Evaluation of Macular Perfusion in Healthy Smokers by Using Optical Coherence Tomography Angiography

Ziya Ayhan, MD; Mahmut Kaya, MD; Taylan Ozturk, MD; Omer Karti, MD; F. Hakan Oner, MD

BACKGROUND AND OBJECTIVE: To investigate the macular perfusion changes in healthy smokers by using optical coherence tomography angiography (OCTA).

PATIENTS AND METHODS: OCTA measurements were taken for the right eyes of 40 smokers (Group 1) and age- and sex-matched control cases (Group 2). Group 1 underwent OCTA scanning with the XR Avanti AngioVue OCTA (Optovue, Fremont, CA) at baseline and at 5 minutes, 30 minutes, and 60 minutes after one standard cigarette smoking. The same scanning protocol was applied without smoking in Group 2. Macular vessel area density, flow index of the choriocapillaris, foveal avascular zone (FAZ), and central macular thickness were evaluated in both groups.

RESULTS: Group 1 consisted of 28 men and 12 women with a mean age of 41.2 years ± 9.1 years (range: 24 years to 63 years). The mean age of Group 2 was 42.6 years ± 8.4 years (range: 21 years to 65 years), which consisted of 28 men and 12 women. The mean baseline vessel area density, flow index of the choriocapillaris, FAZ, and central macular thickness showed no statistically significant differences between Groups 1 and 2. Although the flow index of choriocapillaris values were similar at the repetitive OCTA measurements in the control group (P > .05), it was decreased from 1.94 ± 0.06 to 1.89 ± 0.08, 1.90 ± 0.08, and 1.91 ± 0.07 at 5 minutes, 30 minutes, and 60 minutes following smoking, respectively, in Group 1 (P < .001).

CONCLUSION: Smoking causes a significant decrease in the blood flow index of the choriocapillary area by the acute effects of nicotine and other chemical substances in cigarettes on peripheral vascular structure, as evaluated by OCTA.


INTRODUCTION

Acute and chronic effects of cigarette smoking on ocular circulation have been reported in the literature since the 1980s.1-3 Although mechanisms responsible for sympathetic activation by cigarette smoking in humans have been shown, hemodynamic changes associated with smoking on ocular blood circulation is not yet fully known.4

Ocular blood flow assessment techniques used in humans such as color duplex imaging, laser speckle flowgraphy, laser Doppler velocimetry, and flowmetry are well-documented and provide measurements of different segments of ocular blood flow.5-11

Recently, developments in optical coherence tomography (OCT) technology have provided clearer visualization of microvasculature retinal and choroidal vessels.12-13 This new technology, called OCT angiography (OCTA), allows for the noninvasive and rapid visualization of the retinal and choroidal vascular structures. Therefore, it provides convenience in the diagnosis and follow-up of chorioretinal vascular pathologies such as age-related macular degeneration, diabetic retinopathy, and vascular occlusions.14

Changes of human macular circulation with aging, topical and systemic medications, and caffeine are known to occur.15-18 Cigarette smoking directly causes alterations in retinal blood flow by the peripheral deleterious effect of nicotine.1-3 The acute effect of cigarette smoking on retinal macular blood flow was studied in healthy, habitual smokers using the blue field simulation technique in 1985 by Robinson et al.1 The effects of cigarette smoking on macular microvascular changes in healthy smokers has not yet been shown using OCTA. The aim of this prospective clinical study was to investigate the macular microvascular changes in habitual smokers by using OCTA.

PATIENTS AND METHODS

This study involved the right eyes of 40 chronic smokers (28 men, 12 women) (Group 1) and 40 age-
and sex-matched nonsmoker subjects (Group 2). Neither group had systemic or ocular diseases except for refractive error. This prospective, nonrandomized, interventional case series followed the principles outlined in the Declaration of Helsinki and was approved by the local research ethics committee of the Dokuz Eylul University School of Medicine.

All the study participants underwent detailed ophthalmologic examinations including best-corrected visual acuity assessment, slit-lamp biomicroscopy, intraocular pressure measurement with noncontact tonometry, and dilated fundus examinations. Subjects with refractive errors higher than 3 diopters (D) of myopia, 3 D of hyperopia, and 2 D of astigmatism were excluded from the study.

Smoking package year (number of cigarettes smoked per day × length of smoking history in years) was calculated for Group 1. Group 1 was asked not to

Figure. The flow index of the choriocapillaris at baseline and after 5 minutes, 30 minutes, and 60 minutes in the control group (A) and the smoking group (B).
smoke or consume caffeine and/or nicotine containing drinks or medications for at least 8 hours before the baseline OCTA measurement. Group 2 also underwent OCTA examination following at least 8 hours free of caffeine-containing drinks. All basal OCTA scans were performed at the same period of the day, in the morning (between 9:00 a.m. and 12:00 p.m.), to avoid diurnal fluctuations. This was followed by smoking one standard cigarette (nicotine, 1.3 mg; tar, 15 mg) for Group 1. Patients in Group 1 then underwent OCTA measurements repeated at 5 minutes, 30 minutes, and 60 minutes after the baseline scans. The same scanning protocol without smoking was applied to Group 2. Participants in Groups 1 and 2 were instructed not to consume caffeine- and/or nicotine-containing drinks or medications and not to smoke until the end of measurements.

Before imaging, each subject’s pupils were dilated with a combination of 0.5% tropicamide and 2.5% phenylephrine HCl. OCTA was performed in all participants with a scanning area of 3 mm × 3 mm. Spectral-domain OCTA (SD-OCTA) was performed with the XR Avanti AngioVue OCTA (Optovue, Fremont, CA) — a device with a high speed of 70,000 axial scans per second, using a light source of 840 nm and an axial resolution of 5 mm. The AngioVue OCTA system based on split-spectrum amplitude decorrelation angiography algorithm uses blood flow as intrinsic contrast. Indeed, the flow is detected as a variation over time in the speckle pattern formed by the interference of light scattered from red blood cells and adjacent tissue structure. Both eyes of each participant were examined and scanned within the same visit. Three-dimensional OCTA scans were acquired over 3 mm × 3 mm regions by using five repeated B-scans at 216 raster positions, each B-scan consisting of 304 pixels × 304 pixels in the transverse dimension. With a B-scan frame rate of 270 frames per second, each scan can be acquired in approximately 3.0 seconds. An internal fixation light was used to center the scanning area. The OCT signal position and signal quality were optimized by means of “Auto All” function, which performs in sequence the “Auto Z” to find the best position for obtaining the retina OCT image, the “Auto F” to find the best focus for the particular subject’s refraction, and the “Auto P” to find the best polarization match for the particular subject’s ocular polarization.

One FastX (horizontal raster) set and one FastY (vertical raster) set was performed for each acquisition scan. Each set took approximately 3 seconds to complete. After the completion of the FastX and FastY sets, the software performed the motion correction technology to remove saccades and minor loss of fixation. Scans with low quality were excluded and repeated until good quality was achieved. Three scans for each patient were captured, then the best one in quality (without significant motion artifacts and with a signal strength index of 60) was considered for analysis.

### TABLE 1

<table>
<thead>
<tr>
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<th>Smokers</th>
<th>Nonsmokers</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Vessel Density (%)</td>
<td>52.96 ± 2.23</td>
<td>53.12 ± 3.32</td>
<td>.278</td>
</tr>
<tr>
<td>*Flow Index of Choriocapillaris</td>
<td>1.94 ± 0.06</td>
<td>1.93 ± 0.04</td>
<td>.485</td>
</tr>
<tr>
<td>**FAZ (mm²)</td>
<td>0.30 ± 0.11</td>
<td>0.30 ± 0.17</td>
<td>.593</td>
</tr>
<tr>
<td>**Central Macular Thickness (µm)</td>
<td>254.2 ± 21</td>
<td>255.6 ± 22</td>
<td>.767</td>
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FAZ = foveal avascular zone; CMT = central macular thickness; * = Student’s t-test; ** = Mann-Whitney U test

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>5 Minutes</th>
<th>30 Minutes</th>
<th>60 Minutes</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Flow Index for the Choriocapillaris of the Control Group at Baseline and at 5, 30, and 60 Minutes</td>
<td>1.93 ± 0.04</td>
<td>1.93 ± 0.04</td>
<td>1.93 ± 0.04</td>
<td>1.93 ± 0.03</td>
<td>.26</td>
</tr>
</tbody>
</table>

*: Repeated one-way analysis of variance
Vascular retinal layers were visualized and segmented as previously described. The software then reconstructed the retinal and choroidal microvasculature in three dimensions with the ability to isolate microvasculature in specific layers of customized height in the retina and choroid. The automated retinal layer segmentation algorithm available on the device was used to analyze retinal microvasculature at two different levels: the superficial capillary plexus and the deep capillary plexus. Automatic segmentation individualizes the superficial capillary plexus 3 µm below the internal limiting membrane to 15 µm below the inner plexiform layer (IPL) and the deep capillary plexus 15 µm to 70 µm below the IPL. The outer retina is described as 70 µm below the IPL and 30 µm below the retinal pigment epithelium (RPE). The choriocapillaris is described as being between 30 µm and 60 µm under the RPE. Objective quantification of vessel density was evaluated for each eye using the split-spectrum amplitude decorrelation angiography software. Quantitative analysis was performed on the OCTA to capture en face images for each eye using the AngioVue software. Vessel density was defined as the percentage area occupied by vessels in a 3 mm × 3 mm square region of interest centered over the foveal avascular zone (FAZ). AngioVue software automatically outputs the flow area value within the region of interest. Flow index and vessel density can be determined from the en face maximum projection angiogram. The flow index is calculated as the average decorrelation value (which is correlated with flow velocity) in the selected region. The vessel density is calculated as the percentage area occupied by vessels and microvasculature in the selected region. Avascular area is a significant area (larger than the normal gap between capillaries) devoid of flow signal on an en face angiogram. On a retinal OCTA of the macula, the FAZ produces a normal avascular area. The FAZ is measured automatically by OCTA.

Statistical analyses were performed with SPSS version 17.0 (SPSS, Chicago, IL). Normality for continued variables in groups was determined by the Kolmogorov-Smirnov test. Student’s t-test was used for variables, which showed normal distribution. Mann-Whitney U test was used for variables that did not show normal distribution. To test for a significant difference in means over time, a repeated-measures ANOVA is used. For all tests, P values less than .05 were considered statistically significant.

RESULTS

Group 1 consisted of 28 men and 12 women with a mean age of 41.2 years ± 9.1 years (range: 24 years to 63 years). The mean age of Group 2, which consisted of 28 men and 12 women, was 42.6 years ± 8.4 years (range: 21 years to 65 years). The groups showed no significant difference by means of age and gender (P > .05). The mean smoking package year was 13.3 years ± 9.0 years (range: 1 year to 40 years). The mean baseline vessel area density, flow index of choriocapillaris, FAZ, and central macular thickness of Groups 1 and 2 showed no statistically significant difference (Table 1). Values of flow index

| TABLE 3 | Mean Flow Index for the Choriocapillaris at Baseline and at 5, 30, and 60 Minutes After Smoking (Group 1) |
| Baseline | After Smoking | P Value |
| 1.94 ± 0.06 | 1.89 ± 0.08 | 1.90 ± 0.08 | 1.91 ± 0.07 | <.001 |
| 5 Minutes | 30 Minutes | 60 Minutes |

* = Repeated one-way analysis of variance

| TABLE 4 | Correlation of Smoking Package Year With Flow Index of the Choriocapillaris, Vessel Density, and FAZ |
| Smoking Package Year | Flow Index of Choriocapillaris | Macular Vessel Density | FAZ |
| P Value | r | P Value | r | P Value | r |
| .100 | 0.264 | .156 | 0.229 | .260 | -0.183 |

FAZ = foveal avascular zone; r = Spearman’s correlation
of the choriocapillaris revealed no significant difference when comparing measurements at baseline and at 5 minutes, 30 minutes, or 60 minutes in the control group (Table 2). However; smoking caused a significant reduction in the flow index of the choriocapillaris at 5 minutes, 30 minutes, and 60 minutes compared with baseline in smoking group. The mean flow index of the choriocapillaris at baseline and at 5 minutes, 30 minutes, and 60 minutes following cigarette smoking in Group 1 are shown in Table 3. The Figure shows the flow index of the choriocapillaris at baseline and at 5 minutes, 30 minutes, and 60 minutes in the control group (A) and the smoking group (B).

Linear regression analyses were used to evaluate for effects of age and smoking package year on flow index of the choriocapillaris, vessel density, and FAZ. We found no statically significant difference between age and smoking package year with flow index of choriocapillaris ($P = .06$, $P = .242$) or vessel density ($P = .241$, $P = .838$). Although there was no statistical difference with smoking year and FAZ ($P = .358$), age had significant difference with FAZ ($P = .006$). Smoking package year is not correlated with flow index of choriocapillaris, vessel density, or FAZ (Table 4).

**DISCUSSION**

Impacts of cigarette smoking on the microcirculation include compromised endothelial-dependent vasorelaxation, platelet aggregation, and endothelial cell dysfunction. These changes result in vascular blood flow alterations by causing vasodilatation or vasoconstriction. Deflecterious effects of cigarette smoking on the microcirculation were investigated in human eyes because of having dense microvascular bed readily assessable to noninvasive techniques. Rojanapongpun and Drance found a reduction of ophthalmic artery circulation in response to cigarette smoking by using transcranial doppler ultrasound. Morgado et al. evaluated the effect of smoking on retinal blood flow and autoregulation in smokers with and without diabetes by using laser doppler velocimetry and retinal photography. They showed that smoking reduces retinal blood flow and the ability of the retinal vessels to autoregulate to hyperoxia; these effects are likely to be due to the vasoconstrictive effect of nicotine, which is mediated through activation of the sympathetic system. Robinson et al. studied the acute effect of smoking on macular capillary blood flow using a blue field simulation technique. Velocity of leukocyte flowing through their own macular capillaries was quantified in this procedure. In contrast to other studies, they found that cigarette smoking resulted in a significant increase in macular leukocyte velocity and blood flow. Similarly, increased blood velocity in visible surface vessels of the optic nerve head and choroid-retina were confirmed in habitual smokers by using laser speckle technology in another study. The variation between individuals in the blood flow response to smoking might possibly reflect different levels of nicotine absorption and/or a difference in end-organ responsiveness to the combination of chemicals in tobacco smoke.

The enhanced depth imaging (EDI) mode of the Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany) has been used to evaluate the choroidal thickness since last decade. Effects of smoking and nicotine on choroidal thickness were shown by using EDI OCT in the literature. Ulas et al. found nonsignificant differences for the retinal thickness, choroidal thicknesses, spherical equivalent, intraocular pressure, and central corneal thickness between the smokers and nonsmokers. However, in the measurements taken after smoking, a statistically significant increase was found in choroidal thickness that was observed between 0 minutes and 5 minutes for the central, nasal, and temporal segments. Sizmaz et al. observed that the mean choroidal thickness at the fovea prior to smoking was 301.1 µm ± 63.1 µm, which decreased to 284.2 µm ± 56.7 µm at 1 hour and 270.8 µm ± 80.0 µm at the third hour following smoking. Zengin et al. investigated the effect of nicotine on choroidal thickness by using EDI OCT. They found that nicotine caused a significant decrease in choroidal thickness following oral intake and explained this acute decrease as a result of reduced ocular blood flow due to the vasoconstrictive effect of nicotine. Although EDI OCT helps us evaluate the choroidal region, ratings of blood circulation are made indirectly by measuring choroidal thickness. Besides, measurement of choroidal thickness is performed manually.

OCTA is a new, noninvasive imaging technology that can visualize the retinal and choroidal vasculature. It allows evaluating the retinal vasculature layer by layer and also permits one to obtain images of the deep capillary plexus without dye injection. OCTA also offers a more precise visualization of the superficial capillary plexus thanks to the absence of overlapping with the underlying vascular layers. It seems more sensitive than fluorescein angiography in detecting macular microangiopathy in asymptomatic patients. Also, measurements are made automatically by the software of the system.

In this study, we evaluated the acute and chronic effects of smoking on macular microvascular struc-
ture by using OCTA. Although smoking caused a significant decrease on macular blood flow at the acute stage, we did not find a significant difference in macular blood flow, vessel density, or FAZ measurements between the study and control groups in a chronic period. These results confirmed that smoking causes hypoxia at macula at the acute stage.

There are several limitations to our study. One is that we did not restrict the exercise in both groups. The exercise might have affected the choroidal and retinal blood flow in both groups and we didn’t know how its reflection would be on OCTA measurements. Also, we did not evaluate the blood-nitric oxide levels in the study group. The other limitation of our study is the limited number of cases.

Besides these limitations, to the best of our knowledge, this is the first study to investigate the acute and chronic effects of smoking on macular vascular structure by using OCTA. We believe that the results of this study will be useful in further studies about this issue.

REFERENCES