• Clinical Research •

Choroidal structural changes determined by the binarization method after intravitreal aflibercept treatment in neovascular age-related macular degeneration

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Abstract

● **AIM:** To assess the choroidal structural alterations after intravitreal injection of aflibercept in neovascular age-related macular degeneration (nAMD).

● **METHODS:** Fifty eyes with treatment-naïve nAMD were evaluated at baseline, 3rd, and 12th month. Fifty eyes of 50 healthy subjects were also included as controls. Choroidal thickness (CT) was measured in the subfoveal region. Total circumscribed choroidal area (CA), luminal area (LA), stromal area (SA), and choroidal vascularity index (CVI) was calculated using Image J.

● **RESULTS:** At baseline, subfoveal CT was increased in nAMD patients compared to controls (*P*=0.321). Eyes with nAMD had a significantly increased total circumscribed CA and SA (*P*=0.041, 0.005, respectively). The CVI was decreased (*P*=0.038). In the 3rd month, the subfoveal CT, LA, and CVI revealed a decrease (*P*=0.005, *P*=0.039, 0.043, respectively). In the 12th month, subfoveal CT, LA, and CVI were decreased in comparison to baseline measures (*P*<0.001, 0.006, 0.010, respectively).

● **CONCLUSION:** Significant structural alterations are found after intravitreal aflibercept treatment during the 12-month follow-up, in particular at the third month, in eyes with nAMD.

● **KEYWORDS:** aflibercept; choroidal vascularity index; intravitreal; neovascular age-related macular degeneration
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INTRODUCTION

Age-related macular degeneration (AMD) is a common cause of visual deterioration and legal blindness in patients over the age of 50[1]. It is classified into 2 main subtypes: dry or non-neovascular AMD, and wet or neovascular AMD (nAMD). Main characteristic of nAMD is choroidal neovascularization (CNV), new blood vessels originating from the choroid extending into the region underlying the retina pigment epithelium (RPE) or subretinal area. Generally, AMD is featured by functional loss including the retinal photoreceptors, RPE, Bruch's membrane, and choriocapillaris[2].

Intravitreal (IV) injection of anti-vascular endothelial growth factor (VEGF) agents has been recommended as a first-line therapy for nAMD[3-4]. Aflibercept is a novel fusion protein, which is constructed from portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1[5]. As a soluble decoy receptor, it binds all isoforms of VEGF-A, VEGF-B, and placental growth factor. There is no agreement on the alterations in choroidal circulation in patients with nAMD treated with IV anti-VEGFs. Previous reports[6-9] showed that central choroidal thickness (CT) reduces significantly after IV ranibizumab and aflibercept treatment in nAMD. In addition, there are studies[10-12] reporting no impairment in CT after IV bevacizumab and ranibizumab injections.

Binarization techniques applied to enhanced depth imaging optical coherence tomography (EDI-OCT) allowed quantitative analysis of choroidal vascular tissue. The choroidal vascularity index (CVI), the proportion of the luminal area (LA) to the cross-sectional area of choroid (CA), can be a more valid biomarker to analyze the choroidal vasculature compared to CT. Because it displays lower variation and is modified by a small numer of physiologic factors[13-14]. Recently, Pellegrini *et al*[15] found significantly decreased CT and vasculature after treatment with aflibercept in nAMD eyes.

The objective of the study was to determine the changes in choroidal structure in patients with nAMD following IV aflibercept treatment over a twelve-month duration.

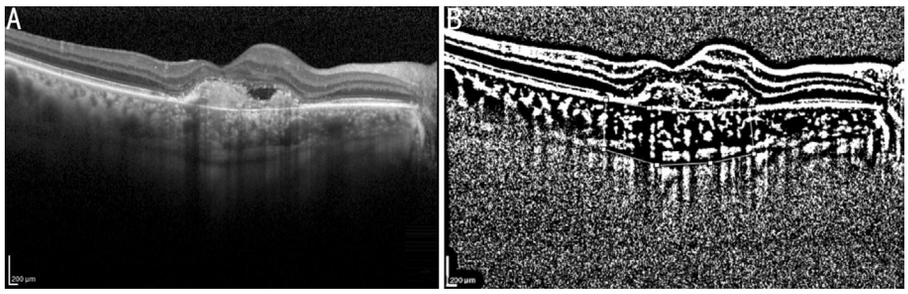

Figure 1 Representative images of a patient with nAMD A: EDI-OCT image of a patient with nAMD; B: Binarized image with the area of interest outlined with white color.

Table 1 Comparison of choroidal structural parameters between the groups

Parameters	Patients (baseline)	Controls	*P*
Subfoveal choroidal thickness (μm)	315.0±37.4	297.3±68.5	0.321
Total circumscribed choroidal area (mm^2)	1.509±0.204	1.345±0.267	0.041[a]
Luminal area (mm^2)	0.979±0.196	0.961±0.212	0.946
Stromal area (mm^2)	0.534±0.209	0.384±0.088	0.005[a]
Choroidal vascularity index (%)	65.13±1.25	71.2±4.56	0.038[a]

[a]Statistically significant.

SUBJECTS AND METHODS

Ethical Approval This retrospective study conducted participants with unilateral treatment-naïve nAMD examined at the Retina Outpatient Clinic of Kırşehir Training and Research Hospital between January 2019 and June 2020. It was in accordance with the tenets of the Declaration of Helsinki and was approved by the Ethics Committee. Informed consent was waived because of the retrospective and anonymous nature of this study.

All participants were treated with 2.0-mg IV injection of aflibercept (Eylea; Regeneron, Tarrytown, NY, USA, and Bayer, Leverkusen, Germany) and underwent an ophthalmic examination including anterior and posterior segment examinations, fluorescein angiography (FA), and OCT imaging (Spectralis®, Heidelberg, Germany). All OCT imagings were captured between 9:00 *a.m.* and 12:00 *p.m.* All measurements were carried out by the same blinded physician.

The eyes with polypoidal choroidal vasculopathy, on the basis of protruded orange-red elevated lesions and/or those with polypoidal vasculopathy findings in OCT, previous retinal disorders like vascular occlusions, diabetic/hypertensive retinopathy, central serous retinopathy, previous intraocular intervention, IV injections, or laser photocoagulation, and presence of glaucoma were excluded.

The subfoveal CT was measured manually with a caliper tool. Binarization was done with Image J (Version 1.50a; National Institutes of Health, Bethesda, MD, USA). The 3000 micrometer wide area with margins of 1500 micrometer temporally to the fovea was selected. The choroid was delineated as the area between the outer RPE and the inner sclera, and the borders were positioned manually with the ROI Manager. Image adjusted by the Niblack auto local threshold (Figure 1). The total circumscribed CA, LA, and stromal area (SA) were automatically calculated. CVI was formulated as the ratio between LA and total circumscribed CA[16]. All measurements were made at baseline, 3rd, and 12th months.

Statistical Analysis Statistical analysis was done with SPSS 11.5 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnow test was used to determine whether continuous variables were distributed normally. The standard deviation was shown for numerical variables with a normal distribution. For variables with normal distribution, difference among groups was determined by a *t*-test in independent groups. For statistical analysis, time points considered were baseline, 3rd, and 12th months. A *P* value lower than 0.05 was determined as significant.

RESULTS

In total, 50 eyes of 50 treatment-naïve participants with nAMD (26 women and 24 men, mean age 64.2±3.1y), and 50 eyes of 50 healthy subjects (26 women and 24 men, mean age 63.9±2.9y) were recruited. The mean number of injections during the 12-month period was 7.8±0.8, the mean number of visits was 11±0.8.

At baseline, subfoveal CT was increased in patients compared to controls, the difference was not significant (*P*=0.321). Eyes with nAMD had a significantly increased total circumscribed CA and SA (*P*=0.041, 0.005, respectively). LA did not reveal a significant difference (*P*=0.946). The CVI was decreased in nAMD eyes (*P*=0.038). Choroidal structural parameters at baseline are given in Table 1.

At 3rd month, in comparison to baseline, subfoveal CT was decreased from 315.0±37.4 to 286.5±19.99 μm (*P*=0.005),

Table 2 Comparison of choroidal structural parameters at baseline and 3rd month

Parameters	Patients (baseline)	Patients (3rd month)	*P*
Subfoveal choroidal thickness (μm)	315.0±37.4	286.5±19.99	0.005[a]
Total circumscribed choroidal area (mm^2)	1.509±0.204	1.471±0.044	0.473
Luminal area (mm^2)	0.979±0.196	0.853±0.130	0.039[a]
Stromal area (mm^2)	0.534±0.209	0.624±0.131	0.119
Choroidal vascularity index (%)	65.1±12.5	57.8±11.1	0.043[a]

[a]Statistically significant.

Table 3 Comparison of choroidal structural parameters at baseline and 12th month

Parameters	Patients (baseline)	Patients (12th month)	*P*
Subfoveal choroidal thickness (μm)	315.0±37.4	279.5±16.1	<0.001[a]
Total circumscribed choroidal area (mm^2)	1.509±0.204	1.467±0.04	0.380
Luminal area (mm^2)	0.979±0.196	0.829±0.108	0.006[a]
Stromal area (mm^2)	0.534±0.209	0.637±0.108	0.050
Choroidal vascularity index (%)	65.1±12.5	56.52±0.73	0.010[a]

[a]Statistically significant.

Table 4 Comparison of choroidal structural parameters at 3rd and 12th months

Parameters	Patients (3rd month)	Patients (12th month)	*P*
Subfoveal choroidal thickness (μm)	286.5±19.9	279.5±16.1	0.362
Total circumscribed choroidal area (mm^2)	1.471±0.044	1.467±0.04	0.901
Luminal area (mm^2)	0.853±0.130	0.829±0.108	0.407
Stromal area (mm^2)	0.624±0.131	0.637±0.108	0.436
Choroidal vascularity index (%)	57.8±11.1	56.52±0.73	0.418

total circumscribed CA from 1.509±0.204 to 1.471±0.044 mm^2 (*P*=0.473), LA from 0.979±0.196 to 0.853±0.130 mm^2 (*P*=0.039), and CVI from 65.1%±12.5% to 57.8%±11.1% (*P*=0.043; Table 2).

At 12th month, subfoveal CT, total circumscribed CA, LA, and CVI were decreased compared to 3mo (279.5±16.1 μm, 1.467±0.04 mm^2, 0.829±0.108 mm^2, and 56.52%±0.73%, respectively). The differences were not significant (all *P*>0.05; Table 3). In comparison to baseline measures, subfoveal CT, LA, and CVI were decreased in the 12th month (*P*<0.001, *P*=0.006, *P*=0.010, respectively; Table 4).

Changes in choroidal structural parameters during the 12-month follow-up are shown in Figure 2.

DISCUSSION

We evaluated the choroidal structural changes in patients with treatment-naïve nAMD at initial visit and after IV aflibercept injection during the 12mo follow-up period.

The choroidal tissue has the main part in the pathogenesis of AMD and there have been several studies evaluating the thickness of the choroid in these patients[6-10]. In our study, as previously reported[17], eyes with nAMD demonstrated increased CT at baseline prior to sequential IV anti-VEGF injections. The difference was not statistically significant compared to normal eyes. CT change is still controversial and there are variations among patients with different types of AMD. These may be due to several causative factors such as the stage of the disease, amount of disease activity, abnormal choroidal blood flow dynamics, and choroidal vascular structural changes. All these parameters could be affected, alone or in combination, in AMD patients. In this study, there were not any characteristic differences in patients other than nAMD which might have affected choroidal tissue, based on OCT findings, color fundus appearance, and FA. According to the results, we propose that increased CT in eyes with nAMD may be associated with increased luminal and stromal components of the choroidal vasculature. This is in agreement with the results of Koh *et al*[18], which showed increased CT and decreased choroidal vasculature in patients with AMD when compared with age-matched healthy subjects.

Part of the previous studies[6,8,19-20] have reported decreased CT after IV anti-VEGF injections both with ranibizumab and aflibercept. Kim *et al*[21] showed that, after the first decline caused by IV anti-VEGF treatment, the CT was progressively increased as the drug weared off and was decreased again with further IV anti-VEGF administrations. Yamazaki *et al*[6] proved that patients under anti-VEGF treatment with *pro re nata* regimen had thinner choroids compared to baseline 12mo after initiating the treatment. In accordance with these findings,

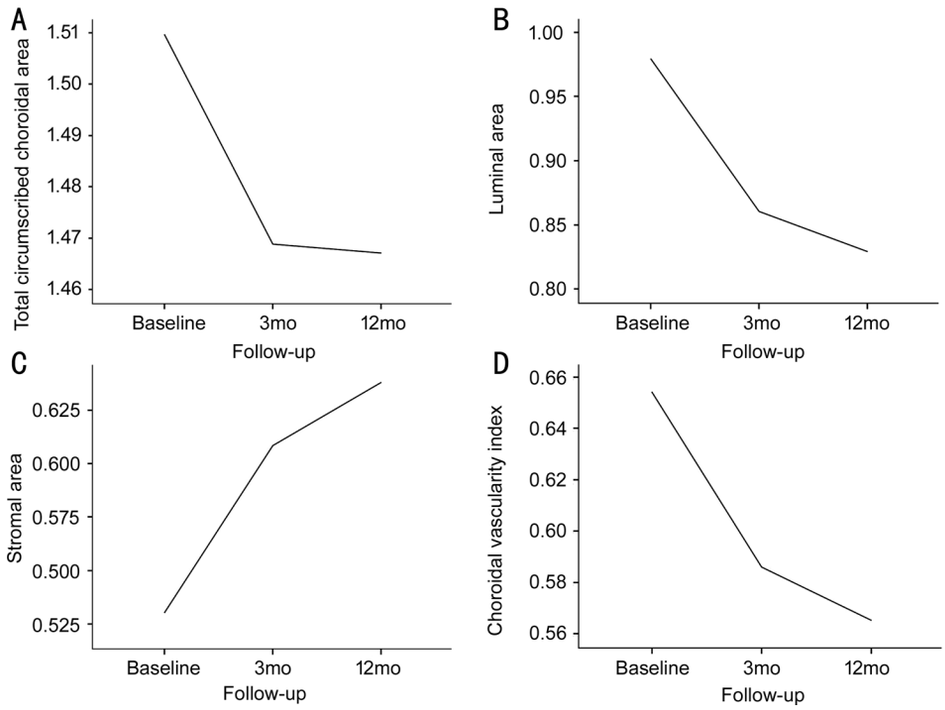

Figure 2 Changes in choroidal structural parameters during the 12-month follow-up A: Alterations in total circumscribed CA during the 12-month follow-up; B: Alterations in LA during the 12-month follow-up; C: Alterations in SA during the 12-month follow-up; D: Alterations in CVI during the 12-month follow-up.

in this study, after 3mo of IV aflibercept injections, the mean subfoveal CT was significantly decreased.

Changes in the choroidal circulation are considered as potentially contributing factors to the development and progression of AMD. There is strong evidence that AMD may be ultimately characterized by the damage of the unit including the photoreceptors, RPE, Bruch's membrane, and choroid[22-24]. In nAMD, new, aberrant vessels grow from the choroid through the Bruch membrane to the sub-RPE and subneurosensory retina, leading to exudation and visual impairment. McLeod *et al*[2] demonstrated choriocapillaris dropout in CNV. Assuming all these situations, evaluating CVI may provide additional insights into choroidal structural and vascular changes in AMD pathogenesis.

In our study, the binarization of the CA showed a significantly decreased LA and CVI. These outcomes are consistent with the study by Pellegrini *et al*[15]. As CVI is the proportion of LA and total circumscribed CA, the decrease indicates a higher depletion in the choroidal vascular portion in comparison to the SA.

The vascular effects of VEGF-A include stimulation of angiogenesis, increase in vascular leakage, and vasodilation. So, it is obvious that suppression of VEGF may be associated with a decline in choriocapillaris endothelial cell fenestrations and may result in decreased CT by reducing choroidal vascular permeability[19]. The decreased CVI after IV aflibercept treatment demonstrated in this study seems to support this hypothesis. Another explanation can be that choroidal structural alterations after anti-VEGF therapy might be secondary to suppression of the CNV activity and leakage.

There were some limitations of the current study. The retrospective design did not allow us to assess the variables such as axial length, systemic blood pressure, and smoking. These variables can impact the choroidal parameters. Also, the retrospective nature limited the regular follow-up examination of the study group. In addition, the CT was measured manually. This method can still be influenced by the skills of the operator.

In conclusion, according to our results, CVI was decreased in patients with nAMD following IV aflibercept. Anti-VEGF agents can have a pharmacologic impact on the choroid, by decreasing the thickness and vascularity index. Though the clinical significance of the vascular alterations is still needed to be clarified, the CVI can be a helpful marker in monitoring the subjects receiving anti-VEGF treatment for nAMD. Future longitudinal studies are needed to approve the findings and to enlighten the long-term impact of aflibercept on choroidal structures.

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