Enhanced Depth Imaging Spectral-Domain Optical Coherence Tomography of the Choroid in Thai Population

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Objective: *To examine subfoveal choroidal thickness (SFCT) in Thai population using enhanced depth imaging spectral-domain optical coherence tomography (EDI-OCT) and to study its correlation with foveal retinal pigment epithelium thickness (FRPE), central neurosensory retinal thickness (CNRT), age, and refraction.*

Material and Method: *Four hundred eighty eyes from 240 subjects without glaucoma, retinal, or choroidal diseases underwent scanning of the retina and choroid using EDI-OCT. SFCT, FRPE, and CNRT measurements were based on the 1:1 micron images and were performed by two independent observers. The reliability of measurements between the observers was evaluated by intraclass correlation coefficient (ICC). The correlations of SFCT with FRPE, CNRT, age, and refractive error were analyzed.*

Results: *The mean age of the subjects was 36.22 years (range 20-81 years). The means (95% reference intervals) of SFCT, CNRT, and FRPE were 294.02 µm (137.14-450.90 µm), 174.22 µm (141.82-206.62 µm), and 41.94 µm (34.65-49.23 µm), respectively. SFCT and CNRT had excellent reliability between the two observers [ICC = 0.947 (95% CI, 0.918-0.963) and 0.929 (95% CI, 0.906-0.945), respectively], while FRPE showed good reliability [ICC = 0.729 (95% CI, 0.637-0.793)]. SFCT had a low positive correlation with FRPE ($r = 0.179$, $p<0.0001$) but not with CNRT ($p = 0.317$). SFCT showed a positive correlation with refraction ($r = 0.338$, $p<0.0001$) and a negative correlation with age ($r = -0.166$, $p<0.0001$). Regression analysis suggested that the SFCT decreased by 12.23 µm per one decade of life and by 11.42 µm per one diopter of myopia.*

Conclusion: *Normal values of SFCT in Thai population were obtained. SFCT significantly decreased with older age and higher myopia. SFCT was associated with FRPE, reflecting the same vascular supply of the choroid and retinal pigment epithelium. When measured with our technique based on the 1:1 micron images, the reliability of SFCT measurement was very high despite highly morphologic inter-individual variations.*

Keywords: *Choroidal thickness, EDI-OCT, Thai*

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Enhanced depth imaging spectral-domain optical coherence tomography (EDI-OCT) is a recent method in which spectral-domain OCT devices (Spectralis®, Heidelberg Engineering, Heidelberg, Germany) are moved closer to the eye to improve visualization of the innermost hyperreflective line of sclera beneath the large vessel layers of the choroid. Therefore, it produces high-resolution, cross-sectional images of the choroid and allows enhanced visualization of choroidal pathology and thickness[1].

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The choroid supports the retinal pigment epithelium and contributes a blood supply to the outer retina, including the photoreceptors[2]. Therefore, the abnormalities of the choroid associate with many retinal diseases. The ability to relay image of the choroid by EDI-OCT makes this an emerging area of study, particularly in ocular diseases that are known to involve the choroid such as choroidal neovascularization (CNV)[3], central serous chorioretinopathy (CSC)[4], polypoidal choroidal vasculopathy (PCV)[5] and high myopia[6].

Previous studies revealed that the choroid was thickest under the fovea and there were variations of the thickness among populations, such as 287 µm in whites[7], 261 µm in the Chinese[8] and 354 µm in the Japanese[9]. Other factors influencing choroidal thickness could be aging and refractive errors, but the data from previous studies were not concordant(7-10). However, these studies did not mention the methods of measurement in detail. Overestimation of the thickness could occur when measured using 1:1 pixel images rather than 1:1 micron images(11).

In the present study, we examined subfoveal choroidal thickness (SFCT) based on 1:1 micron images in 240 Thai subjects to determine the normal values in our population. The correlations of SFCT with foveal retinal pigment epithelium thickness (FRPE) and central neurosensory retinal thickness (CNRT), which have never been studied, were also investigated. The correlations of SFCT with age and refraction were also explored.

Material and Method

Subjects

With informed consent, Thai volunteers aged 20 years or more were recruited and underwent horizontal scanning of retina and choroid using EDI-OCT at the Department of Ophthalmology, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand between October 2011 and May 2013. Exclusion criteria were participants with glaucoma, retinal or choroidal diseases, any retinal or RPE abnormalities detectable with OCT, and poor image quality because of unstable fixation or severe cataract. The study was approved by the Siriraj Institutional Review Board and was conducted in adherence with the tenets of the Declaration of Helsinki.

EDI-OCT

The EDI-OCT technique was previously described(1). Briefly, the technique employed spectral-domain OCT device (Spectralis®, Heidelberg Engineering, Heidelberg, Germany) pushed sufficiently close to the eye to obtain an inverted image. Each section was obtained with eye tracking and 100 b-scans were averaged to improve the signal-to-noise ratio. A 9-mm horizontal image that included the fovea was obtained. The image was re-inverted for displaying purpose.

Retinal and choroidal thickness measurement

The image section of the subfoveal area, identified as the section covering the thinnest area of fovea, was selected. It was then adjusted to a 1:1 micron image (Fig. 1). The three reference lines, upper part RPE (inner border of the RPE interdigitaion hyperreflective band), Bruch's membrane (outermost border of the RPE hyperreflective bands), and chorio-scleral interface were manually drawn under 100% magnification. The image was then enlarged to 400% magnification and thickness measurements were manually performed with a measurement tool implemented in the instrument's software. The CNRT was defined as the vertical distance between the internal limiting membrane (ILM) and the upper part RPE at the foveal center. The FRPE was defined as vertical distance between upper part RPE and the Bruch's membrane. The SFCT was the vertical distance between the Bruch's membrane and the chorio-scleral interface. Two examiners (TW and PN) independently measured the thickness. The data from the two examiners were averaged. The inter-examiner variability was evaluated and in case where there was disagreement between the two, the third examiner (RN) would be required.

Refraction

Refraction was performed using an autorefractor (Nidek, Gamagohri, Japan). The refraction was presented in spherical equivalent.

Statistical analysis

Sample size was calculated using confidence interval 95%, standard deviation (SD) of SFCT 76 µm (the maximum value of SD from the previous studies(7,10,12)) and the desired margin of error of mean SFCT 25 µm. The minimal sample size was 36 eyes. It was planned to recruit subjects in six age groups (20-29, 30-39, 40-49, 50-59, 60-69, and >70 years old) and two refractive status (non-myopia and myopia), resulting in the sample size of at least 432 eyes (216 subjects).

All statistics were calculated using PASW Statistics for Windows version 18.0 (SPSS Inc.,

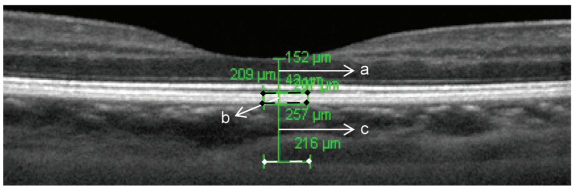

Fig. 1 A horizontal scan EDI-OCT image. Three manually drawn horizontal dotted lines from the uppermost to lowermost are upper part RPE, Bruch's membrane and chorio-scleral interface, respectively. Three vertical lines of measurement from uppermost to lowermost represent CNRT (a), FRPE (b) and SFCT (c), respectively.

Chicago, Illinois, USA). The interobserver variability was evaluated by intraclass correlation coefficient (ICC). Normal values of the thickness of SFCT, FRPE, and CNRT were reported in 95% reference intervals. One-way ANOVA was used to compare SFCT, FRPE, and CNRT values among age groups. Pearson's correlation was used to correlate SFCT values with FRPE, CNRT, age, and refractive error. Regression analysis was performed to study the relationship of SFCT with age and refractive error. A *p*-value of <0.05 was considered statistically significant.

Results

Two hundred and forty healthy volunteers (480 eyes) were enrolled in the present study. The mean ± SD of the age was 36.22±11.55 years (range 20 to 81 years) and the mean refractive error was -1.29 dioptors (D) (range -10.50 to +2.75 D). The distribution of subjects in each age group was shown in Table 1.

The reliability of measurements between the two observers was demonstrated by ICC. The ICC for CNRT and SFCT were 0.947 (95% CI, 0.918-0.963) and 0.929 (95% CI, 0.906-0.945), respectively, showing excellent reliability. The ICC for FRPE was also high at 0.729 (95% CI, 0.637-0.793).

The mean ± SD (95% reference interval) of SFCT was 294.02±80.04 μm (137.14-450.90 μm), and decreased significantly with age (p = 0.001, ANOVA) (Table 1). The means of CNRT and FRPE were 174.22±16.53 μm (141.82-206.62 μm) and 41.94±3.72 μm (34.65-49.23 μm), respectively. There were no significant differences in CNRT and FRPE among age groups (Table 2, 3). In fact, the CNRT and FRPE were very similar among age groups.

Correlation analysis showed that SFCT was negatively correlated with age (r = -0.166, p<0.0001), whereas it was positively correlated with spherical equivalent of refraction (r = 0.338, p<0.0001). Regression analysis suggested that the SFCT decreased by 12.23 μm per decade of life and decreased by 11.42 μm per diopter of myopia. In addition, the SFCT was also positively correlated with FRPE (r = 0.179, p<0.0001) but not with CNRT (p = 0.317).

Discussion

The present study provided the normal reference value of SFCT in Thai population. In addition, to the best of our knowledge, the present study was the first to study the correlation of SFCT with FRPE and CNRT using EDI-OCT technique. These reference data were crucial for further investigations of the choroid in pathologic conditions.

The choroid is involved in the pathophysiology of many diseases. Recent advancements in EDI-OCT technique[7-10,12-14], provided more details of the microarchitecture of the posterior choroid including choroidal thickness. Recent studies showed that the thickness of choroid changed in many retinal and choroidal diseases. This might help us predict the onset and monitor progression of the diseases.

The present study showed that the mean SFCT in 240 healthy Thai subjects was very similar to that reported by Margolis and Spaide[7] using the same EDI system (287 μm). Whereas, the value was thinner than that previously reported by Ikuno et al[9] (354 μm in 43 Japanese subjects, mean age 39.4 years), but thicker than that reported by Ding et al[8] (261.93 μm in 210 Chinese subjects, mean age 49.73 years). The differences could be caused by differences in the measuring software or the OCT light sources. In particular, Ikuno et al[9] used a 1,060-nm-based light source (high-penetration OCT, HP-OCT), however, they later demonstrated that there was no significant difference in measuring SFCT between the two systems (EDI-OCT vs. HP-OCT)[15]. The differences of SFCT among studies may arise from other factors including

Table 1. Subfoveal choroidal thickness (SFCT) among age groups in Thai population

Age (years)	SFCT (μm)				*p*-value (ANOVA)
	n	Mean	Standard deviation	95% reference interval	
20-29	178	312.85	81.41	153.29-472.41	0.001
30-39	148	286.98	79.47	131.22-442.74	
40-49	84	282.85	68.20	149.18-416.52	
50-59	50	280.85	79.51	125.01-436.69	
>60	20	258.45	90.73	80.62-436.28	
Total	480	294.02	80.04	137.14-450.90	

ANOVA = analysis of variance

Table 2. Central neurosensory retinal thickness (CNRT) among age groups in Thai population

Age (years)	CNRT (μm)				p-value (ANOVA)
	n	Mean	Standard deviation	95% reference interval	
20-29	178	172.83	17.26	139.00-206.66	0.231
30-39	148	175.83	17.21	142.10-209.56	
40-49	84	174.58	13.11	148.88-200.28	
50-59	50	171.80	15.62	141.18-202.42	
>60	20	179.22	19.00	141.98-216.46	
Total	480	174.22	16.53	141.82-206.62	

ANOVA = analysis of variance

Table 3. Foveal retinal pigment epithelium thickness (FRPE) among age groups in Thai population

Age (years)	FRPE (μm)				p-value (ANOVA)
	n	Mean	Standard deviation	95% reference interval	
20-29	178	42.11	3.45	35.35-48.47	0.164
30-39	148	42.09	3.64	34.96-49.22	
40-49	84	41.34	3.81	33.87-48.81	
50-59	50	42.48	4.27	34.11-50.85	
>60	20	40.57	4.51	31.73-49.41	
Total	480	41.94	3.72	34.65-49.23	

ANOVA = analysis of variance

differences in patients' profiles such as age, refractive error, axial length, ethnicity, as well as methods of measurement[1,7-11].

Aging was found to be associated with thinning of the choroid[1,7,9]. Two previous analyses showed an approximate decrease in thickness of 14 μm every 10 years[9] and 15.6 μm every 10 years[7]. In contrast, Ding et al[8], with larger sample size (n = 210), did not find the association of age and choroidal thickness. In the present study, with the largest sample size (n = 240), we found a significant negative correlation between SFCT and age, and the regression analysis suggested a decrease in SFCT of 12.23 μm every 10 years of age. Our findings supported that age was a factor affecting the choroidal thickness and that progressive choroidal thinning occurred over time with aging (Table 1). These findings also corresponded to a previous histopathologic study by Curcio and associates showing that decreased choriocapillaris density progressed with age[16].

Refractive error was also found to be associated with choroidal thickness. Ikuno et al[9] presented a borderline positive correlation between SFCT and refractive error (p = 0.086), suggesting that SFCT decreased by 9.3 μm for each diopter of increasing myopia. Ding et al[8] found a significant positive correlation of SFCT and refractive error only in the subgroup younger than 60 years of age (r = 0.307, $p<0.001$), but not in individuals older than 60 years of age (r = 0.074, p = 0.385). Furthermore, they showed that SFCT decreased by 10.87 μm for each diopter of increasing myopia, which was quite similar to that reported by Ikuno et al[9]. Our results confirmed the significant positive correlation between SFCT and refractive error (r = 0.338, $p<0.0001$). Interestingly, our regression analysis revealed that the SFCT decreased by 11.42 μm for each diopter of increasing myopia, a magnitude very similar to the previous studies[9,10].

Kim et al, studied choroidal thickness comparing between measurements based on 1:1 pixel images and those based on 1:1 micron images from 122 patients diagnosed with age-related macular degeneration. They showed a significant overestimation of SFCT on 1:1 pixel images[11]. Since 1:1 pixel setting of Spectralis OCT exhibited a horizontally compressed image for approximately three times, some degrees of measurement variability could occur. Unfortunately, there was limited information regarding the details of measurements of SFCT in previous studies[7,8], which made subsequent comparisons of the results between those studies not straightforward.

Since the reliability of measurements was important, our study proposed a technique for accurate SFCT measurement by using 1:1 micron images, drawing the reference lines under 100% magnification, and magnifying the images to 400% before drawing the perpendicular measurement lines (see Material and Method). With this technique, we found excellent reliability in SFCT and CNRT measurement between two the observers (ICC = 0.929 and 0.947, respectively). The reliability of FRPE measurement (ICC = 0.729) was slightly lower than SFCT and CNRT measurements. This could be explained by the fact that FRPE was thinner than SFCT and CNRT, and a little change of thickness in a thinner structure like FRPE could cause a more error of measurement than that in a thicker one.

The authors found that SFCT had a significant positive correlation with FRPE but not with CNRT. The results might be explained by the fact that choroid and RPE are supplied by the same choroidal circulation. Only the outer part of CNRT is supplied by the choroidal circulation, which could explain why there was no association between SFCT and CNRT.

The authors measured only the choroidal thickness beneath the fovea. The choroidal thicknesses in other areas were variable and it would be difficult to compare them between individuals owing to the lack of landmark for measurements. We believed that SFCT represented a good and practical reference value for comparing the choroid in normal and disease conditions and for comparing the results from different studies.

Conclusion

The present study provided normal reference values of SFCT in Thai population. Our results confirmed that SFCT decreased with older age and higher myopia. The SFCT was associated with FRPE but not with CNRT, reflecting the same vascular supply of the choroid and the retinal pigment epithelium. Furthermore, we proposed that our technique of SFCT measurement could result in good reliability despite highly morphologic inter-individual variations.

What is already known about this topic?

EDI-OCT is a recent technique allowing the visualization of the retina and choroid. Choroidal thickness was then studied and could be used to differentiate some macular diseases such as age-related macular degeneration (AMD), polypoidal choroidal vasculopathy (PCV), and central serous chorioretinopathy (CSC). There were quite a few factors believed to influence the measurement of choriodal thickness by EDI-OCT. These factors included ethnicity, age, refractive error, and detailed methods of measurement. In the literature, the data regarding these factors were still conflicting. Moreover, the normal reference values in Thai population, which was the basis for further studies, were not available.

What this study adds?

The present study provided normal reference value of SFCT in Thai population. Our results confirmed that SFCT decreased with older age and higher myopia. The present study was the first to study the correlation of SFCT with FRPE and CNRT. The SFCT was associated with FRPE but not with CNRT, reflecting the same vascular supply of the choroid and the retinal pigment epithelium. Furthermore, we proposed that our technique of SFCT measurement could result in good reliability despite highly morphologic inter-individual variations.

Potential conflicts of interest

None.

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การศึกษาภาพตัดขวางของชั้นคอรอยด์ในประชากรไทย

นพศักดิ์ ผาสุขกิจวัฒนา, วิไลพันธ์ ทวีรัตนศิลป์, สวรินทร์ เล้าทวีรุ่งสวัสดิ์, ณัฐวุฒิ รอดอนันต์, อภิชาติ สิงคาลวณิช, จุฑาไล ตันฑเทอดธรรม, จักรพงศ์ นะมาตร์, อดิศักดิ์ ตรีนวรัตน์, โสมนัส ถุงสุวรรณ, กฤตพล รัตนวารินทร์ชัย, เกศวรินทร์ ทองอยู่

วัตถุประสงค์: เพื่อทราบความหนาของชั้นคอรอยด์บริเวณใต้จุดรับภาพชัดในประชากรไทยโดยใช้เทคนิค enhanced depth imaging spectral-domain optical coherence tomography (EDI-OCT) และเพื่อทราบความสัมพันธ์ของความหนาของชั้นคอรอยด์ใต้จุดรับภาพชัด กับความหนาของชั้น retinal pigment epithelium ใต้จุดรับภาพชัด ความหนาของชั้น neurosensory retina ใต้จุดรับภาพชัด อายุ และค่ากำลังสายตา

***วัสดุและวิธีการ:** ทำการเก็บตัวอย่าง 480 ตา จากอาสาสมัคร 240 ราย ที่ไม่มีโรคต้อหิน โรคของจอตา หรือ โรคของชั้นคอรอยด์ นำกลุ่มตัวอย่างมาทำการวัดความหนาของชั้นคอรอยด์ ความหนาของชั้น retinal pigment epithelium และความหนาของชั้น neurosensory retina ที่บริเวณใต้จุดรับภาพชัดโดยเทคนิค EDI-OCT โดยมีผู้ทำการวัด 2 คน ซึ่งเป็นอิสระต่อกัน ภาพที่ใช้วัดเป็นภาพที่มีสัดส่วนจริงคือ 1:1 ไมครอน ทำการประเมินความน่าเชื่อถือของการวัดโดยผู้วัด 2 คน โดยใช้ intraclass correlation coefficient (ICC) ทำการวิเคราะห์หาความสัมพันธ์ของความหนาของชั้นคอรอยด์ใต้จุดรับภาพชัด กับความหนาของชั้น retinal pigment epithelium ใต้จุดรับภาพชัด ความหนาของชั้น neurosensory retina ใต้จุดรับภาพชัด อายุ และค่ากำลังสายตา*

***ผลการศึกษา:** กลุ่มตัวอย่างมีอายุเฉลี่ย 36.22 ปี (พิสัย 20-81 ปี) ค่าเฉลี่ย (ช่วงค่าอ้างอิงที่ 95%) ของความหนาของชั้นคอรอยด์ ความหนาของชั้น neurosensory retina และความหนาของชั้น retinal pigment epithelium ที่บริเวณใต้จุดรับภาพชัด มีค่า 294.02 ไมครอน (137.14-450.90 ไมครอน), 174.22 ไมครอน (141.82-206.62 ไมครอน) และ 41.94 ไมครอน (34.65-49.23 ไมครอน) ตามลำดับ ความน่าเชื่อถือของการวัดระหว่างผู้วัด 2 คน อยู่ในเกณฑ์สูง โดยการวัดค่าความหนาของชั้นคอรอยด์ใต้จุดรับภาพชัดมีค่า ICC = 0.947, ช่วงความเชื่อมั่น 95% 0.918-0.963 การวัดความหนาของชั้น neurosensory retina ใต้จุดรับภาพชัดมีค่า ICC = 0.929, ช่วงความเชื่อมั่น 95% 0.906-0.945 และการวัดความหนาของชั้น retinal pigment epithelium ใต้จุดรับภาพชัดมีค่า ICC = 0.729, ช่วงความเชื่อมั่น 95% 0.637-0.793 ความหนาของชั้นคอรอยด์ใต้จุดรับภาพชัดมีความสัมพันธ์ในเชิงบวกกับความหนาของชั้น retinal pigment epithelium ใต้จุดรับภาพชัด ($r = 0.179$, $p<0.0001$) แต่ไม่มีความสัมพันธ์กับความหนาของ neurosensory retina ใต้จุดรับภาพชัด ($p = 0.317$) ความหนาของชั้นคอรอยด์ใต้จุดรับภาพชัดมีความสัมพันธ์ในเชิงบวกกับค่ากำลังสายตา ($r = 0.338$, $p<0.0001$) และมีความสัมพันธ์ในเชิงลบกับอายุ ($r = -0.166$, $p<0.0001$) การวิเคราะห์สมการถดถอยทำนายว่า ค่าความหนาของชั้นคอรอยด์จะลดลงไป 12.23 ไมครอน ทุก ๆ ช่วงอายุ 10 ปี และลดลงไป 11.42 ไมครอน ทุก ๆ 1 ไดออปเตอร์ของค่ากำลังสายตาสั้นที่เพิ่มขึ้น*

***สรุป:** การศึกษานี้ทำให้ทราบค่าความหนาของชั้นคอรอยด์ใต้จุดรับภาพชัดในประชากรไทย ความหนาของชั้นคอรอยด์ใต้จุดรับภาพชัดลดลงอย่างมีนัยสำคัญเมื่ออายุมากขึ้นและค่ากำลังสายตาสั้นมากขึ้น ความหนาของชั้นคอรอยด์ใต้จุดรับภาพชัดมีความสัมพันธ์กับความหนาของ retinal pigment epithelium ใต้จุดรับภาพชัด ซึ่งสะท้อนให้เห็นถึงการที่ทั้งสองส่วนนี้ได้รับเลือดมาเลี้ยงจากระบบหลอดเลือดเดียวกัน การวัดค่าความหนาโดยใช้ภาพ 1:1 ไมครอน และใช้เทคนิคการวัดตามการศึกษานี้ ให้ค่าความน่าเชื่อถือในการวัดสูงถึงแม้ว่าจะมีความผันแปรของชั้นจอตาและชั้นคอรอยด์ระหว่างบุคคลมากก็ตาม*