Int Ophthalmol (2019) 39:2749–2755
https://doi.org/10.1007/s10792-019-01119-3

ORIGINAL PAPER

Reduced vascular perfusion density in idiopathic epiretinal membrane compared to macular pseudohole

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Received: 31 January 2019 / Accepted: 22 May 2019 / Published online: 27 May 2019

Abstract
Purpose To investigate the retinal capillary perfusion density by means of optical coherence tomography angiography (OCT-A) in idiopathic epiretinal membrane (ERM) and macular pseudoholes (MPH).
Methods This observational cross-sectional study examined consecutive patients affected by ERM and MPH presenting between June 2017 and December 2017, as well as the 30 eyes of 30 healthy subjects. All patients underwent swept-source OCT-A examination. For each patient, vessel perfusion density and foveal avascular zone (FAZ) area were measured.
Results Twenty-five eyes of 20 patients with ERM and 28 eyes of 24 patients with MPH were enrolled. Thirty eyes of 30 age-matched healthy controls were included. The perfusion density in the superficial capillary plexus (SCP) of ERM (0.401 ± 0.012) turned out to be inferior that MPH (0.419 ± 0.018) and controls (0.415 ± 0.017) ($p < 0.01$), while no significant differences were evident among the three subgroups in the deep capillary plexus (DCP) ($p = 0.1$). The FAZ area in the SCP was smaller in the ERM group (0.168 ± 0.123 mm^2), respectively, than MPH (0.295 ± 0.013 mm^2) and controls (0.213 ± 0.107 mm^2) ($p < 0.01$), otherwise no difference were noted in the DCP ($p = 0.14$).
Conclusions OCT-A morphological features differ between idiopathic ERM and MPH, showing lower perfusion density in idiopathic ERM compared to MPH.

Keywords Epiretinal membrane · Macular pseudohole · Optical coherence tomography angiography · Perfusion density

Introduction

Epiretinal membranes (ERMs) and macular pseudoholes (MPHs) are both common types of vitreoretinal interface disorders. Epiretinal membrane consists of a fibrocellular proliferation found at the retinal surface, while MPH has been traditionally defined as a macular alteration ophthalmoscopically assuming the appearance of a macular hole, without the loss of foveal tissue [1]. The International Vitreomacular Traction Study (IVTS) group recently proposed a new macular hole classification, providing a specific MPH definition as a condition of: invaginated or heaped foveal edges, the presence of a concomitant ERM with central opening, steep macular contour to the central fovea with near-normal central foveal thickness, and no loss of retinal tissue [2].

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Epiretinal membrane and MPH share similar pathogenic pathways and probably represent two sides of the same spectrum of disease [3]. The contractile properties of ERM may indeed exert traction on the retina, leading to visual changes and metamorphopsia [4]. However, if the contraction process occurs around the fovea, it may contribute to MPH development. Since recent evidences, by mean of optical coherence tomography angiography (OCT-A), highlighted that the retinal microvascular networks are also involved by these traction forces [5–7], vessels morphology and function might be differently impaired in EMRs and MPHs.

This study aims to gain new insights into the vascular abnormalities associated with ERMs as compared to MPHs by means of a quantitative OCT-A analysis.

Methods

In this single-center observational cross-sectional study, we used swept-source OCT (SS-OCT) and OCT-A to compare the foveal avascular zone (FAZ) area and the perfusion density of the superficial capillary plexus (SCP) and deep capillary plexus (DCP) in eyes with idiopathic ERM and MPH. Data were then compared with an age- and sex-matched control group. We certify that every applicable institutional and governmental regulation concerning the ethical use of human volunteers was followed during this study. All patients gave written general informed consent to take part in observational studies approved by the Institutional Review Board of the San Raffaele Scientific Institute. The study was carried out in accordance with the ethical standards laid down in the 1964 Helsinki Declaration.

Patient selection

All patients fulfilling the inclusion/exclusion criteria were consecutively recruited at the Vitreoretinal Surgery Service of the Ophthalmology Department, San Raffaele Scientific Institute between June and December 2017.

Inclusion criteria were age $\geq$ 18 years, axial length between 22.5 and 24.5 mm (measured with IOL Master, Zeiss), and a diagnosis of idiopathic ERM or MPH. Diagnosis was obtained using biomicroscopy and was confirmed with SS-OCT. Only ERM in stage 1–3 was included, according to Govetto et al. classification [8]. MPH was defined as abovementioned, according to the IVTS group classification [2].

Exclusion criteria for the study eyes were ERM in stage 4, any concurrent ocular disease other than ERM of MPH involving the posterior segment (*e.g.,* uveitis, retinal vein occlusion, glaucoma, optic neuropathy, diabetic retinopathy, age-related macular degeneration), any previous posterior segment surgical or laser treatment, cataract surgery within the past 6 months, past complicated cataract surgery, and any previous intravitreal injection of anti-VEGF agents or corticosteroids. Eyes with optical media opacities that could interfere with good-quality imaging acquisition were also excluded. Patients with a history of arterial hypertension, diabetes mellitus, systemic vasculopathies (*e.g.,* vasculitides), or connective-tissue disease were also excluded from the study.

Study protocol

Patients underwent complete ophthalmic examination, including best-corrected visual acuity (BCVA) on Early Treatment Diabetic Retinopathy Study (ETDRS) charts, anterior segment biomicroscopy, applanation tonometry, indirect fundus examination, and SS-OCT and OCT-A scans of the macula (OCT-A; DRI OCT Triton, Topcon, Inc., Tokyo, Japan). BCVA was converted into the logarithm of the minimal angle of resolution (logMAR) for the sake of calculations.

We also enrolled a group of healthy subjects as controls. Inclusion criteria were a silent ophthalmic pathological history, BCVA of 20/20 or better, axial length of 24 $\pm$ 1 mm, normal optic nerve with no neuroretinal rim alterations, anterior chamber with open angle, normal fundus biomicroscopy examination, SD-OCT scan within normal limits, and no previous surgery other than uncomplicated cataract extraction and intraocular lens (IOL) implantation.

OCT measurements

We obtained SS-OCT and OCT-A scans with DRI OCT Triton, Topcon Corporation, Japan.

Mean foveal thickness (mFT, defined as the mean retinal thickness within the central 1-mm-diameter area, which corresponds to the central circle on the

ETDRS map) and the diameter of MPH were registered.

Macular OCT-A was acquired in the 3 × 3 mm macular area. Each examination was carried out by a single experienced masked operator (AM). We used only automatically segmented images of SCP and DCP to prevent any subjective influence during the final analysis.

All 3 × 3 mm OCT-A images were exported from the OCT database as a Joint Photographic Experts Group (JPEG) file and then transferred to ImageJ 1.48 software (National Institutes of Health, Bethesda, Maryland, USA) for calculation purposes.

To calculate the vessel density coefficient, images were binarized through a threshold strategy as in previous studies [9–11]. We generated a novel macro that automatically: (i) converts the image from 8-bit into red green blue (RGB) color type; (ii) splits it into the three channels (red, green, and blue) keeping the red one open as a reference; (iii) applies a mean threshold to convert the image from gray scale to binary; (iv) converts the processed images back to RGB; (v) restores the FAZ area and colors it with pure blue. White pixels were considered as vessels, black pixels as the background, and blue pixels were automatically excluded from the analysis (Fig. 1). Vessel density was expressed as the ratio between measured vessel pixels and the total 3 × 3 mm area after subtracting the FAZ area. We used this method to analyze the SCP and DCP of patients and healthy controls. Since any segmentation error would be a significant pitfall for the purpose of this study, the same operator checked each B-scan of every OCT-A acquisition to avoid any rough or subtle segmentation errors, and all inaccurately segmented examinations were excluded. Only good-quality and correctly segmented images were included in the analysis.

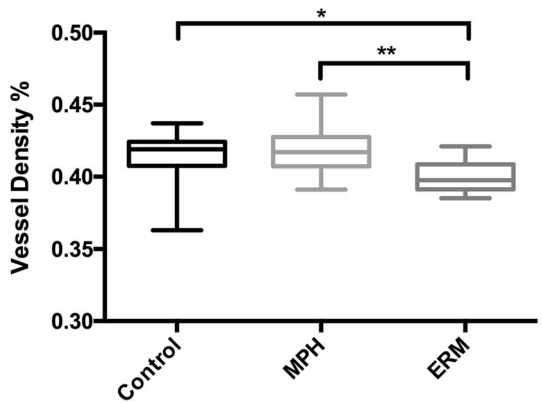

Fig. 1 Perfusion density in the superficial capillary plexus (SCP) of controls, macular pseudoholes (MPH), and idiopathic epiretinal membranes (ERM)

The FAZ area in the SCP was manually outlined with a polygon selection tool, and its area (in mm^2) was calculated using a previously described method [9].

Morphological features of the macula were also registered, including intraretinal fluid (judged as present of absent) and the status of the ellipsoid zone (EZ) (judged as regular, whether the hyper-reflective line was uninterrupted and homogeneous, or irregular).

Statistical analysis

Variables included in the analysis were: age, sex, eye (right/left), BCVA, FAZ area in the SCP, and vessel density. All data are presented as mean ± standard deviation. All variables were tested for normal distribution using the D'Agostino-Pearson test. Differences between groups for age, BCVA, FAZ area, and vessel density in every layer were assessed with one-way analysis of variance (ANOVA), and Duns correction was used as a post hoc test. Differences between sex were assessed using the Chi-square test. Statistical analysis was carried out with GraphPad Prism software 6.0 (GraphPad Software, San Diego, California, USA). All tests were two-sided; p values < 0.05 were considered significant. All results are presented as average value ± standard deviation.

Results

We enrolled 56 eyes of 44 patients that fulfilled the inclusion/exclusion criteria. Three eyes were excluded, two for optical media opacities, and one due to impaired glucose tolerance pending investigation. Hence, we finally considered 53 study eyes. Twenty-five eyes of 20 patients had idiopathic ERM, while 28 eyes of 24 patients had MPH. Thirty eyes of 30 age- and gender-matched healthy controls were included.

Intraretinal fluid was never found in any of the investigated eye, whereas EZ irregularities were found in few eyes, which rate was similar between ERM and

MPH. Macular pseudohole average diameter was 289 ± 56 μm. Both ERM and MPH disclosed similar mFT, which was higher than controls. Similarly, both study eye groups showed similar BCVA, which was inferior to controls. Demographic and morphological data are summarized in Table 1. All continuous studied variables showed normal distribution.

All patients had disturbing metamorphopsia, which were reflected in distorted and nonuniform lines in the Amsler grid test. Average symptoms duration was 6.4 ± 2.1 months before surgery.

The perfusion density varied greatly among the three subgroups in the SCP ($p < 0.01$). In the post hoc analysis, the SCP density of ERM (0.401 ± 0.012) turned out to be inferior that MPH (0.419 ± 0.018) and controls (0.415 ± 0.017) (Fig. 1). No significant differences were evident among the three subgroups for DCP vessel density (0.418 ± 0.016 for ERM, 0.426 ± 0.015 for MPH, 0.415 ± 0.017 for controls; $p = 0.1$) (Fig. 2).

The FAZ area in the SCP was different among groups ($p < 0.01$). The post hoc analysis showed that ERM had the smaller FAZ (0.168 ± 0.123 mm^2), respectively, than MPH (0.295 ± 0.013 mm^2) and controls (0.213 ± 0.107 mm^2) (Fig. 3), otherwise no difference was noted in the DCP among groups ($p = 0.09$; Fig. 4).

The perfusion density of both SCP and DCP did not correlate with BCVA in the ERM group (respectively, $p = 0.578$ and 0.798) nor in the MPH group (respectively, $p = 0.801$ and 0.899). Similar results were achieved for the correlation between FAZ area and BCVA (p not significant for all).

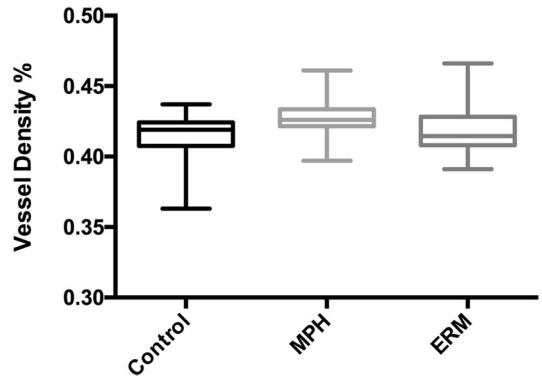

Fig. 2 Perfusion density in the deep capillary plexus (DCP) of controls, macular pseudoholes (MPH), and idiopathic epiretinal membranes (ERM)

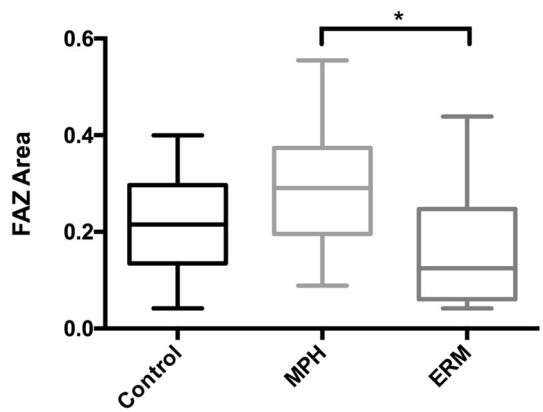

Fig. 3 Foveal avascular zone (FAZ; mm^2) in the superficial capillary plexus (SCP) of controls, macular pseudoholes (MPH), and idiopathic epiretinal membranes (ERM)

Table 1 Clinical features of the investigational groups and controls

	Idiopathic epiretinal membrane	Macular pseudohole	Control	*p*
Number of eyes	25	28	30	–
Age (years)	57.8 ± 13.9	58.2 ± 11.8	56.7 ± 13.1	0.971
Sex (f / m)	11:9	12:12	14:16	0.846
BCVA (logMAR)	0.32 ± 0.21	0.35 ± 0.31	0.0 ± 0.0	0.788*
mFT baseline (micron)	341 ± 43	316 ± 16	215 ± 19	0.873*
Ellipsoid zone irregularity	3 (12%)	3 (11%)	0 (0%)	0.999*
Intraretinal fluid	0 (0%)	0 (0%)	0 (0%)	–

f female, *m* male, *BCVA* best-corrected visual acuity, *mFT* mean foveal thickness, *logMAR* logarithm of the minimal angle of resolution

*Comparison between idiopathic epiretinal membrane and macular pseudohole

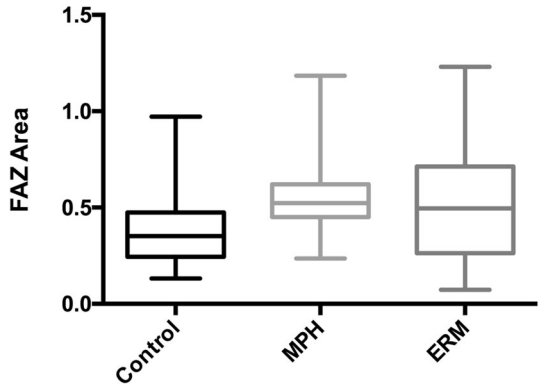

Fig. 4 Foveal avascular zone (FAZ; mm^2) in the deep capillary plexus (DCP) of controls, macular pseudoholes (MPH), and idiopathic epiretinal membranes (ERM)

Discussion

Idiopathic ERMs and MPHs, despite their clinical divergence, are thought to represent similar aspects of the same pathogenetic entity. Thorough investigation of these two coupled disorders, including the use of OCT-A, gave us the opportunity to highlight intriguing evidences.

It has been already demonstrated that full-thickness macular hole, lamellar macular hole, and MPHs can alter the retinal vascular networks, and OCT-A-based methodologies already described in detail such changes [5–7]. Similarly, OCT-A studies reported the vascular alterations secondary to ERM development [12]. Although ERMs are strongly associated with the onset of MPHs [13], with this study, we selectively investigated the retinal microvascular morphological features and the differences between these two conditions.

Here, we showed the major SCP involvement in eyes selectively affected by ERMs. Specifically, they disclose a lower perfusion density than both MPHs and healthy controls. Accordingly, also the FAZ area in the SCP resulted altered in the ERM group, being smaller than MPHs and controls. Therefore, ERMs and MPHs differ in terms of vascular network changes, suggesting that the traction forces may engender different effects on the retinal vessels. Specifically, the ERM seems to produce a direct effect on the SCP, thus leading to vessel density reduction.

Different hypotheses may be raised. It may be speculated that a linear and continuous ERM over the entire macula area can exert stronger mechanical effects onto the superficial retinal vessels, thus possibly leading to the SCP reduction. These assumptions agree with Sebag's anomalous posterior vitreous detachment theory [3]. Specifically, centripetal vitreous traction displacement of an otherwise healthy SCP could explain our results, furthermore justifying also the FAZ reduction at the SCP in eyes with ERM. On the other hand, as previously published [14], MPHs may be produced by centrifugal tractions, limited to the fovea and not to the entire retina. The latter forces may preserve the SCP perfusion density, which appears normal. The DCP density preservation is not in contrast with the abovementioned theory, since both conditions substantially involve the inner retina.

A recent research assessed the OCT and OCT-A characteristics of eyes with epiretinal membrane [8], describing the presence of continuous ectopic inner foveal layers, which were defined as a continuous band extending from the inner nuclear and plexiform layers across the foveal region, and that were also negatively associated with visual function. Despite a quantitative OCT-A analysis was not performed, the authors qualitatively evaluated the macular en-face OCT-A images and observed abnormalities of the FAZ area ranging from a slight decrease to its absence in eyes with continuous ectopic inner foveal layers. Our study is in agreement with these results, reinforcing the fact that eyes with idiopathic ERM present the smallest FAZ area.

We intentionally decide to exclude stage 4 ERMs from our research. Despite OCT-A studies about the characteristics of ERM in the different stages are not available, it is reasonable to assume that the hamartomatous features of end-stage ERMs (loss of layer segmentation) may have altered the retinal capillary plexuses, therefore jeopardizing our analysis.

We did not find any significant morpho-functional correlation between perfusion density and visual acuity. Despite this aspect would have been of great clinical value, we were limited by the cross-sectional design of the study. We believe further clinical implication might be inferred from a prospective observational or interventional postsurgical study. We are aware that this study has other different limitations, including the number of patients analyzed and the use of relatively new techniques. The analysis of the DCP may suffer from projection artifacts and beam absorption. Moreover, all the images analyzed the same angiocube size, which has been shown to be

important for macular perfusion parameters and inter-rater reliability [15, 16]. Future studies should explore the application in this setting of larger angiocubes, which have now become available [17]. They might be able to provide more information on vessels abnormalities associated with wider epiretinal traction forces.

In addition, owing to the design of our study, we cannot ascertain whether ERMs and MPHs represent consequential stages of the same pathogenetic process, or substantially different entities. Further prospective studies may clarify this aspect.

In conclusion, the present study describes using OCT-A the quantitative vascular feature differences between ERMs and MPHs, showing how these pathological mechanisms can affect differently the retinal vascular networks.

Compliance with ethical standards

Conflict of interest Luisa Pierro, Lorenzo Iuliano, Alessandro Marchese, Alessandro Arrigo, and Alessandro Rabiolo declare no conflict of interest. Francesco Bandello is a consultant for: Alcon (Fort Worth,Texas, USA), Alimera Sciences (Alpharetta, Georgia, USA), Allergan Inc (Irvine, California, USA), Farmila-Thea (Clermont-Ferrand, France), Bayer Shering-Pharma (Berlin, Germany), Bausch And Lomb (Rochester, New York, USA), Genentech (San Francisco, California, USA), Hoffmann-La-Roche (Basel, Switzerland), Novagali Pharma (Évry, France), Novartis (Basel, Switzerland), Sanofi-Aventis (Paris, France), Thrombogenics (Heverlee,Belgium), Zeiss (Dublin, USA).

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Human and animal rights This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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