the study, including the relatively small sample size, the limited age range of the patients which did not allow an age-wise comparison of epithelial characteristics, and the lack of evaluation of epithelial distribution characteristics beyond 7 mm. Though reproducibility of readings was not evaluated in the current study, previous studies have shown good reproducibility of epithelial thickness mapping by using SD-OCT.[25,26,29,30] Additionally, the ultrafast scanning speed of the SD-OCT device used with a very short image acquisition time of 0.3 s could be expected to minimize variations as a result of involuntary eye movements and tear film thinning/break up and evaporation.

Conclusion

In conclusion, we report the corneal epithelial and total corneal thickness distribution in healthy eyes and between gender comparison of these parameters from a predominantly North Indian cohort. Consistent with published literature, the pattern of epithelial distribution was confirmed to be nonuniform over the 7-mm area studied, with a distinct superior-inferior asymmetry and a lack of correlation with underlying stromal thickness. The central epithelial thickness of 60.48 ± 8.37 µm was approximately 6 µm greater than that reported from the western part of India recently. Further studies with a larger sample size are suggested to confirm or refute these differences to be able to truly "customize" corneal epithelial thickness-based diagnostic or therapeutic algorithms for our population.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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