Original Article

Retinal and Choroidal Vascular Changes in Eyes with Pseudoexfoliation Syndrome: a Comparative Study Using Optical Coherence Tomography Angiography

Çınar et al. Vascular Changes in Eyes with Pseudoexfoliation

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Background: Optical coherence tomography angiography (OCTA) allows the detailed evaluation of retinal and choroidal microvascular structures with no need for a contrast agent. Pseudoexfoliation (PEX) is a condition that leads to anatomical and functional losses due to the accumulation of degraded abnormal fibrillar material in the intraocular and extraocular tissues. Histopathological studies have shown that the accumulation of PEX material in vascular structures may play a role in different pathologies such as retinal vein occlusion, iris hypoperfusion, anterior segment hypoxia, retinal arterial occlusion, and neovascular glaucoma.

Aims: To evaluate and compare flow and vascular density in the retina and choroid in eyes with pseudoexfoliation (PEX), non-PEX fellow eyes, and healthy eyes using optical coherence tomography angiography (OCTA).

Study Design: Cross-sectional case control study

Methods: The study included 35 PEX eyes of 35 PEX patients, 32 non-PEX fellow eyes of 32 unilateral PEX patients, and 35 eyes of healthy control subjects. Flow area and vessel density (VD) in the superficial capillary plexus (SCP) and deep vascular plexus (DCP) were measured by OCTA as three separate parameters: total, parafoveal, and foveal. Choroidal thickness (CT) and foveal avascular zone (FAZ) area were measured for each patient.

Results: There were significant differences between the PEX eyes and control eyes in total, parafoveal, and foveal flow and VD in the SCP (P<0.05 for all), while there were no significant differences between these groups in any of the flow or VD values in the DCP (P>0.05). None of the SCP and DCP flow and VD values showed significant differences between PEX eyes and non-PEX fellow eyes or between fellow eyes and control eyes (P>0.05). CT was significantly lower in PEX eyes compared to control eyes. FAZ area was significantly enlarged in PEX eyes compared to control eyes in both the superficial and deep layers (P<0.05).

Conclusion: PEX eyes have significant damage to the retinal and choroidal vascular structures associated with PEX material.

Keywords: Optical coherence tomography angiography, pseudoexfoliation, retinal vascular structure

Although retinal veins can be directly visualized, measuring retinal flow noninvasively was not possible before the development of Doppler techniques.1 Optical coherence tomography angiography (OCTA) is a new imaging modality that measures on red blood cell velocity. OCTA allows the detailed evaluation of retinal and choroidal microvascular structures with no need for a contrast agent. To date, OCTA has been used in various retinal vascular pathologies such as diabetic retinopathy, age-related macular degeneration, retinal vein occlusion, macular telangiectasia, and choroidal neovascularization.2

Pseudoexfoliation (PEX) is a condition that leads to anatomical and functional losses due to the accumulation of degraded abnormal fibrillar material in the extracellular spaces of intraocular and extraocular tissues. Although it
is frequently associated with glaucoma, the accumulation of PEX material in the retinal vasculature is known to cause vasculopathy as well as disrupt perfusion by damaging the walls of the posterior ciliary arteries and vortex vein.3-5 Histopathological studies have shown that the accumulation of PEX material in vascular structures may play a role in different pathologies such as retinal vein occlusion, iris hypoperfusion, anterior segment hypoxia, retinal arterial occlusion, and neovascular glaucoma.6-8

In the present study, we sought to identify PEX-related changes in the retinal vasculature by evaluating retinal and choroidal flow and vessel density using OCTA in eyes with biomicroscopically detected PEX material and comparing them with the non-PEX fellow eyes of these patients and a healthy control group.

**MATERIALS AND METHODS**

This Cross-sectional study included 35 patients (19 females, 16 males; mean age 69.8±6.9 years) diagnosed with PEX and 35 healthy individuals (18 females, 17 males; mean age 68±5.5 years) with no known ocular disease. Three of the 35 PEX patients had bilateral involvement, while the other 32 had unilateral involvement. Therefore, the eyes were divided into 3 groups: eyes with PEX (35 eyes), non-PEX fellow eyes of unilateral PEX patients (32 eyes), and healthy control eyes (35 eyes). For the bilateral PEX patients and healthy subjects, one eye was randomly selected for the study. When both eyes had PEX and healthy subjects, random number tables were used to select the eye for analysis (odd number= left; even number= right).

All patients were informed about the study in accordance with the Declaration of Helsinki and provided informed consent. The study was approved by the ethics committee (ethics committee no:5-9). All patients had visual acuity of 1.0 according to Snellen chart, and slit-lamp anterior segment and dilated fundus examinations were normal. In order to avoid affecting OCTA measurements, all participants selected for the study had spherical refractive error values less than or equal to ±3.00 diopters (D) and cylindrical values less than or equal to ±3 D. Exclusion criteria included history of ocular surgery, presence of systemic disease (diabetes mellitus, hypertension, etc.), use of systemic medication or eye drops other than artificial tears, and presence of corneal opacity or cataract higher than grade 1 in severity. Those with grade 1 cataract and those using tear eye drops were included in the study.

All OCTA measurements and imaging procedures were performed with the pupil dilated, using an AngioRTVue XR (Optovue Inc., Freemount, CA) with version 2015.1.1.98 software, by a qualified technician who was trained on the equipment.

**OCTA imaging**

An area map of 6 x 6 mm was used in all images. Optovue AngioVue system technology allows for quantitative analysis. The inner boundary for SCP (superficial capillary plexus) was assumed as 2.6μm below the internal limiting membrane and the outer boundary 15.6μm below the inner plexiform layer, the inner boundary was 15.6μm and the outer boundary was 70.2μm below the inner plexiform layer for DCP (Deep capillary plexus). (Figure 1A, B). The area 15.6–70.2 microns from the retinal surface was considered the deep vascular plexus (DCP) and its flow was also measured. For each patient, the area of the central macula with no detected vascular structures was automatically measured and recorded as the foveal avascular zone (FAZ) (Figure 1D). The DCP starts from the internal limiting membrane and extends to the retinal pigment epithelium (RPE). For measurements of the choroidal layer, the RPE was used as a reference point and the areas under RPE were measured (Figure 1C). Flow and vessel density (VD) in the SCP and DCP were evaluated as three different parameters: total, parafoveal, and foveal. A total of 5 choroidal thickness (CT) measurements were obtained by manually measuring at the fovea (subfoveal) and at distances of 500 and 1000 μm nasal and temporal to the fovea. Retinal thickness was also measured at the fovea.

To avoid diurnal fluctuations in CT, measurements were performed in the same time interval (10:00 am–12:00 pm) for all participants. Only those with good image quality (>72 of 100) were included in the study.

**STATISTICAL ANALYSIS**

We calculated the number of patients needed to obtain the desired effect size. Taking into account the results of a previous study that detected a significant difference in mean peripapillary capillary density between PXG and POAG using Angiovue OCTA, and assuming an α error of 0.05% and power of 85%, the required sample size was calculated as 32 patients per group. In this study, one eye per subject was enrolled. Continuous variables are described as mean and standard deviation (SD). The distribution of numerical data was tested for normality using the one-sample Kolmogorov–Smirnov test. Significant differences between continuous variables across groups were tested with one-way analysis of variance (ANOVA) with Bonferroni post hoc analysis. SPSS software (ver. 16.0 for Windows; SPSS Inc., Chicago, IL, USA) was used for all analyses. A p value <0.05 was considered statistically significant.

**RESULTS**

There was no difference in age or sex ratio between the PEX patient group and control group (age; p=0.814, sex; p=0.943). Comparisons of the PEX patients’ affected eyes and unaffected fellow eyes and the healthy control eyes are shown in Table 1.

PEX eyes had significantly lower total, parafoveal, and foveal SCP flow compared to healthy control eyes (p<0.05, for all), but there were no differences in these parameters when compared with the non-PEX fellow
eyes (p>0.05, for all). Total, parafoveal, and foveal VD in the SCP were also significantly reduced in PEX eyes compared to healthy control eyes (p<0.05, for all), while no significant differences were detected compared to fellow eyes (p>0.05, for all). Details are shown in table 1. The box plot analysis representing the superficial and deep capillary plexus vessel density in the PEX patients and control subjects are shown in figure 2.

The PEX eyes showed significantly lower flow in the total, parafoveal, and foveal area in DCP when compared to control eyes (p<0.05, for all) but there was no significant difference in flow compared to the fellow eyes in any of the areas (p>0.05, for all). In addition, VD in the total, parafoveal, and foveal area in DCP was significantly lower in PEX eyes compared to healthy eyes (p<0.05, for all), whereas no significant differences emerged between PEX eyes and non-PEX fellow eyes (p>0.05, for all) Details are shown in table 1. PEX eyes exhibited significant FAZ enlargement in both the SCP and DCP compared to the non-PEX fellow eyes and healthy control eyes (p<0.05, for all).

There were no significant differences between the groups in terms of subfoveal retinal thickness (p>0.05, for all). Subfoveal CT did not differ statistically between PEX and fellow eyes (p=0.413), but was significantly lower in PEX eyes when compared with healthy control eyes (p=0.021).

**DISCUSSION**

PEX is characterized by the accumulation of abnormal fibrillar material in the extracellular matrix of the ocular tissues, which has been shown to cause clinically observable pathologies such as glaucoma, zonular weakness, and iris atrophy, as well as vascular dysfunction due to its accumulation on vessel walls, which is not directly observable.4,5

In the present study, OCTA measurements demonstrated significant decline in superficial retinal blood flow and vessel density in eyes with PEX compared to healthy eyes. Although significant disruption in deep retinal flow and deep vessel density was not observed, enlargement of the FAZ in both superficial and deep retinal layers supports the existence of a PEX-related vascular pathology.

The role of PEX in the retinal vasculature has been debated for many years. A retrospective study evaluating 332 patients with branch retinal vein occlusion (BRVO) and 159 patients with central retinal vein occlusion (CRVO) revealed PEX material in 6% of the BRVO patients and 6.9% of the CRVO patients, providing important evidence that PEX material creates a tendency for vascular thrombosis.2 Yüksel et al.10 used color Doppler ultrasound to measure peak systolic and diastolic flow in the ophthalmic artery, central retinal artery, short posterior ciliary arteries, and temporal ciliary arteries of 14 eyes with PEX glaucoma, 14 PEX eyes without glaucoma, and 14 healthy eyes, and reported a significant reduction in blood flow in eyes with PEX regardless of the presence of glaucoma. Ocakoglu et al.11 compared 22 eyes with unilateral PEX with the patients’ fellow eyes and 22 healthy eyes and found that both PEX and non-PEX fellow eyes had significantly disrupted flow in the optic nerve head and peripapillary area when compared with the healthy control eyes. Studies have not only demonstrated that PEX material is correlated with vascular dysfunction, but have also identified it as an independent risk factor.6 In a recent study comparing ophthalmic arterial hemodynamics and vascular resistance in eyes with PEX and healthy eyes, Kocatürk et al.12 reported an increase in vascular resistance and disruption of hemodynamic parameters in PEX eyes. The results of our study corroborate previous studies demonstrating retinal blood flow abnormalities in PEX eyes.8,10-12

In a histological study conducted using electron microscopy, comparison of the posterior segment vascular structures in 120 eyes enucleated due to CRVO-related complications and 107 eyes enucleated due to melanoma, PEX material was detected in 10% of the CRVO eyes and 1.9% of the melanoma eyes, and the authors indicated that PEX may play a role in vascular pathologies.13 In another recent study, Karagiannis et al.14 detected PEX material in 29.17% of eyes with CRVO and 8.5% of eyes with BRVO, showing that these rates were higher than in healthy eyes. Moreover, they proposed that the high amount of PEX material in eyes with CRVO may be an independent risk factor in the etiology of CRVO.14

Park et al.15 compared peripapillary optic nerve VD and retinal nerve fiber layer (RNFL) thickness in 39 eyes with PEX glaucoma and 39 eyes with primary open-angle glaucoma (POAG). Their study showed that although peripapillary VD was significantly lower in patients with PEX, there was no significant difference between the two groups in terms of RNFL thickness. The authors stated that in addition to glaucoma, PEX material may have precipitated an ischemic event in the peripapillary area due to the damage it caused, particularly in the endothelium of the small vessels. Kromer et al.16 evaluated macular VD and flow in 30 individuals with POAG and 21 healthy individuals using OCTA. Global and nasal VD were reduced in POAG patients compared to the healthy individuals, but no difference was observed in terms of flow. However, they did not perform an additional subgroup analysis between eyes with PEX glaucoma, eyes with other types of glaucoma, and healthy eyes. The results from Park, Kromer, and our study suggest that although having POAG alone causes a decrease in both macular and peripapillary vascular density, the presence of PEX material in addition to POAG also leads to flow disruption. This may be a result of the PEX material inducing ischemia, as stated by Park. Our finding that PEX material is an independent risk factor supports the study by Park et al.15

The superficial retinal vascular plexus is closely related to the ganglion cell layer and has a critical role in supplying it. Eltutar et al.17 compared OCT findings in 45 eyes with PEX and 29 healthy non-PEX eyes and
demonstrated that the ganglion cell layer and superficial retinal layer were significantly thinner in PEX eyes, and that this thinning may be an early sign of PEX glaucoma in patients with PEX. The reduced flow and VD in the superficial retinal layers observed in our study are important findings that may explain the thinning of the ganglion cell layer reported by Eltutar et al.

Another finding of our study was significant enlargement of the FAZ measured in both the superficial and deep layers compared with healthy individuals. Freiberg et al.18 compared FAZ area in the superficial and deep layers in 29 patients with diabetic retinopathy and 25 healthy eyes using OCTA and found that eyes with diabetic retinopathy had significant FAZ enlargement and irregularity in both the superficial and deep layers. The authors emphasized the importance of altered FAZ area in diseases of vascular etiology. Wons et al.19 indicated that visual acuity was associated with FAZ diameter in patients with CRVO and BRVO and that destruction of vascular pathologies in FAZ may be an important indicator. The FAZ enlargement shown in our study supports the correlation between FAZ and vascular dysfunction demonstrated by Freiberg and Wons. Assessing FAZ with OCTA may be useful for identifying PEX-associated vasculopathy or as an indicator of ocular tissue damage due to PEX, and further studies on this subject are needed.

Our comparison of PEX eyes, non-PEX fellow eyes, and healthy eyes revealed no significant differences in the deep vascular plexus in the outer plexiform layer. Although our data indicate that both flow and vascular density in the deep vascular plexus were affected by PEX, these differences did not reach statistical significance. PEX is known to generally show unilateral involvement, although it can also appear in the fellow eye in later years.20 Histopathologic studies using electron microscopy have demonstrated that a certain amount of PEX material is present in the conjunctiva, iris, and especially the iris dilator muscles of eyes in which PEX involvement is not detected biomicroscopically.21 In addition, clinical studies have clearly demonstrated vascular dysfunction in fellow eyes with no apparent PEX material.18,20 Both clinical and histological studies have shown that although PEX material may appear biomicroscopically unilateral, it is actually present in the fellow eye at a microscopic level. We also observed lower retinochoroidal flow and vascular density in the non-PEX fellow eyes of PEX patients in the current study. Although the differences were not statistically significant, our findings support the possibility of undetected PEX material in apparently unaffected fellow eyes.

In this study we observed no significant differences among the eyes in terms of retinal thickness at the fovea. However, there was a significant reduction in mean CT in the PEX eyes compared with the healthy eyes. Demircan et al.25 compared CT in 43 eyes with PEX glaucoma, 45 PEX eyes without glaucoma, and 48 healthy eyes using enhanced depth imaging (EDI)-OCT. They demonstrated significant choroidal thinning in eyes with PEX material regardless of the presence of glaucoma, and stated that this may be due to ischemia in the choroidal vessels caused by PEX material.25 Eroğlu et al.23 also demonstrated significant choroidal thinning in the eyes of patients with bilateral PEX compared to healthy eyes and eyes with unilateral PEX. In the same study, they showed that patients with unilateral PEX also had significant choroidal thinning compared to the healthy individuals, indicating that PEX material significantly affects CT. Our findings of reduced CT in eyes with PEX suggest that this may be due to changes in choroidal blood flow resulting from existing vasculopathy, which both confirms the choroidal thinning observed in previous studies and sheds light on its possible causes. However, more data are needed on this subject.

Rebolleda et al.9 compared peripapillary retinal nerve fiber layer thickness and peripapillary capillary density values obtained by two different OCTA devices in 20 eyes with PEX glaucoma, 20 eyes with primary open-angle glaucoma, and 20 healthy eyes. They reported that only Angiovue detected significantly lower capillary density in PEX glaucoma compared to primary open-angle glaucoma at similar levels of glaucoma damage, while both Angiovue and Angioplex demonstrated decreased capillary density in glaucoma eyes compared to healthy eyes. Zengin et al.26 evaluated the relationship between age-related macular degeneration (AMD) and clinically unilateral PEX syndrome and showed that PEX was associated with a lower prevalence of wet AMD. As demonstrated in both glaucoma and AMD studies, PEX material may be involved in the etiopathogenesis of these conditions. Histopathologic studies are needed to clarify the causal relationship between PEX material and retinochoroidal diseases.

It has been shown that PEX patients are prone to thrombosis and vascular pathologies because they have higher blood homocysteine levels compared to healthy individuals, and that PEX biomarkers such as amyloid β peptide accumulate and lead to dysfunction in various tissues.25,26 It has also been suggested that increased levels of various local mediators of vasoconstriction, such as endothelin-1, and decreased aqueous levels of strong mediators of vasodilatation, such as nitric oxide, may play a role in vascular occlusion in eyes with PEX.25,26 In our study, both flow and vascular density were reduced. All of these findings indicate that PEX patients have multiple risk factors, and the decrease in retinochoroidal flow may not be solely attributable to PEX material, but intermediate mediators may also be involved.

Limitations of our study are that it included a small number of patients and was cross-sectional in design. Data from patients with PEX can be better demonstrated through longitudinal studies. In addition, there is potential for human error in manual CT measurement. Strengths of our study are the use of OCTA, which enables a rapid
and noninvasive measurement of vascular flow, and the inclusion of a control group of healthy eyes for comparison. In conclusion, we believe that the decrease in retinal and choroidal vascular density observed in our study may help elucidate the pathophysiology of glaucoma and other retinochoroidal diseases associated with PEX. Future studies investigating systemic (e.g. homocysteine, amyloid beta peptide) and local factors (e.g. endothelin, lysyl oxidase-like 1, nitric oxide) together with OCTA evaluation of retinochoroidal circulation may further advance our understanding of the pathophysiology of PEX.

REFERENCES
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<th>PEX EYES No:35</th>
<th>FELLOW EYES No:32</th>
<th>CONTROL EYES No:35</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
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<tr>
<td>Superficial retinal flow area (mm²)</td>
<td>13.478±0.22</td>
<td>15.307±0.64</td>
<td>16.245±0.51</td>
<td><strong>0.011</strong></td>
<td>0.071</td>
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<td>Deep retinal flow area (mm²)</td>
<td>13.284±0.21</td>
<td>13.814±0.26</td>
<td>13.912±0.64</td>
<td>0.775</td>
<td>0.603</td>
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<td>CC flow area (mm²)</td>
<td>16.483±0.37</td>
<td>17.745±0.54</td>
<td>18.274±0.27</td>
<td><strong>0.022</strong></td>
<td>0.074</td>
<td>0.242</td>
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<td>FAZ area (Superficial, mm²)</td>
<td>0.384±0.011</td>
<td>0.351±0.018</td>
<td>0.312±0.021</td>
<td><strong>0.010</strong></td>
<td>0.284</td>
<td>0.537</td>
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<td>FAZ area (Deep, mm²)</td>
<td>0.375±0.014</td>
<td>0.332±0.010</td>
<td>0.319±0.027</td>
<td><strong>0.013</strong></td>
<td>0.344</td>
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**Superficial vessel density (%)**

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<td>Fovea</td>
<td>32.154±1.44</td>
<td>34.039±1.37</td>
<td>34.445±1.23</td>
<td><strong>0.026</strong></td>
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<td>Parafoveal</td>
<td>55.610±1.92</td>
<td>56.751±1.33</td>
<td>57.847±1.84</td>
<td><strong>0.013</strong></td>
<td>0.102</td>
<td>0.228</td>
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<td>Total</td>
<td>54.935±0.32</td>
<td>56.838±1.85</td>
<td>56.974±1.52</td>
<td><strong>0.014</strong></td>
<td>0.773</td>
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**Deep vessel density (%)**

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<td>Fovea</td>
<td>34.651±0.64</td>
<td>35.072±0.37</td>
<td>35.143±0.51</td>
<td>0.573</td>
<td>0.202</td>
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<tr>
<td>Parafoveal</td>
<td>56.946±1.39</td>
<td>57.369±0.51</td>
<td>57.854±0.93</td>
<td>0.096</td>
<td>0.363</td>
<td>0.408</td>
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<tr>
<td>Total</td>
<td>52.993±1.52</td>
<td>58.193±1.06</td>
<td>58.336±1.83</td>
<td>0.312</td>
<td>0.274</td>
<td>0.295</td>
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**Central Foveal thickness**

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<td>243.112±5.3</td>
<td>249.279±4.8</td>
<td>249.937±5.4</td>
<td>0.777</td>
<td>0.734</td>
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**Subfoveal choroidal thickness**

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<td>301.094±9.8</td>
<td>308.042±10.4</td>
<td>322.957±10.7</td>
<td><strong>0.031</strong></td>
<td>0.077</td>
<td>0.049</td>
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CC: Choriocapillaris, FAZ: Foveal avascular area. P1 value is shown to compare PEX eyes and control eyes. P2 value is shown to compare PEX eyes and fellow eyes. P3 value is shown to compare fellow eyes and control eyes.
Figure 1. Macular perfusion parameters of a 6 mm×6 mm angiography scan size using OCTA. A. The flow area within a 3-mm radius is represented by the color yellow. B. The vessel density of five areas of interest, including the fovea (1-mm diameter) and temporal, inferior, nasal, and superior quadrants (1-mm annular ring); (C) the choroidal capillary flow area within a 3-mm radius is represented by the color yellow; (D) the FAZ is automatically delineated using the included software and represented by the color yellow.

Figure 2. The box plot analysis representing the superficial and deep capillary plexus vessel density in the PEX patients and control subjects.