CHOROIDAL STRUCTURAL CHANGES AND VASCULARITY INDEX IN STARGARDT DISEASE ON SWEPT SOURCE OPTICAL COHERENCE TOMOGRAPHY

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Purpose: To evaluate structural changes in the choroid of patients with Stargardt disease using swept source optical coherence tomography scans.

Methods: A retrospective comparison cohort study was conducted on 39 patients with Stargardt disease, and on 25 age and gender matched-healthy controls. Subfoveal choroidal thickness (SFCT) was computed from the swept source optical coherence tomography machine, and the scans were binarized into luminal area and stromal areas, which were then used to derive choroidal vascularity index (CVI). Choroidal vascularity index and SFCT were analyzed independently using linear mixed effects model.

Results: There was no significant difference in SFCT between the 2 groups (347.20 ± 13.61 μm in Stargardt disease vs. 333.09 ± 18.96 μm in the control group, $P = 0.548$). There was a significant decrease in the CVI among eyes with Stargardt disease as compared with the normal eyes (62.51 ± 0.25% vs. 65.45 ± 0.29%, $P < 0.001$). There was a negative association between visual acuity and CVI (correlation coefficient = −0.75, $P < 0.001$) and a positive association between visual acuity and SFCT (correlation coefficient = 0.21, $P = 0.035$).

Conclusion: Choroidal vascularity index appears to be a more robust tool compared with SFCT for choroidal changes in Stargardt disease. Choroidal vascularity index can possibly be used as a surrogate marker for disease monitoring. A decrease in CVI was associated with a decrease in visual function in eyes with Stargardt disease.

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Stargardt disease is the most common juvenile-onset retinal dystrophy.[1] It is caused by the mutation of the ABCA4 gene that results in an error in the processing and transportation of the metabolic byproduct from the photoreceptor visual cycle.[2] The byproduct, also known as lipofuscin, accumulates in the retinal pigment epithelium (RPE). This causes the RPE to degenerate, resulting in secondary atrophy of the photoreceptor cells and choriocapillaris.[2–5] The RPE and the choroid are interdependent. The choroid provides vascular support to the RPE, which in turn maintains choriocapillaris by secreting vascular endothelial growth factor isomers. Animal studies have shown secondary choriocapillaris atrophy after RPE atrophy apparently because of the lack of the RPE specific vascular endothelial growth factor isomers.[6] Histopathological examination has also shown the loss of RPE, overlying photoreceptors, and choroidal circulation—in particular the choriocapillaris.[7,8]

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It has become easier to study the choroidal structure with the advent of newer optical coherence tomography (OCT) techniques such as the enhanced depth imaging and the swept source OCT. Swept source optical coherence tomography uses longer wavelength and faster scanning speed which allows for deep range imaging. Various studies have compared the subfoveal choroidal thickness (SFCT) in patients with Stargardt disease compared with matched normal controls.[9–11] However, the studies could not reach a consensus.

In our study, we looked at the structural changes of the choroid in patients with Stargardt disease instead. We studied the choroid with our previously validated tool of choroidal vascularity index (CVI)—a novel technique that indirectly analyzes changes in the choroidal vasculature.[12,13] This technique has been used to study other eye disease that affect the choroidal layer and has been shown to be more robust and less variable as compared with SFCT.

Methods

Study Population

We conducted a retrospective cohort study of patients diagnosed with Stargardt disease between January 2015 and December 2016. Patients were recruited from two tertiary eye centers in Southern India. Normal controls were patients recruited from the clinics with nonretina-related issues. Approval by Institutional Ethical Committee Board of both institutes was obtained before the study. The study adhered to the tenets of the Helsinki Declaration.

We studied both eyes of 39 patients with Stargardt disease (study group) and 25 normal patients (control group), with a total of 128 eyes. Patients were diagnosed as Stargardt disease based on dilated fundus examination. Color fundus photograph, enhanced depth imaging-OCT scans using swept source machine, fundus fluorescein angiography, electroretinography, and fundus autofluorescence were performed as per the physician's discretion. Patients with pathological myopia (−6 diopters or more) or clinical features of any other retinal pathology such as age-related macular degeneration, diabetic retinopathy, or retinitis pigmentosa were excluded from this study. Age, sex, logarithm of the minimum angle of resolution visual acuity (VA), CVI, and SFCT were recorded for each patient.

Imaging of Choroid

Imaging of the choroid was performed using swept source OCT (DRI-OCT "Triton"; Topcon Corporation, Tokyo, Japan) for all 128 eyes. Horizontal scans of 9 mm were centered over the fovea. The SFCT was then measured using the in-built software calipers tool at the fovea. Subfoveal choroidal thickness was taken as the distance between the Bruch membrane (located at the lower edge of the RPE) and the choroid–scleral interface.

Image Binarization

The entire length of the same foveal OCT scans (used for SFCT measurements) was used for binarization. The protocol previously described by our group was used for image binarization.[12] Image segmentation was performed by one of the authors (N.K.) who was a trained grader and was masked to the patients' information. Briefly, the images were processed on a public domain software Fiji. After uploading the images (Figure 1A) on Fiji, a polygon tool was used to select the total choroid area (TCA), with the RPE as the anterior boundary of TCA and the choroidal scleral interface as the posterior boundary of TCA, across the entire length of the scan. Total choroid area was added to the regions of interest manager. After converting the image into eight bit, Niblack auto local thresholding was subsequently applied, which gives the mean pixel value with Stargardt disease for all the points.[12] Color threshold tool was then applied to the TCA. The dark pixels represented the luminal area, whereas the light pixels represented the stromal or interstitial area (Figure 1B). Choroidal vascularity index was then calculated using the ratio of luminal area/TCA. The images with inconspicuous choroid scleral junction were excluded from the image binarization and further analysis.

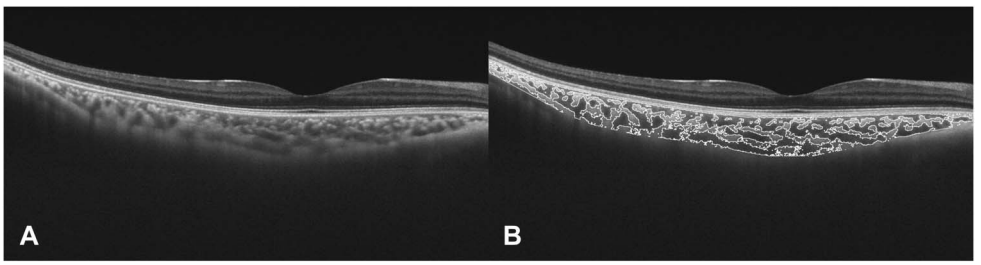

Fig. 1. Swept source optical coherence tomography before binarization (**A**) and after binarization (**B**).

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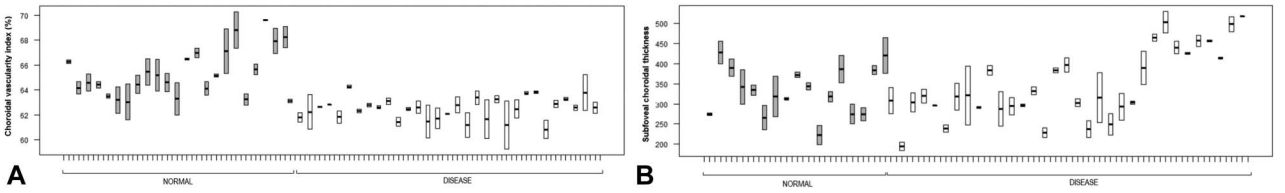

Fig. 2. A. Boxplots showing choroidal vascularity indices values in two eyes of patients from the normal and disease group. **B.** Boxplots showing choroidal thickness values in two eyes of patients from the normal and disease group.

Statistical Analysis

Choroidal vascularity index and SFCT were analyzed independently using linear mixed effects model, treating age and gender as covariates, control and study groups as fixed factor, and individual patients as random factor. For each patient, CVI and SFCT were obtained for both the eyes. Thus, a two-eye design was followed with an aim to partition the variance of CVI and SFCT contributed by patients and that resulting from differences in eyes. Visual inspection of both CVI and SFCT did not show much deviation from normality. Statistical significance of difference of estimated marginal means of both CVI and SFCT between two groups was obtained after adjusting for covariates. In addition, correlation of CVI and SFCT was independently performed with VA using scatter plots, obtaining the correlation coefficient. All analyses were performed in SPSS version 20.0 (IBM Corp, Armonk, New York) and statistical significance was evaluated at 5% level.

Results

Seventy-eight eyes from 39 patients with Stargardt disease (study group) were compared with 50 eyes from 25 normal controls (control group). In the Stargardt disease group, there were 22 (56.4%) men and 17 (43.6%) women, whereas in the control group, there were 10 (40%) men and 15 (60%) women. The gender distribution was not significantly different between the two groups (*P*-value: 0.305). The mean age was 23.82 ± 11.31 years in the Stargardt disease group and 23.44 ± 8.5 years in the control group. The difference in mean age of the two groups was statistically insignificant (*P*-value 0.88). The mean best-corrected VA was logarithm of the minimum angle of resolution 0.89 (Snellen's VA: 20/160) for the eyes with Stargardt disease and logarithm of the minimum angle of resolution 0.00 (Snellen's VA: 20/20) for the normal eyes (*P*-value < 0.0001).

Table 1. Estimated Means for CVI and SFCT in Two Groups

	CVI (%)		SFCT (μm)	
Parameter	Normal	Stargardt Disease	Normal	Stargardt Disease
Mean	65.45	62.51	333.09	347.20
SE	0.29	0.24	18.96	13.61

The SFCT for both eyes in both groups of patients was analyzed and its distribution depicted in Figure 2A. Table 1 shows the estimated mean SFCT for the control group (333.09, SE: 18.957 μm) and disease group (347.203, SE: 13.607 μm). The control group had a lower mean SFCT than study group by 14.12 μm. This difference was statistically insignificant with *P*-value of 0.548, after adjusting for age and sex.

The CVI for both eyes in both groups of patients is shown through a boxplot in Figure 2B. It is evident that the control group had higher CVI compared with the study group. The effects of various confounding variables on CVI were determined through linear mixed model. The linear mixed model was chosen to address the inclusion of two eyes from one subject and random factors (individual patient factors such as age, sex, and SFCT). The estimated marginal mean CVI for the control group was 65.45 ± 0.29%, while that of the study group was 62.51 ± 0.25%, as presented in Table 1.

The inclusion of random factors in the linear model characterizes variation due to individual subjects. The unstructured covariance type was selected suggesting that two eyes had unequal variances of CVI in the model. Regarding fixed effect due to the 2 groups (control and study), the coefficient for the control group indicated that the mean CVI for the control group was higher than the study group by 2.94%, which was statistically significant as revealed by *P*-value <0.001, after adjusting for covariates age and sex (Table 2).

In addition, correlation analysis was performed between VA and CVI as well as VA and SFCT, with scatter plots as shown in Figure 3. The correlation between logarithm of the minimum angle of resolution transformed VA and estimated CVI was strongly negative with a correlation coefficient of -0.75 and statistically significant with a *P*-value of <0.001. The correlation between estimated SFCT and VA showed a positive relationship with a correlation coefficient of 0.21, which was statistically significant with *P*-value of 0.035.

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Table 2. Factor Effects on CVI and SFCT

	CVI				SFCT			
Factor	Estimate	SE	*t*	*P*	Estimate	SE	*t*	*P*
Fixed								
Intercept	62.51	0.25	253.64	<0.001	347.20	13.61	25.52	<0.001
Group: normal	2.94	0.38	7.72	<0.001	−14.18	23.33	−0.60	0.548

Discussion

Several recent studies have attempted to analyze the choroidal status in patients with Stargardt disease and comparing that with those in normal patients.[9–11] However, no definite conclusion can be drawn from it. In 2015, 2 studies were published with disagreeing results. The study by Nunes et al[9] showed that the SFCT of patients with Stargardt disease had insignificant difference compared with normal patients, whereas another study by Adhi et al[10] showed that patients with Stargardt disease had significantly reduced SFCT and significantly reduced subfoveal large choroidal vessel layer thickness, compared with healthy eyes. However, the latter study was conducted using OCT without enhanced depth imaging. A more recent study, published this year, by Vural et al,[11] studied 30 eyes and showed that patients with Stargardt disease have thinner SFCT as compared with normal control. The heterogeneity of the results in these studies may suggest that SFCT does not directly correlate with the presence of Stargardt disease and might be dependent on many other factors. Muller et al[14] reported no significant difference in the SFCT of patients carrying the common ABCA4 mutation, namely p.Gly1961Glu. Also these patients had no evidence of RPE atrophy and normal scotopic as well as photopic responses on electroretinography. However, in the presence of RPE atrophy, the choroid was thinner, with the inner choroid being more affected.

In our study, we found that SFCT did not show significant variation between Stargardt disease and normal eyes. However, CVI was significantly reduced in Stargardt disease as compared with normal eyes. This suggests that in patients with a clinical diagnosis of Stargardt disease, CVI appears to be a more sensitive biomarker in detecting choroidal changes as compared with SFCT. This was also seen in previous studies on CVI in diseases involving the choroid.[13,15–19] However, the difference in CVI is only 2.94% and may not be visible clinically. A similar difference was also seen in the previous published literature, among patients with age-related macular degeneration (2.61%)[15] and diabetic retinopathy (2.1%).[17] Currently, this helps us to better understand the structural changes in the choroid. To determine whether this difference will be clinically relevant or not, the data need to be validated with a large scale longitudinal prospective study. A decrease in the luminal area but with a compensatory increase in stromal area would lead to a decrease in CVI, but not a decrease in SFCT. A decrease in the overall size of the choroidal vessels would decrease the luminal area, but increase the stromal area as there is a larger ratio of vessel wall and interstitial tissue compared with vessel lumen. Adhi

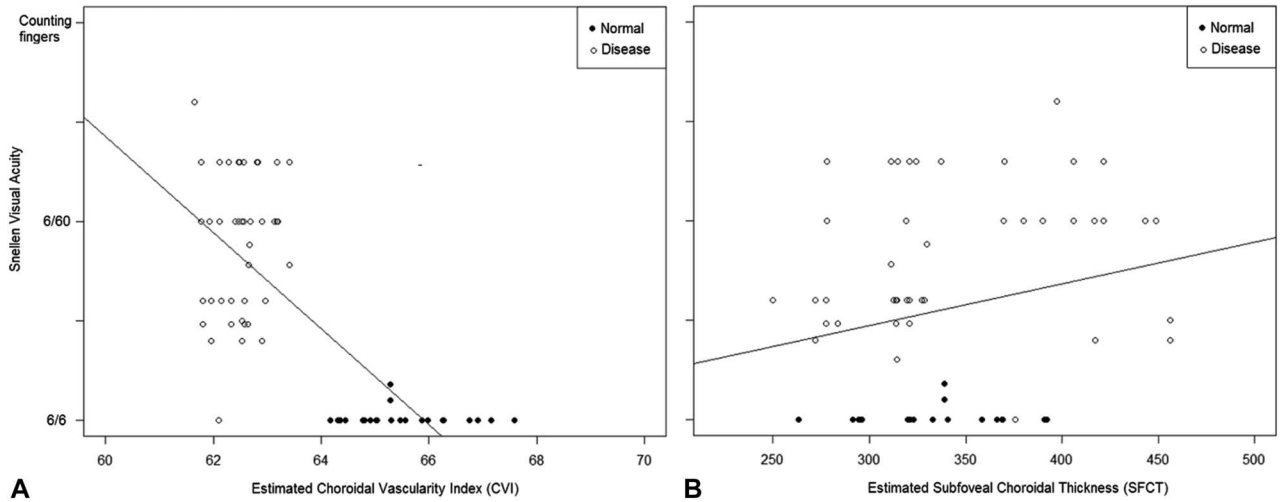

Fig. 3. Regression analysis of VA against CVI (**A**) and choroidal thickness (**B**).

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et al[10] studied the thickness of large vessels (>100 μm) choroidal layer, medium vessels choroidal layer, and choriocapillaris layer in patients with Stargardt disease, and showed that a subset of patients with Stargardt disease had a significant decrease in the large choroidal layer thickness as compared with normal control. This may account for the reduction in CVI but not SFCT.

A significant difference in CVI, among patients with Stargardt disease compared with normal, also leads to the conclusion that not only the retina and RPE are affected but also the choroidal layer. The choroidal angiopathy could either be part of the pathogenesis of Stargardt disease[10,20] or a consequence of RPE atrophy. Further cohort studies would be needed to determine this correlation. The lipofuscin deposits in the RPE cells in Stargardt disease is nearly seven times more than the normal limit. Lipofuscin has been shown to alter the secretion of vascular endothelial growth factor from the RPE cells, which might be responsible for the choroidal vascular changes.[21]

Our study also showed that reduction of CVI correlates with worsening VA, but has no correlation with age, sex, or SFCT. As the disease in patients with Stargardt disease progresses, the VA worsens.[22,23] Hence, CVI can possibly be used as a biomarker to monitor progression of disease and worsening of vision. In the study by Adhi et al,[10] there was a correlation between the reduction in thickness of the large choroidal vessels and reduction in VA.

Over the past few years, many researchers have analyzed the possibility of RPE transplantation or stem cell therapy, as a treatment modality for retinal atrophy.[24–28] Knowledge of the pathogenesis of choroidal angiopathy in Stargardt disease will be able to guide this treatment. In addition, selection of suitable patients may be required for future RPE transplantation or stem cell therapy, as these treatments address the issue of RPE atrophy, but not of choroidal angiopathy. A compromised choroid may lead to further progression and worsening of the RPE and photoreceptor degeneration.

Some limitations of this study include a smaller sample size and the absence of genetic testing for the confirmation of Stargardt disease. Genetic testing was not performed due to the financial constraints. Choroidal vascularity index was measured using one horizontal scan across the fovea. We might have obtained additional information if the same technique was applied to a volume scan over a broader area of the macula. However, in our recent publication, we have demonstrated statistically insignificant influence of scanning area on CVI in normal eyes.[29] It would have also been interesting to correlate the degree of RPE and photoreceptor atrophy seen in the horizontal scan used for CVI calculation. However, CVI is a very generalized measurement across the entire single scan and does not give us the exact specific area of loss of choroidal vasculature.

Conclusion

Choroidal vascularity index is a novel and noninvasive imaging tool, which is both a sensitive and a robust surrogate marker to monitor the choroidal angiopathy in patients with Stargardt disease. Choroidal vascularity index showed a negative trend with decreasing VA in patients with Stargardt disease and hence can be considered in future prospective studies as one of the end points for clinical trials. Choroidal vascularity index has a potential to be a surrogate marker for future studies to analyze more in depth, the pathogenesis and progression of Stargardt disease. Further studies can be conducted to verify our findings on patients with genetically proven Stargardt disease.

Key words: stargardt disease, choroidal thickness, choroidal structural changes, choroidal vascularity index, EDI-OCT, swept source OCT.

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