Choroidal Vascularity Index among Filipinos with Non-Neovascular and Neovascular Age-Related Macular Degeneration using Binarization of Enhanced Depth Imaging Optical Coherence Tomography

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ABSTRACT

Objective: This study compared the choroidal vascularity indices (CVI) among eyes with neovascular, non-neovascular age-related macular degeneration (AMD) and healthy controls.

Methods: Spectral-domain optical coherence tomography (OCT) with enhanced depth imaging (EDI) from 52 eyes of 33 subjects were analyzed and designated into 3 groups: control, non-neovascular AMD, and neovascular AMD. Using Image J software, a 1.5 mm subfoveal choroidal area was segmented and binarized to measure total and luminal choroidal areas. The CVI was calculated as the ratio of luminal to total choroidal area. Correlation studies were done to assess relationship of CVI with best-corrected visual acuity (BCVA) and disease severity.

Results: The overall median CVI was 0.66 (IQR = 0.62 – 0.69), with overall median total choroidal area of 696,707.60 (IQR = 530,776.80 – 806,348.00), overall median luminal choroidal area of 442,884.60 (IQR = 351,612.80 – 549,540.30), and an overall median choroidal thickness of 237.10 (IQR = 178.43 – 270.25). The overall median LogMAR BCVA was 0.30 (IQR = 0.10 – 0.54). Statistical comparisons showed no significant differences in the median CVI, median total choroidal area, median luminal choroidal area, and median choroidal thickness among the three groups ($p>0.05$). However, median BCVA was significantly different among the groups ($\chi2=35.98$, $p=0.001$). Specifically, those with non-neovascular AMD and neovascular AMD had significantly worse visual acuity compared to the control group ($p<0.05$).

Conclusion: The study found that CVI, as measured by binarization of EDI-OCT images, was not significantly different among AMD and control groups. BCVA, however, was significantly affected by AMD. These findings suggest that while CVI may not vary with AMD severity, BCVA remains a crucial diagnostic factor. Further research is needed to explore the relationship between choroidal vascularity and AMD using advanced imaging techniques.

Keywords: choroidal vascularity index, optical coherence tomography, retinal imaging, image binarization, age-related macular degeneration

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The choroid is the vascular layer of the posterior segment of the eye, responsible for supplying blood to the other retina and the central macula. It has three distinct layers: the innermost choriocapillaris consisting of capillaries with fenestrated endothelial cells that facilitate nutrients and oxygen exchange; Sattler's layer which contains medium-sized vessels; and the outermost Haller's layer, housing larger blood vessels. These layers work together to provide the essential nutrients and oxygen to the outer retina and macula, supporting the maintenance and health of these critical ocular structures.[1]

Age-related macular degeneration (AMD) is a chronic and progressive disease of the retina that affects the macula, responsible for sharp, central vision. Vision loss from this condition occurs in the advanced stages of its two forms: non-neovascular (dry) and neovascular (wet). In the non-neovascular form, geographic atrophy represents the late stage and is characterized by progressive atrophy of the retinal pigment epithelium (RPE), photoreceptors, and choriocapillaris. Neovascular age-related macular degeneration is characterized by choroidal neovascularization, leakage of fluids and/or blood, and subsequent fibrous scarring. [2]

There are several population-based research on AMD. These studies reported variations in disease prevalence and characteristics across different ethnicities and regions. According to two meta-analyses focusing on individuals aged 40 to 79 years, Europeans exhibit a higher prevalence of early-stage AMD (8.8%) than Asians (6.8%), while prevalence of late-stage AMD is similar in both groups, 0.59% and 0.56%, respectively. [3]

Diagnostic advancements in retinal imaging have significantly impacted the treatment for AMD. Traditionally, fundus fluorescein angiography (FFA) has been the primary method to visualize and characterize choroidal neovascularization (CNV) and other retinal abnormalities. However, FFA is an invasive procedure requiring the intravenous injection of a dye, which may cause side effects such as nausea or allergic reactions. In comparison, optical coherence tomography (OCT) is a non-invasive imaging modality that uses interferometry and light waves to generate high-resolution cross-section images of the retina and choroid.[1] Spectral-domain OCT (SD-OCT) has gained prominence due to its ability to provide in-depth imaging with faster acquisition time. However, the signal strength of the SD-OCT diminishes as the distance from the vitreoretinal surfaces increases, leading to reduced clarity in deeper layers.[4] To overcome these limitations, enhanced depth imaging (EDI) mode has been developed, allowing for improved visualization of deeper structures, such as the choroid.

In addition, binarization of OCT images provides quantifiable assessment of the choroidal structure using a public domain software. This technique enables the calculation of choroidal vascularity index (CVI) which is a novel imaging parameter defined as the ratio of the choroidal luminal area to the total choroidal area. CVI has been reported to be a reliable indicator of choroidal vascularity and is less affected by physiologic influences.[5]

This study compared the choroidal vascularity indices (CVI) among eyes with neovascular, non-neovascular AMD and healthy controls. In order to gain better understanding of the role of choroidal vascularity in AMD progression and visual function, CVI values were also correlated to the best-corrected visual acuity (BCVA) and disease severity.

METHODS

This cross-sectional study involved a retrospective review of EDI-OCT images from Filipino patients diagnosed with AMD, including both non-neovascular (dry) and neovascular (wet) forms, as well as a control group of patients without AMD. These patients were seen at either the clinical or private division of a tertiary hospital. Approval for the study was obtained from the institutional ethics review committee, and the study was conducted in accordance with the Declaration of Helsinki.

Medical records of patients aged 50 years and older who were diagnosed with AMD by OCT or FFA from November 2021 to June 2022 were reviewed. Subjects must have an OCT scan of the macula employing the EDI to be included in the study. Subjects who had other chorio-retinal conditions (e.g. pathological myopia, pachychoroid disease spectrum, uveitis, and uveal malignancy) that were not AMD-related, systemic disease (i.e. infectious, collagen vascular diseases, demyelinating

conditions, and malignancies) that could affect the vision and the posterior pole of the eye, and eyes that underwent surgery or anti-vascular endothelium growth factor injection or any form of treatment on the eye were excluded.

Eyes in the control group were identified through a thorough review of existing EDI-OCT image archives within the study period from the hospital's eye care facility. The control group was age-matched and showed no clinical evidence of AMD or other retinal conditions. A purposive sampling process was employed to select the study eyes in the control and AMD groups.

Medical records were reviewed, and the following data were collected: age, sex, and best-corrected visual acuity. Eyes meeting the inclusion criteria were categorized according to disease severity (neovascular vs non-neovascular AMD) or absence of disease (control).

Image Acquisition and Analysis

Images were gathered from the records of the subjects who underwent OCT with EDI using Cirrus HD-OCT 6000 (Carl Zeiss Inc, California, USA). The macula was scanned with 5-horizontal lines 6 mm in length and spaced apart by 0.25 mm centered on the fovea. The third raster scan with a signal strength of at least 6/10 was selected for analysis. Two qualified retina specialists (C.S.L. and M.F.F.) independently analyzed and measured the images.

This study applied image binarization described by Iovino *et al.* using a public domain software, Image J (National Institutes of Health, USA).[5] Choroidal boundaries were outlined to determine the total choroidal area. The image was converted to 8-bit type and was then binarized using Niblack auto-local threshold. Previous studies have shown that this thresholding method was efficient for isolation of vascular components.[6] The color threshold tool was used to select the dark pixels, representing the luminal area.[5] Segmentation followed the protocol by Agrawal *et al*, wherein a 1.5 mm segmentation block of the subfoveal choroidal area was taken. Upper boundary was at the choroidal-retinal pigment epithelium junction and lower boundary was along the sclero-choroidal junction[7] (**Figure 1**). The values for the total choroidal area and the luminal area were measured by the software. The CVI was calculated by dividing the luminal area by the total choroidal area.[7] Choroidal thickness was determined by using the straight tool.

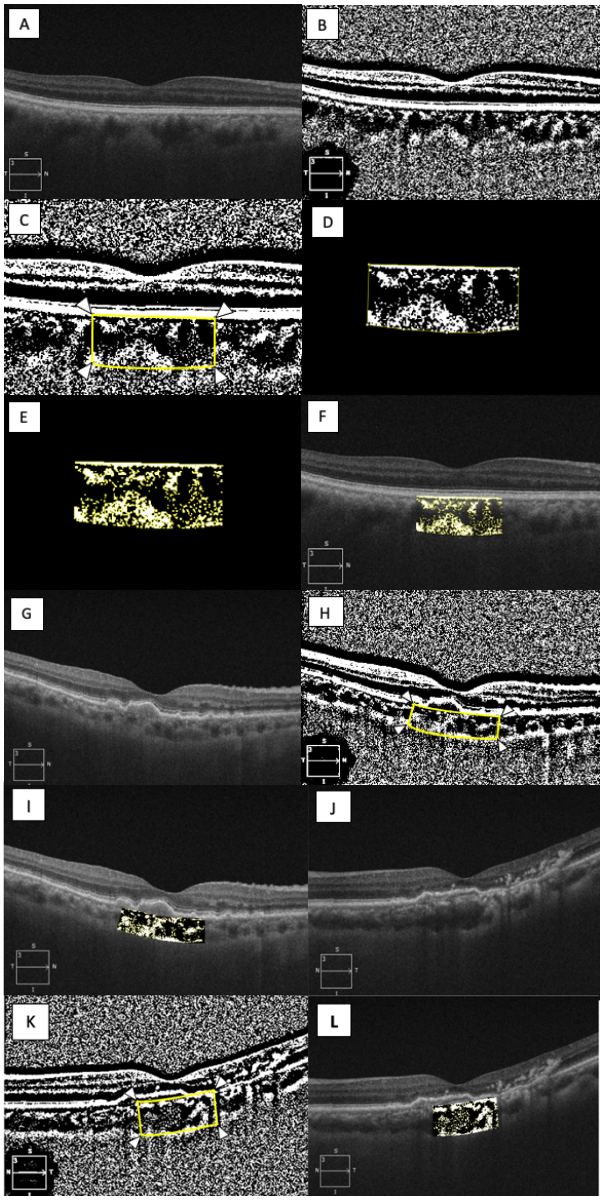

Figure 1. EDI-OCT image binarization of representative photos from eyes in the control (A-F), dry (G-I), and wet (J-K) AMD groups. (**A**) Original SD-OCT image of a healthy eye. (**B**) Binarized image using Niblack auto-local threshold. (**C**) 1.5mm segmentation block of the subfoveal choroidal area (yellow lines, corners indicated by white arrowheads) using polygonal tool of Image J. (**D**) Focus on region of interest with outside areas removed. (**E**) Color threshold tool was used to delineate choroidal areas indicated by dark (luminal) and white (stromal) pixels. (**F**) Overlay of region of interest performed after image binarization to the original SD-OCT image (**G**) Original SD-OCT image of an eye with dry AMD. (**H**) 1.5 mm segmentation block of the subfoveal choroidal area. (**I**) Overlay of region of image performed after

image binarization to the original SD-OCT image. (**J**) Original SD-OCT image of an eye with wet AMD. (**K**) 1.5 mm segmentation block of the subfoveal choroidal area. (**L**) Overlay of region of image performed after image binarization to the original SD-OCT image.

Sample Size Computation

Sample size computation was conducted using GPower version 3.1.9.4. Using data from the study by Koh *et al.* wherein CVIs of AMD and non-AMD eyes were 64.04% and 66.07%, respectively, and assuming an effect size of 0.456, a power of at least 80.00%, a significance level of 5.00%, the calculated minimum sample size was 39 eyes.[8] This sample size was inflated to accommodate at least 10% incomplete charts. Hence, the final sample size of the study was a total of at least 45 eyes.

Statistical Analyses

Statistical analyses were conducted using STATA version 13 statistical software (StataCorp LP, College Station, TX). Descriptive statistics included mean and standard deviation for normally distributed, continuous data; median and interquartile range (IQR), for ordinal and non-normal, continuous data; and frequency and percentage for nominal data.[9] Data normality was evaluated using Shapiro-Wilks test. Comparative analyses of the study variables including total choroidal area, luminal choroidal area, choroidal thickness, choroidal vascularity index, and visual acuity were conducted using Kruskall-Wallis H test.[9] In addition, the correlation of CVI with total choroidal area, luminal choroidal area, choroidal thickness, and BCVA was evaluated using Spearman Rho correlation.[9] Inter-rater reliability between the raters was analyzed using intra-class correlation (ICC), with values ≥0.75 indicating good reliability.[8] A p-value of ≤0.05 was considered statistically significant.

RESULTS

A total of 33 patients (52 eyes) were included in the study: 11 patients (18 eyes) with non-neovascular AMD, 10 patients (15 eyes) with neovascular AMD, and 12 patients (19 eyes) in the control group. **Table 1** shows the demographic characteristics of the patients. The mean age of the control group was 64.67 ± 6.14 years, which was significantly younger than the patents with non-neovascular AMD and neovascular AMD, at 73.27 ± 5.85 (p=0.037) and 75.60 ± 10.76 years old (p=0.008) respectively. There were no significant differences in the sex distribution among the 3 groups (p=0.556).

Table 1. Demographic Characteristics according to Study Group

Demographic Characteristics	Group (N = 33)				Test Statistic	*p*-value (Two-Tailed)
	Non-AMD (n = 12)	Non-Neovascular AMD (n = 11)	Neovascular AMD (n = 10)	Total (N = 33)		
Mean age ± SD (in years)	64.67 ± 6.14	73.27 ± 5.85	75.60 ± 10.76	70.85 ± 8.92	6.24	0.005
Sex (*n*, %)					1.30	0.556
Male	5(42)	6 (55)	3 (30)	14(42)		
Female	7(58)	5 (45)	7 (70)	19 (58)		

Table 2 illustrates the inter-rater reliability of the two raters in evaluating total choroidal area, luminal choroidal area, and choroidal thickness. The intra-class correlation (ICC) coefficients for total choroidal area, luminal choroidal area, and choroidal thickness were 0.7815, 0.7533, and 0.7677, respectively, which can be interpreted as good reliability.

Table 2. Intra-Class Correlation (ICC) Coefficients between Raters in the Evaluation of Total Choroidal Area, Luminal Choroidal Area, and Choroidal Thickness

Outcomes	Rater Evaluations		ICC Coefficient (95% CI)	Interpretation
	Rater 1 (N = 52)	Rater 2 (N = 52)		
Total Choroidal Area ($\bar{x}$, SD)	770,372.90 (271,896.30)	637,250.20 (201,862.50)	0.7815 (0.6193 – 0.8746)	Good Reliability
Luminal Choroidal Area ($\bar{x}$, SD)	505,615.30 (186,554.00)	412,273.80 (133,521.00)	0.7533 (0.5702 – 0.8584)	Good Reliability
Choroidal Thickness (μm; $\bar{x}$, SD)	267.57 (92.59)	209.40 (69.00)	0.7677 (0.5953 – 0.8667)	Good Reliability

ICC – Intra-Class Correlation; CI – confidence interval; $\bar{x}$ - mean; SD – standard deviation

Table 3 shows the CVI, total choroidal area, luminal choroidal area, choroidal thickness, and BCVA. The overall median CVI, total choroidal area, luminal choroidal area, choroidal thickness, and BCVA were 0.66 (IQR 0.62 – 0.69), 696,707.60 (IQR 530,776.80 – 806,348.00), 442,884.60 (IQR 351,612.80 – 549,540.30), 237.10 (IQR 178.43 – 270.25), and 0.30 (IQR 0.10 – 0.54), respectively. Median CVI, total choroidal area, luminal choroidal area, choroidal thickness scores were not statistically different among the three groups (p>0.05). However, there was statistically significant difference in the medians of BCVA among the 3 groups (p=0.001). Post-hoc analysis showed that the median BCVA in LogMAR of those with non-neovascular AMD and

neovascular AMD were significantly higher than those in the control group (p<0.05).

Table 3. Choroidal Vascular Index (CVI), Total Choroidal Area, Luminal Choroidal Area, Choroidal Thickness, and Visual Acuity of Control Group and Eyes with Age-Related Macular Degeneration (AMD)

Outcomes	Group (N = 52)				Test Statistic	*p*-value (Two-Tailed)
	Non-AMD (n = 19)	Non-Neovascular AMD (n = 18)	Neovascular AMD (n = 15)	Total (N = 52)		
Median Choroidal Vascular Index (IQR)	0.68 (0.65 – 0.69)	0.64 (0.62 – 0.70)	0.63 (0.54 – 0.69)	0.66 (0.62 – 0.69)	4.33	0.115
Median Total Choroidal Area (IQR)	743,693.20 (529,723.40 – 809,442.30)	640,044.10 (497,243.90 – 711,082.00)	772,134.80 (577,916.00 – 1,152,057.00)	696,707.60 (530,776.80 – 806,348.00)	5.08	0.079
Median Luminal Choroidal Area (IQR)	524,807.60 (359,820.40 – 551,800.70)	391,122.40 (326,068.00 – 494,786.30)	511,201.30 (343,405.10 – 709,677.50)	442,884.60 (351,612.80 – 549,540.30)	3.65	0.161
Median Choroidal Thickness (IQR), in μm	245.51 (188.96 – 269.91)	221.97 (169.48 – 243.60)	264.95 (175.99 – 390.87)	237.10 (178.43 – 270.25)	3.16	0.207
Median Visual Acuity (IQR), in LogMAR	0.10 (0.00 – 0.20)	0.40 (0.30 – 0.54)	0.60 (0.50 – 2.00)	0.30 (0.10 – 0.54)	35.98†	0.001

AMD – age-related macular degeneration; IQR– interquartile range

Table 4 illustrates the correlation of CVI with total choroidal area, luminal choroidal area, total choroidal thickness, and BCVA. Among the control group, CVI had a positive, moderate correlation with luminal choroidal area (r_s=0.52, p=0.023). No correlation was observed between CVI and total choroidal area, choroidal thickness, and BCVA. Among those with non-neovascular AMD, CVI had a positive, moderate correlation with both total choroidal area (r_s=0.51, p=0.032) and luminal choroidal area (r_s=0.62, p=0.007). Lastly, among those with neovascular AMD, CVI was not significantly correlated with any of the OCT-EDI indices except with BCVA, wherein it had a negative and moderate correlation (r_s=–0.62, p=0.015).

DISCUSSION

Our study utilized binarization of EDI-OCT images to assess the CVI among Filipino patients with neovascular and non-neovascular AMD and a control group without AMD. We found that CVI, total choroidal area, luminal choroidal area, and choroidal thickness did not significantly differ between eyes with and without AMD.

The mean CVI for the control eyes was 68% which is consistent with previous studies. In particular, Agrawal *et al.* reported a mean CVI of 66% in healthy eyes using a similar binarization technique.[7] Our findings align with this, suggesting a comparable level of choroidal vascularity in non-AMD eyes.

Table 4. Correlation Analyses of the Association of Total Choroidal Area, Luminal Choroidal Area, Choroidal Thickness, and Visual Acuity (LogMAR) with Choroidal Vascular Index (CVI) According to Study Group

Outcomes	Group (N = 52)					
	Non-AMD (n = 19)		Non-Neovascular AMD (n = 18)		Neovascular AMD (n = 15)	
	r_s-value	*p*-value (Two-Tailed)	r_s-value	*p*-value (Two-Tailed)	r_s-value	*p*-value (Two-Tailed)
Total Choroidal Area	0.32	0.185	0.51	0.032	0.03	0.910
Luminal Choroidal Area	0.52	0.023	0.62	0.007	0.30	0.283
Choroidal Thickness	0.26	0.276	0.43	0.078	0.07	0.800
Visual Acuity	0.01	0.997	–0.15	0.558	–0.62	0.015

Interpretation of r_s-value: ±1.00 = Perfect Correlation; ±0.90 to ±0.99 = Very Strong Correlation; ±0.70 to ±0.89 = Strong Correlation; ±0.50 to ±0.69 = Moderate Correlation; ±0.26 to ±0.49 = Weak Correlation; ±0.01 to ±0.25 = Very Weak Correlation; 0.00 = No Correlation

In contrast, our results differ from those of Wei *et al.,* who reported reduced CVI in eyes with exudative AMD compared to fellow eyes with dry AMD, suggesting that CVI could serve as a non-invasive marker for monitoring choroidal disease in exudative AMD.[10] Invernizzi *et al.* also reported increased choroidal thickness and vascularity index in eyes with active neovascular AMD, indicating that these parameters can reflect disease activity.[11] Similarly, Giannaccare *et al.* observed that CVI decreased in geographic atrophy associated with AMD over time, with a more profound reduction after a mean follow-up of 18 months.[12] These studies suggest that CVI can vary with disease activity. However, our study findings did not find significant differences in CVI between AMD and control eyes, indicating a divergence from these reported outcomes.

The discrepancies between our findings and those of previous research can be attributed to several key factors, including variations in study populations, methodologies, and the subjective nature of image analysis and thresholding techniques. For instance, Agrawal *et al.* studied a population-based cohort of Malay adults aged 45-85 years in Singapore, which may differ demographically from our Filipino cohort, potentially impacting CVI

results. Additionally, Agrawal used a specific 7-horizontal line scan protocol with distinct imaging parameters that might differ from those in our study.[7] Wei *et al.* focused on a prospective case series of 42 patients with unilateral exudative AMD, presenting a different clinical context compared to our study, while Invernizzi *et al.* focused on both inactive and active stages of neovascular AMD which introduces dynamic elements not captured by our cross-sectional design.[10,11] The longitudinal approach of Giannaccare *et al.* with baseline and follow-up scans of patients with GA, offers insights into CVI changes over time.[13] Despite using similar binarization techniques, discrepancies in CVI measurements can be attributed to differences in scanning protocols, subjective image analysis, and methodological approaches. These factors highlight the need for standardized imaging and analysis protocols to improve the consistency and comparability of CVI measurements in future research.

Utilizing automated techniques to outline and measure areas of interest could result in more standardized and objective assesment of choroidal vascularity. Although differences were noted, our study emphasized that the BCVA was notably worse in AMD eyes, specifically in the neovascular type, indicating that factors other than choroidal vascularity may affect visual acuity. Though CVI is helpful in providing information about choroidal structure, it may not completely account for the differences in visual acuity related to AMD.

The retrospective nature of this study, utilizing previously collected data, may have introduced potential biases and confounding variables. Variations in the quality of EDI-OCT images could also influence the accuracy of CVI measurements. Additionally, the cross-sectional study design offers only a single temporal assessment of CVI, without accounting for disease progression. Future research should explore in-depth association of the choroidal vascularity and AMD using image binarization and other advanced imaging methods. The adoption of automated techniques for CVI measurements could enhance its utility as a reliable marker of choroidal vascularity, both in healthy eyes and those affected by chorioretinal diseases.

This study involved the utilization of image binarization using EDI-OCT image for evaluating choroidal vascularity in persons with AMD. Our findings show that choroidal vascularity measured by CVI did not significantly differ between eyes with and without AMD. Furthermore, thorough ophthalmologic examinations, including visual acuity measurement, remain essential for the detection and evaluation of AMD.

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