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*Original Article*

# Correlation of vascular change and cognitive impairment in age-related macular degeneration patients

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**Abstract:** Age-related macular degeneration (AMD) is one of the leading causes of blindness among the elderly. However, the correlation between vascular change and cognitive impairment in AMD disease is still unknown. In our study, we investigate the blood flow change among different layers of the retina in the AMD eye and normal fellow eye of AMD patients and its influence with patients' cognition. Our study applies optical coherence tomography angiography (OCTA) to assess the blood flow of the retina in AMD patients and the healthy controls (HCs). Magnetic resonance imaging (MRI) and Montreal Cognitive Assessment (MoCA) were performed to evaluate the cognitive change of the individuals. The results showed that deep capillary plexus density, superficial capillary plexus density, retina thickness and retinal nerve fiber layer thickness deduction existed in both eyes of the AMD patient compared with the HCs. The reduced vessel density in the choroidal layer only existed in the AMD eye of the patients while the fellow eye of patients and HCs did not change much. Furthermore, the AMD patient got a lower MoCA score compared to the HCs. Our results illustrate that the fellow eye of the AMD patient underwent vessel density change, which may lead to the early stage of AMD. The lower score of the MoCA test in AMD patients refers to the cognitive impairment. These findings show the significance of taking actions to prevent the progress of AMD in the fellow eye, as well as paying more attention to the development of cognitive impairment of these patients.

**Keywords:** Optical coherence tomography angiography, cognitive impairment, age-related macular degeneration

## Introduction

Age-related macular degeneration (AMD), which is characterized by irreversible and severe central vision loss, is one of the leading causes of blindness among the elderly [1]. AMD has already resulted in almost 10% of blindness in the world, disabling individuals from reading, driving and living. Wet AMD, which is characterized by neovascularization and capillary leakage, causes much more severe vision damage than dry AMD [2]. Studies show that the thickening of Bruches membrane, accumulation of drusen and decreasing of blood flow exist in the wet AMD retina [3].

A novel non-invasive technology named optical coherence tomography angiography (OCTA) enables us to visualize the blood flow within the retina vessels by targeting moving blood cells [4]. It reduces the artifact motion effect by performing multiple repeated B-scans in the same place [5]. The OCTA's capability of depth-resolved and enhanced microvascular resolution provides a great opportunity for studying the evolution of neovascular disease like wet AMD [6]. It has been used in large amounts of research to assess the alteration of blood flow in variant neovascular diseases such as diabetic retinopathy, AMD, uveitis, etc. [7-9]. To date, there are several reports that illustrate the alteration of retina blood flow in AMD patients with the application of OCTA [10, 11]. However, they do not clarify optic nerve head (ONH) microvascular change in the AMD eye in detail as well as blood flow change in the fellow eye of AMD patients.

There are several types of cognitive impairment, including Alzheimer's disease (AD) which is a representative one (60%-70%) in the world. Cognitive impairment including AD is mostly caused by synapses and neuronal loss in the cerebral cortex and is characterized by decreased capacity of memory, learning, calculating, and thinking [12]. However, these classic symptoms are only apparent in the late stage of cognitive impairment when irreversible neuronal loss has already happened. The diagnosis of cognitive impairment, especially AD, nowadays mainly includes testing the biomarkers, such as β-amyloid and tau protein in cerebrospinal fluid (CSF) and performing positron emission tomography (PET). However, these tests are either invasive or expensive, making them hard to implement in routine clinical screening [13].

Cognitive impairment, such as AD, has been known to be associated with the visual system since the 1970s. During the last two decades, it has been specifically connected with the decreased blood flow, vascular diameter and vascular density that existed in the eye of AD patients [14]. However, it is still unknown whether the blood flow change caused by AMD would cause certain damage to the brain. Though there are several clinical trials still in progress which evaluate new drugs in preventing neuronal loss, the clinical strategy can be taken earlier than this stage. According to recent studies, preclinical AD is a vital period that undergoes in which key pathophysiologic changes occur within the brain [15]. Thus, the diagnosis of preclinical AD with cognitive impairment would be significant for potential therapy. The purpose of our study is analyzing the change of retina and disk blood flow in AMD patients by OCTA as well as finding the correlation of flow change and cognitive impairment, which provide a promising method for early diagnosis of AD.

## Materials and methods

### *Ethics*

Ethical approval was obtained from the local ethics committee of the People's tenth hospital, Tongji University, where the study was conducted.

### *Study design*

This perspective study enrolled 30 AMD patients along with 30 cataract patients who had one healthy eye as healthy controls (HCs) from August 2019 to February 2020 according to the inclusion/exclusion criteria indicated below. All patients have signed consent to use their data for research after the nature of the study had been explained to them before participation. In this study, OCTA, magnetic resonance imaging (MRI) scanning and Montreal Cognitive Assessment (MoCA) were performed among different groups, whose data was acquired for further analysis.

### *Retina diagnosis and patient eligibility*

One eye of all patients was diagnosed as wet AMD by slit lamp biomicroscope, optical coherence tomography (OCT), digital fundus photography, OCTA, and fluorescein angiography (FFA). Fluorescence staining in the early stage and fluorescence leakage around the macular area in the late stage was showed in FFA. The fellow eye of the patients was free of any neovascular diseases. Investigator determination was based on blinded review of treatment and medical histories.

The inclusion criteria are as follows: 1) the age of all individuals is more than 55 years old and less than 80 years old; 2) patients are newly diagnosed with AMD in one eye while the fellow eye is defined as lacking signs of AMD.

The exclusion criteria for all group were as follows: 1) poor image quality <60 because of severe cataract or unstable fixation; 2) patients accepted any intraocular treatment or photocoagulation in the past 3 month; 3) patients diagnosed with any kind of dementia including AD; 4) intraocular pressure >21 mmHg; 5) pre-existing other macular diseases or ocular diseases such as diabetic retinopathy, macular hole optic neuropathy, etc.

### *OCTA measurements*

The images of all patients' retina were obtained by applying the commercial spectral domain OCTA (Optovue, Inc., Fremont, CA, USA). Scans were performed in a 6*6 mm area centered on the macular. As for each fixed position, two B-scans were captured to minimize the motion artifact.

To evaluate the retina vascular structures, the utilized software automatically divided the full retina into 4 parts: the superficial retina, the deep retina, the outer retina and the choriocap-

**Table 1.** Basic information of participants

| | HC (n=30) | AMD eye (n=30) | Fellow eye (n=30) | $P_1$ value | $P_2$ value | $P_3$ value |
|---|---|---|---|---|---|---|
| Age | 63.6±6.5 | 67.8±5.4 | 67.8±5.4 | N/A | 0.054 | 0.054 |
| Gender Male to Female | 12:18 | 18:12 | 18:12 | N/A | N/A | N/A |
| VA | 0.95±0.1 | 0.35±0.25 | 0.89±0.22 | 0.000* | 0.000* | 0.1791 |
| IOP (mmHg) | 14.55±3.68 | 13.1±2.5 | 14.0±2.5 | 0.169 | 0.079 | 0.501 |

Note: All data is shown as mean ± standard deviation; *stands for significant difference existed between the group; $P_1$: *P* value between AMD eye group and fellow eye group; $P_2$: *P* value between AMD eye group and HC eye group; $P_3$: *P* value between fellow eye group and HC eye group. 0.000 means *P* value less than 0.0001; N/A: Not Applicable.

illaries. This software calculated the vascular density and retina thickness of the superficial and deep retina automatically. The foveal avascular zone (FAZ) and flow index rate in the center circular zone of 36 $mm^2$ of the choriocapillaris segments were all automatically calculated by software.

To assess the optic nerve head microvascular change, a 4.5*4.5 mm scan centered on the ONH was obtained, in which peripapillary and inside disk vessel density as well as thickness were automatically calculated by software.

*MRI scanning and MoCA testing*

MRI scanning was performed by an experienced and blinded technician. Specifically, structure MR images were performed using a 3-Tesla MR scanner (Philips, Ingenia, Netherland) with a 15 channel head coil. The parameters of three-dimension T1 turbo field echo (3D T1TFE) sequences were voxel size =0.9×0.9×0.9 $mm^3$; field of view =230×230×180 mm; repetition/echo times =6.2 ms/2.7 ms; flip angle =8 degrees; slice orientation = sagittal; slice thickness =0.9 mm; number of slices =200. We used the Statistical Parametric Mapping analysis package (SPM12, http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) on the MATLAB® R2018a platform together with the Computational Anatomy Toolbox (CAT12, http://www.neuro.uni-jena.de/cat/) for SPM for Voxel-based morphometry (VBM) processing and analysis.

Structure MR images in DICOM format were converted to images in NIFTI format (Neuroimaging Informatics Technology Initiative) for morphological processing. VBM processing included denoising, segmentation, spatial normalization, and smoothing. As a result, all original structure data were segmented into gray matter, white matter and CSF volumes based on MNI152 template. Then we ran regions of interest (ROI) tools in CAT12 to estimate mean values inside the ROI and export hippocampal volume files in XML format for external statistics.

MoCA is one test which can reflect individual's memory, thinking, orientation, comprehension, calculation, learning capacity and language. MoCA testing was conducted by a well-trained and blinded doctor. We combined the MoCA score with the MRI results to analyze both the structure and function change of the brain in AMD patients.

*Statistics*

The statistical analysis was performed using one-way ANOVA. Results were expressed as mean ± SD. The independence between the variables was analyzed with Least-Significant Difference test. Correlation analysis was performed by Pearson correlation test. Differences were considered significant at $P<0.05$.

## Results

*General characteristics of AMD patients and healthy controls*

We included 30 AMD patients and 30 cataract patients who had one healthy eye as healthy controls (HCs) in our study. The basic information of all involved individuals are shown as follows (**Table 1**). Both groups were similar in age, visual acuity (VA), intraocular pressure (IOP) and gender. All patients enrolled in this study were diagnosed with AMD by OCT, color of the fundus (CF), and FFA (**Figure 1**). The fellow eye of patients showed normal fundus without any damage to the VA.

*Vessel density of superficial capillary plexus (SCP) in AMD patients versus HCs*

With the help of the in-built algorithm, we obtained 6*6 mm optical OCTA scan images,

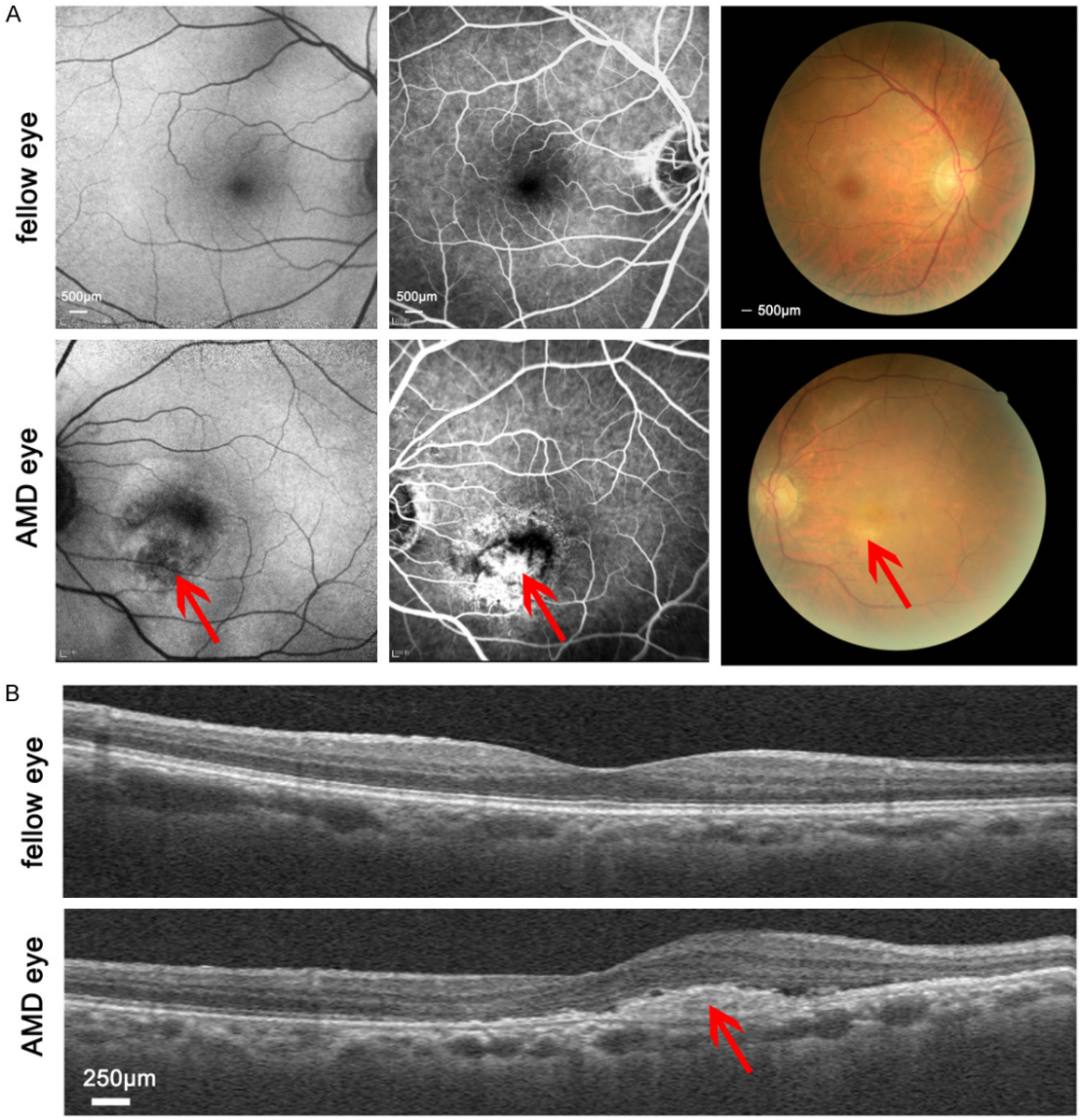



**Figure 1.** Diagnosis of AMD by FFA and OCT. A. Representative auto-florescence and FFA results of the AMD patient. (Scale bar, 500 μm). B. Representative OCT results of the fellow eye and AMD eye in the AMD patient. (Scale bar, 250 μm).

which include the SCP, deep capillary plexus (DCP) and choroidal layer (**Figure 2A**). The machine divided SCP and DCP into several parts (**Figure 2B**). By analyzing the vessel density of each part, we found that SCP vessel density in HC group was much smaller in the AMD group (44.71±0.85, $P<0.01$) and fellow eye group (45.45±0.69, $P<0.05$) compared with healthy control (HC) group (47.74±0.64). According to detailed data, the difference mainly existed in the superior part of perifovea section (Figure S1A). However, the fovea vessel density did not show significant difference between AMD eye and HC eyes (**Table 2**). Additionally, there was no significant difference of SCP vessel density between AMD eye group and fellow eye group.

*Vessel density of DCP in AMD patients versus HCs*

In our study, a decrease in the vessel density in the DCP layer could be observed in the AMD group (44.71±1.07, $P<0.05$) and fellow eye group (44.51±0.96, $P<0.05$) compared with the HC group (47.03±0.85) respectively.

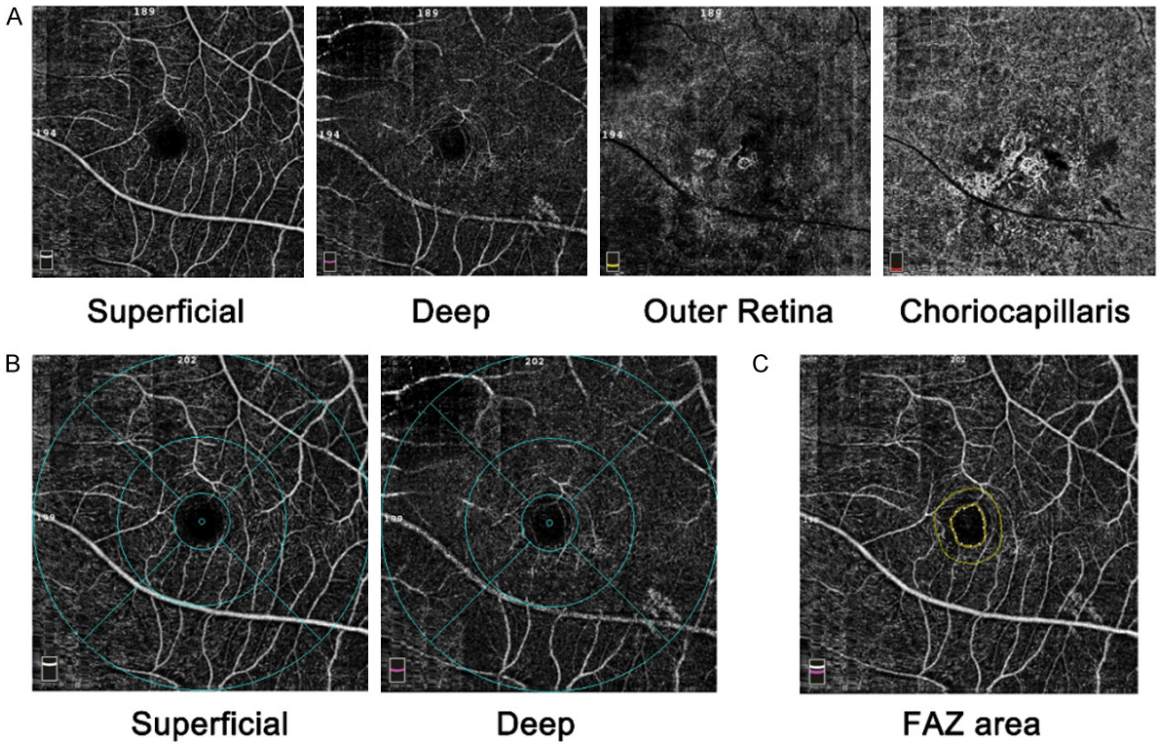



**Figure 2.** OCTA scan of the retina. A. Different layers including superficial, deep, outer retina and choriocapillaris are divided by the OCTA machine automatically. B. Both the superior and deep layer are separated into several parts. C. The foveal avascular zone (FAZ) is outlined in yellow.

**Table 2.** Comparison of SCP vessel density change

| SCP | AMD eye (n=30) | Fellow eye (n=30) | HC (n=30) | $P_1$ value | $P_2$ value | $P_3$ value |
|---|---|---|---|---|---|---|
| Whole image | 44.71±0.85 | 45.45±0.69 | 47.74±0.64 | 0.496 | 0.003* | 0.025* |
| Sup-Hemi | 45.20±0.75 | 45.72±0.76 | 48.18±3.88 | 0.631 | 0.003* | 0.014* |
| Inf-Hemi | 44.13±1.04 | 45.21±0.69 | 47.26±0.66 | 0.369 | 0.005* | 0.066 |
| Fovea | 19.17±2.20 | 17.58±1.41 | 16.88±0.97 | 0.485 | 0.272 | 0.734 |
| Parafovea | 45.40±1.30 | 47.40±0.92 | 49.79±0.74 | 0.177 | 0.002* | 0.078 |
| -Sup-Hemi | 46.26±1.17 | 48.13±1.08 | 50.70±4.37 | 0.198 | 0.001* | 0.056 |
| -Inf-Hemi | 44.53±1.58 | 46.66±1.20 | 48.86±0.85 | 0.233 | 0.009* | 0.182 |
| -Tempo | 47.13±1.07 | 48.00±1.03 | 50.44±0.80 | 0.548 | 0.014* | 0.067 |
| -Sup | 46.21±1.30 | 48.54±1.28 | 51.17±0.75 | 0.154 | 0.001* | 0.079 |
| -Nasal | 43.40±1.78 | 46.62±1.10 | 49.32±0.84 | 0.084 | 0.001* | 0.114 |
| -Inf | 44.85±1.90 | 46.45±1.50 | 46.91±1.44 | 0.513 | 0.360 | 0.838 |
| PeriFovea | 45.30±0.88 | 45.95±0.79 | 48.37±0.67 | 0.576 | 0.005* | 0.026 |
| -Sup-Hemi | 45.77±0.78 | 46.07±0.83 | 48.77±0.67 | 0.794 | 0.005* | 0.012* |
| -Inf-Hemi | 44.78±1.13 | 45.83±0.80 | 47.97±0.69 | 0.422 | 0.009* | 0.079 |
| -Tempo | 41.89±0.92 | 41.87±0.83 | 44.90±0.77 | 0.987 | 0.011* | 0.011* |
| -Sup | 45.76±0.85 | 46.06±0.96 | 48.83±0.72 | 0.814 | 0.009* | 0.019* |
| -Nasal | 49.61±1.03 | 49.71±0.84 | 51.82±0.65 | 0.934 | 0.057 | 0.060 |
| -Inf | 44.18±1.38 | 46.21±1.06 | 47.89±0.70 | 0.190 | 0.009 | 0.232 |

Note: All data is shown as mean ± standard deviation; *stands for significant difference existed between the group; Abbreviations: Sup-Hemi: Superior hemisphere; Inf-Hemi: Inferior hemisphere; Tempo: Temporal; Sup: Superior; Inf: Inferior; $P_1$: *P* value between AMD eye group and fellow eye group; $P_2$: *P* value between AMD eye group and HC eye group; $P_3$: *P* value between fellow eye group and HC eye group.

**Table 3.** Comparison of DCP vessel density

| DCP | AMD eye (n=30) | Fellow eye (n=30) | HC (n=30) | $P_1$ value | $P_2$ value | $P_3$ value |
|---|---|---|---|---|---|---|
| Whole image | 44.71±1.07 | 44.51±0.96 | 47.03±0.85 | 0.803 | 0.044* | 0.038* |
| Sup-Hemi | 44.92±1.07 | 45.10±0.94 | 47.47±0.78 | 0.897 | 0.049* | 0.067 |
| Inf-Hemi | 44.50±1.15 | 43.37±0.97 | 46.59±0.97 | 0.477 | 0.045* | 0.028* |
| Fovea | 30.90±1.58 | 31.58±1.42 | 30.93±1.09 | 0.737 | 0.984 | 0.734 |
| Parafovea | 49.62±0.75 | 50.90±0.76 | 53.26±0.69 | 0.253 | 0.001* | 0.025* |
| -Sup-Hemi | 50.66±0.79 | 51.89±0.85 | 53.85±0.65 | 0.284 | 0.003* | 0.063 |
| -Inf-Hemi | 48.57±0.92 | 49.91±0.90 | 52.67±0.81 | 0.313 | 0.001* | 0.026* |
| -Tempo | 51.19±0.79 | 52.71±0.88 | 67.14±13.13 | 0.981 | 0.239 | 0.286 |
| -Sup | 49.35±0.97 | 50.68±1.15 | 53.34±0.72 | 0.341 | 0.003* | 0.042* |
| -Nasal | 50.69±0.98 | 52.29±0.99 | 54.58±0.68 | 0.221 | 0.002* | 0.057 |
| -Inf | 47.24±0.96 | 48.02±0.94 | 51.12±0.96 | 0.598 | 0.005* | 0.025* |
| PeriFovea | 45.34±1.21 | 45.70±1.02 | 48.21±0.94 | 0.688 | 0.053 | 0.052 |
| -Sup-Hemi | 45.62±1.13 | 45.69±1.09 | 48.72±0.85 | 0.966 | 0.029* | 0.032* |
| -Inf-Hemi | 45.03±1.37 | 43.70±1.07 | 47.69±1.09 | 0.459 | 0.110 | 0.017* |
| -Tempo | 47.65±1.21 | 48.44±1.20 | 51.80±0.85 | 0.623 | 0.006* | 0.026* |
| -Sup | 44.95±1.36 | 44.61±1.34 | 47.67±0.96 | 0.853 | 0.103 | 0.068 |
| -Nasal | 45.77±1.24 | 43.45±1.22 | 46.67±1.08 | 0.194 | 0.586 | 0.044* |
| -Inf | 43.03±1.68 | 42.58±1.23 | 46.43±1.26 | 0.832 | 0.083 | 0.050* |

Note: All data is shown as mean ± standard deviation; *stands for significant difference existed between the group; Abbreviations: Sup-Hemi: Superior hemisphere; Inf-Hemi: Inferior hemisphere; Tempo: Temporal; Sup: Superior; Inf: Inferior; $P_1$: *P* value between AMD eye group and fellow eye group; $P_2$: *P* value between AMD eye group and HC eye group; $P_3$: *P* value between fellow eye group and HC eye group.

**Table 4.** Comparison of choroidal vessel density (CVD) and FAZ area change

| | AMD eye (n=30) | Fellow eye (n=30) | HC (n=30) | $P_1$ value | $P_2$ value | $P_3$ value |
|---|---|---|---|---|---|---|
| CVD | 0.62±0.01 | 0.65±0.01 | 0.67±0.01 | 0.003* | 0.000* | 0.073 |
| FAZ | 0.30±0.02 | 0.32±0.03 | 0.33±0.01 | 0.56 | 0.27 | 0.64 |

Note: All data is shown as mean ± standard deviation; *stands for significant difference existed between the group; $P_1$: *P* value between AMD eye group and fellow eye group; $P_2$: *P* value between AMD eye group and HC eye group; $P_3$: *P* value between fellow eye group and HC eye group.

However, no significant vessel density change happened between the two eyes of AMD patients. In detail, the vessel density of the parafovea section was greatly decreased in both the AMD eye group (49.62±0.75, P<0.001) and fellow eye group (50.90±0.76, P<0.05) compared with the HC group (53.26±0.69). In the AMD eye group, the vessel density in both of superior (44.92±1.07, P<0.05) and inferior (44.50±1.15, P<0.05) parts of the retina was reduced compared with the HC group (47.47±0.78, 46.59±0.97). However, only the inferior (43.37±0.97, P<0.05) part of the retina was affected in the fellow eye group compared with the HC group (46.59±0.97) (**Table 3**; Figure S1B).

*Choroidal vessel density and FAZ area in AMD patients versus HCs*

After acquiring OCTA scans, the software analyzed the images automatically. To underline the inter-individual variety of size, the foveal avascular zone was outlined in yellow (**Figure 2C**). According to the data (**Table 4**), the choroidal vessel density changed a lot in the AMD eye group (0.62±0.01, P<0.001), while it did not change much in the fellow eye group (0.65±0.01, P>0.05) compared with the HC group (0.67±0.01). Besides, the FAZ area did not change much in both of AMD eye group and fellow eye group compared with HC group (Figure S1C).

*Retina thickness analysis in AMD patients versus HCs*

Overall, the thickness of the retina was declined in both the AMD eye group (274.62±7.15, P<0.05) and fellow eye group (274.00±3.51, P<0.01) compared with the HC eyes (289.5± 10.8). The reduced thickness was mainly from the perifovea region, where the AMD eye

**Table 5.** Comparison of Retina thickness

| | AMD eye (n=30) | Fellow eye (n=30) | HC (n=30) | $P_1$ value | $P_2$ value | $P_3$ value |
|---|---|---|---|---|---|---|
| Whole image | 274.62±7.15 | 274.00±3.51 | 289.5±10.8 | 0.923 | 0.012* | 0.009* |
| Sup-Hemi | 276.27±8.11 | 276.31±3.73 | 292.37±1.94 | 0.996 | 0.015* | 0.016* |
| Inf-Hemi | 273.27±7.75 | 271.73±3.4 | 286.76±1.67 | 0.818 | 0.030* | 0.016* |
| Fovea | 301.69±31.86 | 247.27±5.84 | 247.16±2.65 | 0.03* | 0.018* | 0.996 |
| Parafovea | 322.62±15.99 | 311.92±22.31 | 325.37±2.12 | 0.405 | 0.815 | 0.255 |
| -Sup-Hemi | 326.54±18.28 | 312.15±4.21 | 326.42±2.18 | 0.321 | 0.993 | 0.248 |
| -Inf-Hemi | 318.38±14.65 | 311.92±4.65 | 324.29±2.12 | 0.588 | 0.590 | 0.260 |
| -Tempo | 312.92±18.73 | 303.77±4.63 | 316.71±2.09 | 0.538 | 0.781 | 0.344 |
| -Sup | 328.54±18.45 | 314.96±4.06 | 329.37±2.29 | 0.353 | 0.951 | 0.283 |
| -Nasal | 332.12±16.77 | 316.50±4.80 | 330.08±2.24 | 0.249 | 0.869 | 0.274 |
| -Inf | 316.12±13.25 | 313.04±4.70 | 325.39±2.19 | 0.779 | 0.358 | 0.221 |
| PeriFovea | 270.50±7.42 | 272.77±3.53 | 288.32±1.90 | 0.731 | 0.004* | 0.012* |
| -Sup-Hemi | 273.12±9.03 | 276.92±3.54 | 292.50±2.07 | 0.622 | 0.007* | 0.030* |
| -Inf-Hemi | 267.62±7.48 | 268.42±3.65 | 284.13±1.75 | 0.903 | 0.008* | 0.011* |
| -Tempo | 253.81±6.68 | 262.04±3.98 | 271.92±1.56 | 0.187 | 0.002* | 0.086 |
| -Sup | 268.62±11.37 | 276.46±3.24 | 292.61±2.25 | 0.402 | 0.006* | 0.062 |
| -Nasal | 288.26±8.63 | 293.65±5.52 | 309.95±2.24 | 0.499 | 0.004* | 0.023* |
| -Inf | 261.28±8.19 | 262.68±3.64 | 278.55±1.95 | 0.844 | 0.009* | 0.016* |

Note: All data is shown as mean ± standard deviation; *stands for significant difference existed between the group; Abbreviations: Sup-Hemi: Superior hemisphere; Inf-Hemi: Inferior hemisphere; Tempo: Temporal; Sup: Superior; Inf: Inferior; $P_1$: *P* value between AMD eye group and fellow eye group; $P_2$: *P* value between AMD eye group and HC eye group; $P_3$: *P* value between fellow eye group and HC eye group.

(270.50±7.42, P<0.01) and fellow eye (272.77±3.53, P<0.05) thickness greatly decreased compared with the HC eye (288.32±1.90). Specially, the thickness of the fovea part of AMD eyes (301.69±31.86, $P_1$<0.05, $P_2$<0.05) was increased when compared with fellow eyes (247.27±5.84) and HC eyes (247.16±2.65) (**Table 5**; Figure S1D).

*Optic nerve head (ONH) area analysis in AMD patients versus HCs*

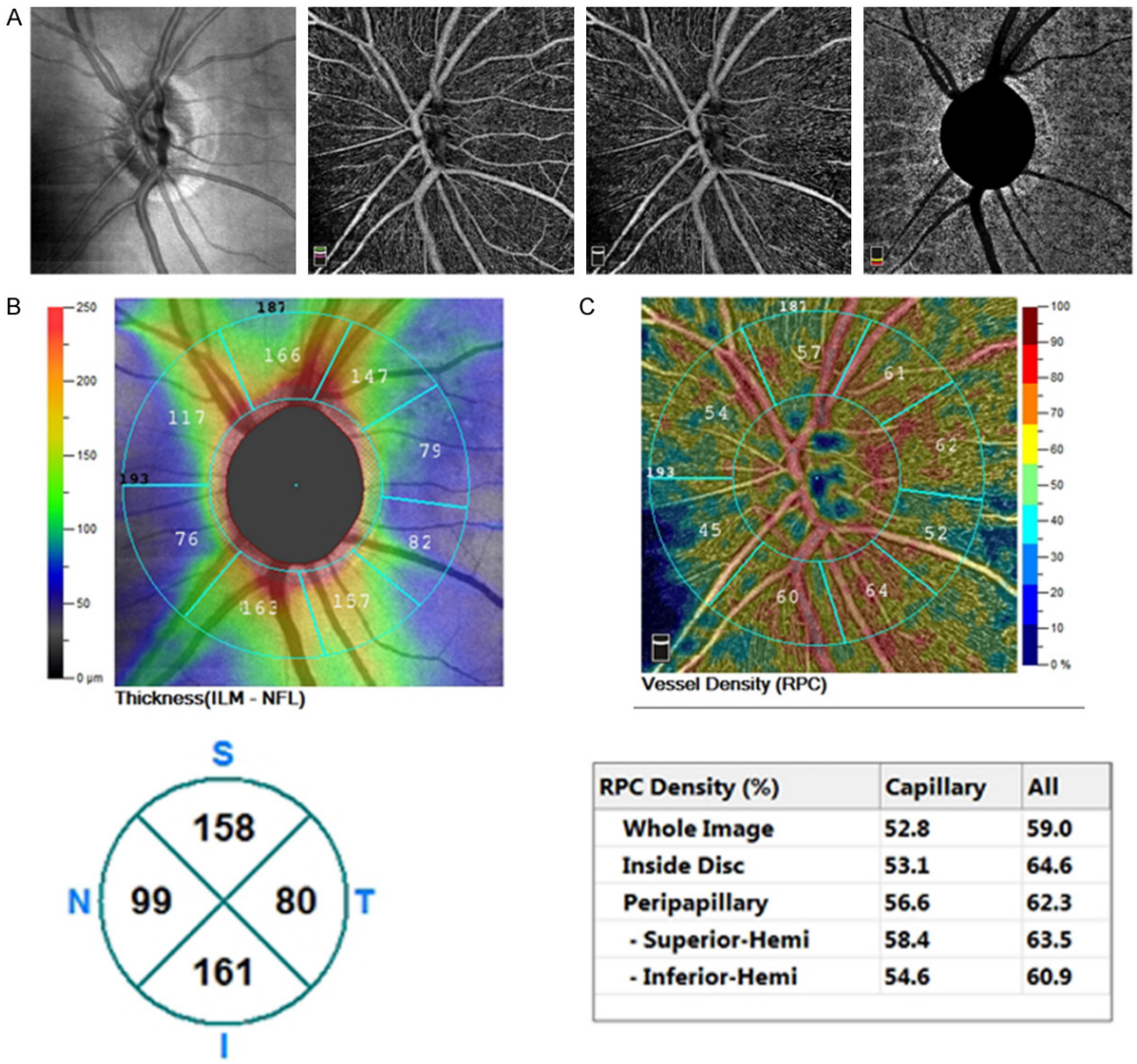
We used a 4.5*4.5 mm ONH scan to assess the retinal nerve fiber layer (RNFL) thickness change and the vessel density change (**Figure 3**; **Table 6**). ONH scan analysis showed that RNFL thickness of the AMD eye group (108.42±4.32, P<0.05) and fellow eye group (107.68±3.40, P<0.05) was much thinner than the HC eye group (116.18±1.70), which was typically reflected in nasal and inferior parts. Peripapillary capillary density was significantly lower in the AMD eye group (50.20±0.84, P<0.01) and fellow eye group (50.76±0.70, P<0.05) compared with the HC group (52.61±0.45). However, there was no difference of inside disc capillary density among all three groups (**Table 6**; Figure S1E).

*MoCA and MRI in AMD patients versus HCs*

MoCA was designed for testing patients' ability of memory, comprehension, calculation, learning, etc. MRI was done to analyze the volume of hippocampus tissue (HIP) which was related with people's memory and learning. Therefore, we applied these tests for exploring the connection between AMD and cognitive impairment. Our results showed that the MoCA score was lower in the AMD patients compared with the HC group (**Figure 4A**). However, the volume of HIP area showed no significant difference between these two groups (**Figure 4B**). Most importantly, we applied Pearson correlation test with OCTA data and MoCA scores on AMD patients, in which peripapillary capillary density on ONH scan was significantly and positively correlated with MoCA scores (r=0.523, P<0.01; **Figure 4C**).

## Discussion

This study presents the detailed OCTA features of AMD patients' retina by scanning a large area (6*6 mm area centered on the macular), which was divided into more specific subregions for further study. We compare AMD eyes




| RPC Density (%) | Capillary | All |
|---|---|---|
| Whole Image | 52.8 | 59.0 |
| Inside Disc | 53.1 | 64.6 |
| Peripapillary | 56.6 | 62.3 |
| - Superior-Hemi | 58.4 | 63.5 |
| - Inferior-Hemi | 54.6 | 60.9 |

**Figure 3.** 4.5*4.5 mm ONH scan. A. Photos presented by ONH scan. B. The thickness of the ONH area. We assessed four parts that include superior, inferior, nasal and temple parts of the ONH area. C. Vessel density of the ONH area. The data in the lower table is generated by software automatically, which is used to assess the vessel density change in the AMD patient.

with fellow eyes, which shows decreased vessel density in SCP and DCP in the AMD eye group. This result is also supported by Toto and Matt Trinh's study, which report lower SCP vessel density in the intermediate AMD patients [16, 17]. This specific results may be related with progression of AMD, such as outer retina atrophy and reticular pseudodrusen [18], and reduced demand of nutrition from inner retina. However, Cicinelli illustrates that only the DCP vessel density is deducted in the AMD patient [19], which holds a different opinion than us. According to recent studies, different OCTA machine used in study may produce different results [20]. Thus, controlling variables and keeping consistency in one study are vital for reliability of the data. Despite the different results in aforementioned studies, the decreased vessel density is related with AMD progression determinately. Thus, similar results in the fellow eyes suggest that they may be suffering from the early stage of AMD.

The choroid is the vascular bed that lies under the retinal pigment epithelium (RPE), of which the blood flow provides nutrition and oxygen to the outer retina. Moreover, the change of choroidal blood flow is reported to be related to the progression of AMD [21]. Our results refer to the decreased vessel density of the choroid in

**Table 6.** Comparison of ONH scan

| | | AMD eye (n=30) | Fellow eye (n=30) | HC (n=30) | $P_1$ value | $P_2$ value | $P_3$ value |
|---|---|---|---|---|---|---|---|
| RNFL thickness | | 108.42±4.32 | 107.68±3.40 | 116.18±1.70 | 0.872 | 0.048* | 0.041* |
| | S | 129.44±5.28 | 128.20±3.61 | 138.82±2.41 | 0.824 | 0.049* | 0.039* |
| | I | 131.54±4.97 | 129.48±5.36 | 144.82±3.19 | 0.757 | 0.034* | 0.012* |
| | T | 96.46±11.39 | 79.44±2.20 | 82.89±1.97 | 0.057 | 0.096 | 0.665 |
| | N | 92.63±3±.15 | 94.04±4.06 | 102.16±1.73 | 0.750 | 0.021* | 0.044* |
| whole image | C | 47.92±0.59 | 48.06±0.75 | 49.42±0.46 | 0.870 | 0.073 | 0.097 |
| | All | 54.08±0.62 | 54.27±0.78 | 55.87±0.45 | 0.829 | 0.037* | 0.057 |
| Inside disk | C | 49.12±1.34 | 49.05±1.47 | 47.86±0.72 | 0.967 | 0.429 | 0.445 |
| | All | 58.73±1.07 | 58.70±1.18 | 58.05±0.62 | 0.982 | 0.602 | 0.611 |
| Peripapillary | C | 50.20±0.84 | 50.76±0.70 | 52.61±0.45 | 0.566 | 0.008* | 0.036* |
| | All | 56.05±0.87 | 56.74±0.73 | 58.76±0.46 | 0.498 | 0.004* | 0.028* |
| -Sup-Hemi | C | 50.40±0.86 | 51.03±0.69 | 52.57±0.50 | 0.532 | 0.020* | 0.093 |
| | All | 56.38±0.90 | 57.20±0.68 | 59.19±3.07 | 0.422 | 0.003* | 0.034* |
| -Inf-Hemi | C | 49.49±1.05 | 50.25±0.80 | 52.62±0.43 | 0.493 | 0.003* | 0.019* |
| | All | 55.29±1.01 | 55.92±0.85 | 58.37±0.46 | 0.575 | 0.004* | 0.017* |

Note: All data is shown as mean ± standard deviation; *stands for significant difference existed between the group; Abbreviations: Sup-Hemi: Superior hemisphere; Inf-Hemi: Inferior hemisphere; S: Superior; I: Inferior; T: Temporal; Sup: Superior; C: capillary density; All: all vessel density; $P_1$: *P* value between AMD eye group and fellow eye group; $P_2$: *P* value between AMD eye group and HC eye group; $P_3$: *P* value between fellow eye group and HC eye group.

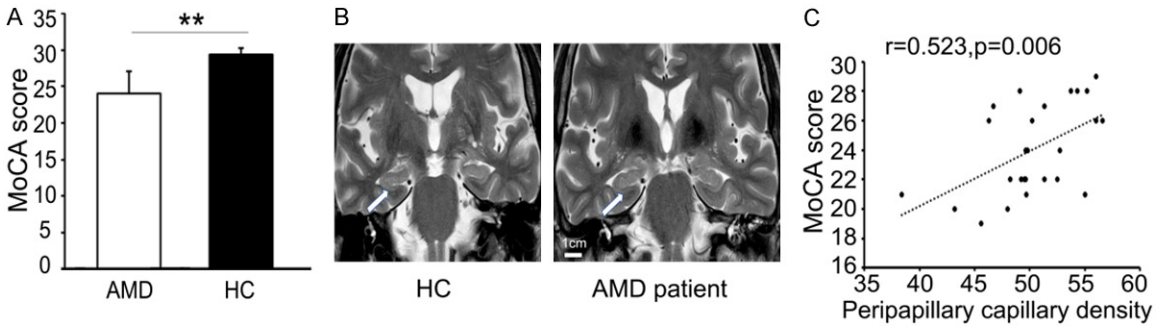



**Figure 4.** MoCA and MRI in AMD patient versus HC. A. MoCA score of the AMD patient versus HCs. Data are presented as mean ± SD. **P<0.01. B. MRI scan of the AMD patient and HCs. The HIP area is showed in red arrow. (Scale bar, 1 cm). C. Correlation between peripapillary capillary density on ONH scan and MoCA score.

the AMD eyes compared with fellow eyes. Besides, HC eyes have the highest the vessel density among all groups. According to related studies, the vessel density in the choroid is parallel with AMD progression [22, 23]. Thus, fellow eyes with lower vessel density compared with HC eyes may experience the early pathogenesis of AMD.

Furthermore, we investigate the thickness change in the retina. We find that the thickness of retina in AMD patients is thinner than HC group, while the thickness of fellow eyes is similar with AMD eyes. Based on our data, the reduced thickness mainly exists in the perifovea area. Though we do not clarify the specific layer which contributes to the thinner retina in this study, other published researches mention that ganglion cell layer (GCL), inner plexiform layer (IPL) and ganglion cell complex (GCC) are involved in this decreased thickness of AMD patients. They also attribute this change to the altered needs of vascular supplying [24-26]. In addition, we observe increasing thickness of fovea part of AMD eye, which may result from fluid accumulation in the sub-retina space.

By analyzing the ONH area related data, we find reduced RNFL thickness in both eyes of AMD patients as well as decreased peripapillary cap-

illary density in AMD patients compared with the HC group. Because tiny branching vessels or peripheral arcade may differentiate an active lesion from a quiescent one, they are considered as a biomarker of active exudative lesions that may need intravitreal injection treatment. Moreover, the peripheral neovascularization is one of the reason for frequent recurrence of AMD [27]. Thus, AMD patients in our study with lower peripapillary capillary density in both eyes may need an anti-VEGF therapy in not only the AMD eyes but also the fellow eyes to improve the vision. Though there is no evidence directly supporting this theory now, the prognosis of AMD patient accepting an anti-VEGF treatment in both eyes is worth exploring in the future.

Because the vessels of retina and cerebral are anatomically and physiologically homologous [28], it is reasonable to explore changes in the brains of AMD patients. Based on our knowledge, this is the first report that explores cognitive impairment progression in AMD patients. To assess the mental changes of AMD patients, we apply the questionnaire of MoCA that is widely used to evaluate the cognitive state of patients. Besides, HIP area measured by MRI is the region that was first reported to be related to AD, which is one representative type of cognitive impairment [29]. Due to the high sensibility of these methods, they can provide clues to decide whether patients are suffering from cognitive impairment, even early stage of AD [30, 31]. Based on our data, lower scores in the AMD group suggests that patients have mild cognitive impairment (MCI). However, MRI shows no significant difference in the volume of HIP between AMD patients and the HC group, which indicates that the patients with MCI mentioned above do not meet the AD diagnosis criteria. Apart from our study, some works also try to uncover the relationship between AMD and AD. Bliss Elizabeth O'Bryhim's findings imply that in the early stage of AD, the retina of patients already undergoes retinal neuronal loss and vessel modification, which means vascular dysfunction happens far earlier than we believe. The Aβ accumulation within the inner retina may be one explanation for retinal degeneration observed in AMD eyes [13]. Seulggie Choi et al. depicts that AMD patients have a higher risk of AD when lifestyle is taken into consideration. They also point out that this relationship may come from three aspects including amyloid β-peptide in the AMD retina drusen, increased inflammatory response, and the oxidizing radicals released from the damaged mitochondrial [32]. Along with our findings, these results connect AMD with cognitive impairment in disease pathology and progression. Nevertheless, their studies do not clarify the specific blood flow change of the retina along with HIP change measured by MRI scan which may reveal underlying mechanisms between the two diseases. Thus, our study is the first one exploring the risk of cognitive impairment in AMD patients which may provide a promising method for related disease prediction and prognosis evaluation. In addition, a strong correlation between peripapillary capillary density on ONH scan and MoCA score was found in our study which may suggest paralleling degeneration of optic nerve and central nerve system. This is also the first study indicating the correlation of blood flow change around the optic nerve and cognitive impairment, which may provide great insight for future diagnosis of cognitive impairment in AMD patients by OCTA test. To further confirm these results, we will collect aqueous humour from AMD patients and test related biomarkers in the future. By doing this, we want to prove that AMD can be regarded as a hallmark of the development of cognitive impairment and even the early stage of AD. Thus, the intervention in the early stage of AMD will improve the prognosis of cognitive impairment.

We have to admit the limitations of our study. Firstly, OCTA may be regarded as a safe option compared with traditional angiography because of the adverse reactions of intravenous dye injection. However, OCTA scan may miss some small lesions with slow blood flow, which may produce some artifacts [33]. Secondly, the relatively small number of patients involved in this study is another potential set back. However, the sample size will be amplified on our ongoing research along with aqueous humour collection and related biomarkers test. In this way, we could further explore the related underlying mechanisms of these two diseases and providing a potential diagnosis method for cognitive impairment in AMD patients. Lastly, whether the fellow eye is developing into AMD still needs to be followed up, which may help us understand the disease progression from another

angle. However, our data provides a possible direction that the fellow eye may be in the early stage of AMD. Thus, the patients would pay more attention to the development of their fellow eye, which may remind them to go to the hospital and receiving prompt treatment once their vision decrease. What's more, our study provides a new possible early-diagnosis marker of cognitive impairment, which would benefit the patients with regards to their prognosis.

In conclusion, our study proves that the fellow eye with good vision condition of the AMD patients are probably in the early stage of AMD. In addition, we find that AMD patient may have a greater chance suffering from a cognitive impairment, which may help us interfere with the disease in the early stage and improve the life quality for patients.

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## Disclosure of conflict of interest

None.

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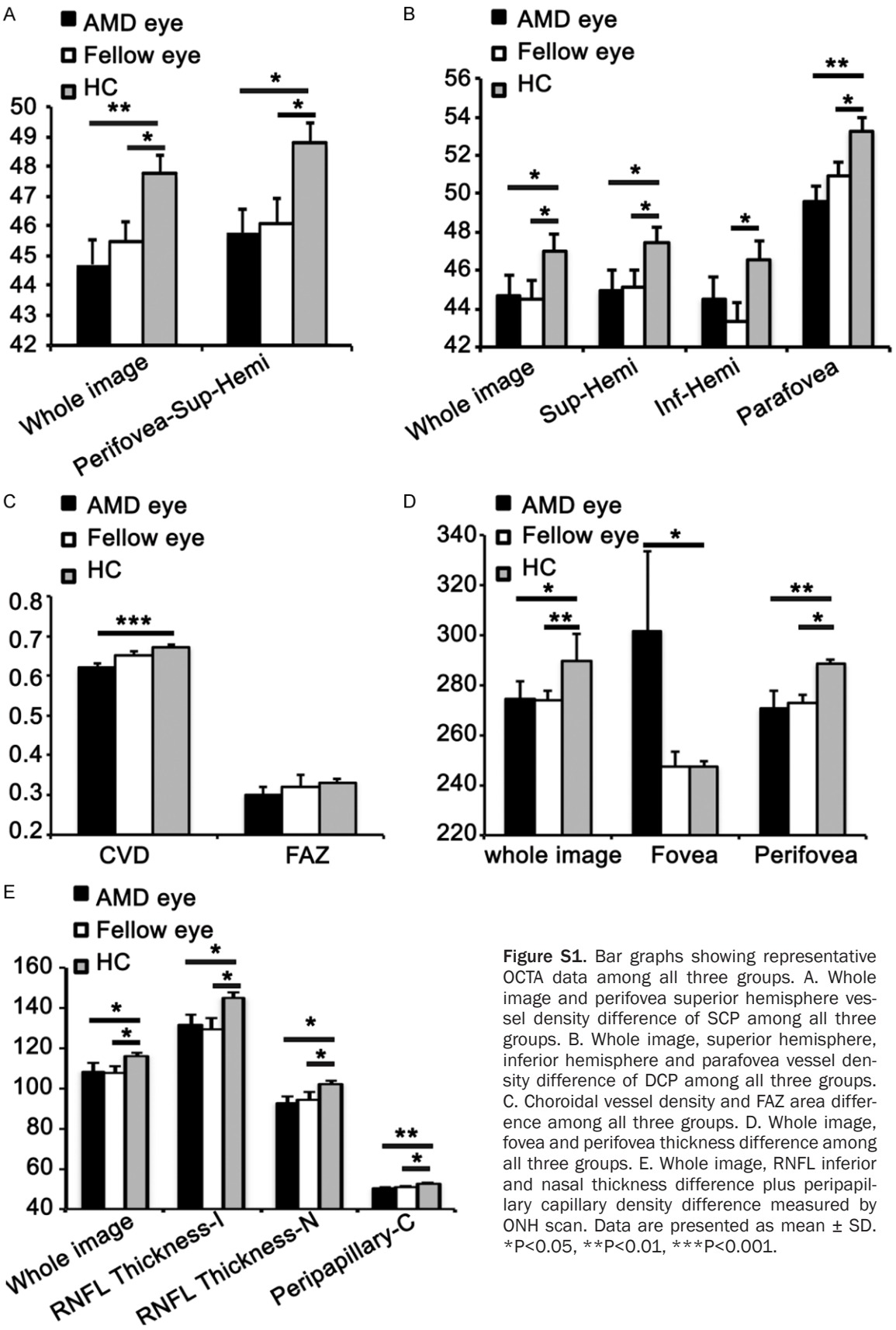


**Figure S1.** Bar graphs showing representative OCTA data among all three groups. A. Whole image and perifovea superior hemisphere vessel density difference of SCP among all three groups. B. Whole image, superior hemisphere, inferior hemisphere and parafovea vessel density difference of DCP among all three groups. C. Choroidal vessel density and FAZ area difference among all three groups. D. Whole image, fovea and perifovea thickness difference among all three groups. E. Whole image, RNFL inferior and nasal thickness difference plus peripapillary capillary density difference measured by ONH scan. Data are presented as mean ± SD. *P<0.05, **P<0.01, ***P<0.001.