Correlation of optical coherence tomography angiography and microperimetry (MP3) features in wet age-related macular degeneration

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Purpose: To evaluate and correlate the functional treatment response using microperimetry (MP3) with the morphological findings on optical coherence tomography angiography (OCTA) in wet AMD pre- and post-treatment with anti-vascular endothelial growth factor (VEGF). This was a single-centre prospective, interventional study. **Methods:** Patients with wet AMD were treated with 3 injections of intravitreal anti-VEGF at monthly intervals for 3 months and followed at 1, 2, 3, and 6 months postinjection. Using "overlay" features, morphologic characteristics of OCTA at the site of choroidal neovascular membrane (CNVM) lesion were analyzed and correlated functionally with MP3. Data were collected including visual acuity at presentation and follow-up with multimodal imaging features, treatment details, complications (if any), and treatment given for that complication. Descriptive observational analysis and paired *t*-test was used to compare the appearance of the neovascular network on OCTA imaging with retinal sensitivity on MP3. **Results:** OCTA in the pretreatment phase revealed CNVM as an abnormal vascular network arising from the choroid and invading the subretinal space. On MP3, decreased retinal sensitivity was observed corresponding to the area of CNVM. Post-treatment, OCTA revealed reduction in abnormal vascular network in 51 (91.07%) eyes that correlated with increased retinal sensitivity at the corresponding area on MP3. Statistical analysis showed baseline mean retinal sensitivity at the site of CNVM as 320.07 dB, which improved to 521.53 and 730.20 dB at 1 and 3 months postinjection follow-up, respectively. **Conclusion:** Combining the findings of OCTA and MP3 using "overlay" features gives us precise information of structure–function correlation at presentation and also in response to treatment. It also helps to improve patient's compliance, confidence to treatment, and their understanding of the disease process as well.

Key words: Age-related macular degeneration, anti-VEGF, microperimetry, neovascular network, optical coherence tomography angiography, retinal sensitivity

Wet age-related macular degeneration (WAMD), an advanced form of macular degeneration, is the leading cause of visual impairment in older adults related to AMD.[1] The presence of abnormal blood vessels, known as choroidal neovascular membrane (CNVM), can induce hemorrhage, fluid exudation, and fibrosis, resulting in photoreceptor damage and vision loss.[2] Fluorescein angiography (FA) along with optical coherence tomography (OCT) are gold standard tools to diagnose WAMD. Optical coherence tomography angiography (OCTA; AngioScan OCT Angiography software on the RS-3000 Advance OCT; Nidek, Gamagori, Japan) is a novel imaging modality that allows direct visualization of the retinal and choroidal vasculature *in vivo*. In OCTA, high frequency and dense volumetric scanning is applied to detect blood flow by analyzing the signal decorrelation between scans.[3] Compared with stationary areas of the retina, the movement of erythrocytes within a vessel generates a decorrelated signal. Unlike traditional angiography, OCTA is a noninvasive procedure and does not require the use of exogenous dyes, thus

avoiding potential side effects, such as nausea or other more serious adverse events.

There have been significant advances in the assessment of retinal morphology due to introduction of FA, OCT, and OCTA into clinical practice; however, there exists an inequilibrium between the structural and functional evaluation of retinal pathology in WAMD. This is overcome by the use of newer noninvasive microperimetry (MP3) which measures local retinal sensitivity for functional assessment of the retina. It is a subjective, quantitative, noninvasive diagnostic examination aimed at assessing retinal functionality and puts it in strict correlation with retinal morphology.[4] MP3, otherwise known as fundus-driven perimetry, has evolved into a robust tool to evaluate the retinal function in recent years.[5] In particular, MP3 with real-time fundus imaging along with eye tracking technology and a quicker

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acquisition time is considered to have an advantage over the conventional perimetry.[6] The fundus view allows the user to position the stimuli in the retinal area of interest enabling specific structure–function associations to be evaluated.

The aim of this study is to evaluate and correlate the functional treatment response using MP3 with the morphological findings on OCTA in WAMD pre- and post-treatment with anti-VEGF.

Method

This was a single-center prospective interventional study approved by the Institutional Review Board. All cases provided informed consent to be enrolled in the study. Data were collected from patients diagnosed as WAMD in the retina clinic from April 2017 to April 2018.

Eligibility criteria and data collection

Data were collected under the following headings: demographic details, history of presenting illness, eye laterality, history of associated systemic comorbidities, clinical examination details including visual acuity at presentation and follow-up with multimodal imaging features, treatment details, complications (if any), and treatment given for that complication. Inclusion criteria were the following: (1) age >50 years, (2) best corrected visual acuity (BCVA) >6/60, (3) clinical findings consistent with WAMD (subfoveal or juxtafoveal) pretreatment at presentation, (4) follow-up of at least 6 months with regular medications, and (5) complete availability of documentation till the last follow-up. If any one of the above-mentioned inclusion criteria was not met, patients were excluded from the study.

Exclusion criteria were diabetic retinopathy, retinal vascular diseases, intraocular tumors, prior intraocular infections, inflammations, ocular surgery except uncomplicated cataract surgery or extensive laser treatments (posterior to the equator or covering more than 3 clock hours of periphery), WAMD lesions with retinal angiomatous proliferation, or

hemorrhage with the largest diameter >2.5 disc diameters. For this analysis, out of 72 eyes, 16 eyes were excluded because of unstable fixation (6) or co-operational problems (7) in MP3 and problems with autoalignment of MP3 and OCTA scans because of the lack of clear fundus landmarks in the central part of SLO images (3), thereby amounting to a total of 56 eyes in the study.

Treatment regime

All patients were treated with 3 injections of intravitreal anti-VEGF at monthly intervals for 3 months. They were followed at 1, 2, 3, and 6 months from the baseline. Further injections were given on pro re nata (PRN) basis.

Study parameters

Patients who met inclusion criteria were enrolled in the study. All cases underwent a complete eye examination at presentation and also at every follow-up visit. The examination protocol included BCVA with the help of Snellen chart and converted to logarithm of the minimum angle of resolution, dilated fundoscopic examination, color fundus images (TOPCON 50 Dx; Medical Systems Inc., Oakland, NJ, USA), fundus fluorescein angiography, indocyanine green angiography (ICGA) (SPECTRALIS; Heidelberg Engineering Inc., Heidelberg, Germany), spectral domain-optical coherence tomography (SD-OCT) (SPECTRALIS; Heidelberg Engineering Inc.), OCTA (RS-3000 Advance OCT; Nidek) centered on the fovea covering 3 × 3 mm area (10° × 10° field of view), and Microperimetry (MP3) (Nidek) centered on fovea covering 12° field of view.

Data analysis

Using "overlay" features, morphologic characteristics of OCTA at the site of CNVM lesion were analyzed and correlated functionally with MP3.

Color fundus photography, optical coherence tomography

All cases at presentation and at each follow-up visit underwent (a) color fundus photography (TOPCON 50 Dx; Medical Systems Inc.) and a fundus mosaic was made in cases where lesions extended beyond arcades, and (b) SD-OCT (SPECTRALIS®; Heidelberg Engineering) using the dense line-scan protocol with the enhance depth imaging (EDI) function activated. The size of the scan was based on the size and location of the lesion. The minimum averaged number of frames per B-scan was 100; (c) Fundus autofluorescence (FAF) imaging prior to FA/ICGA using Heidelberg SPECTRALIS®.

Fluorescein angiography and indocyanine green angiography

FA and ICGA images were obtained once adequate pupillary dilation (minimum of 7 mm) was achieved, at baseline (prior to initiating therapy) and at subsequent follow-up

Table 1: Demographic data

Parameters	*n* (%)
Number of patients	44
Number of eyes	56
Male	34 (60.7)
Female	22 (39.2)
Age (mean)	67.54±2.3
Age (range)	55-85
Unilateral	32 (72.7)
Bilateral	12 (27.2)

Table 2: Correlation of OCTA with MP3 features from baseline to follow up

	OCTA	MP3
Baseline (prior to first injection)	Abnormal neovascular network	Decreased retinal sensitivity
1 month of follow up	Decrease in size of neovascular network	Retinal sensitivity started to increase
3 months of follow-up	Further reduction in size of neovascular network	Further increase in retinal sensitivity
6 months of follow-up	Almost complete resolution of neovascular network	Stabilized retinal sensitivity

OCTA: Optical coherence tomography angiography, MP: Microperimetry

visits (on the discretion of the treating clinician). Sequential fundus images were obtained from early to late frames in all cases. Heidelberg SPECTRALIS® (Heidelberg Engineering) was used to acquire the angiography images.

Table 3: Functional characterstic features from baseline to follow up

	Mean retinal sensitivity (db)	SD	P
Baseline (prior to first injection)	320.07	177.97	
1 month of follow-up	521.53	164.52	<0.0001
3 months of follow-up	730.20	215.21	<0.0001

SD: Standard deviation

OCT-Angiography

For all cases, at presentation and follow-up, OCTA images were obtained by AngioScan OCT Angiography software on the RS-3000 Advance OCT (Nidek, Japan). The en face images obtained with OCTA allowed to visualize individual retinal vascular plexuses and choriocapillaris. For all cases, OCTA scan covering an area of 3 × 3 mm equivalent to 10° × 10° field of view was used at presentation and also on each follow-up.

Microperimetry (MP3)

The automatic microperimeter with a nonmydriatic fundus camera has a dynamic range of 0–34 db. The patients were provided with clear instructions about the testing, and all tests were conducted/supervised by two experienced MP3 examiners. There was no specific practice session prior to final testing on the MP3, but subjects were given the opportunity
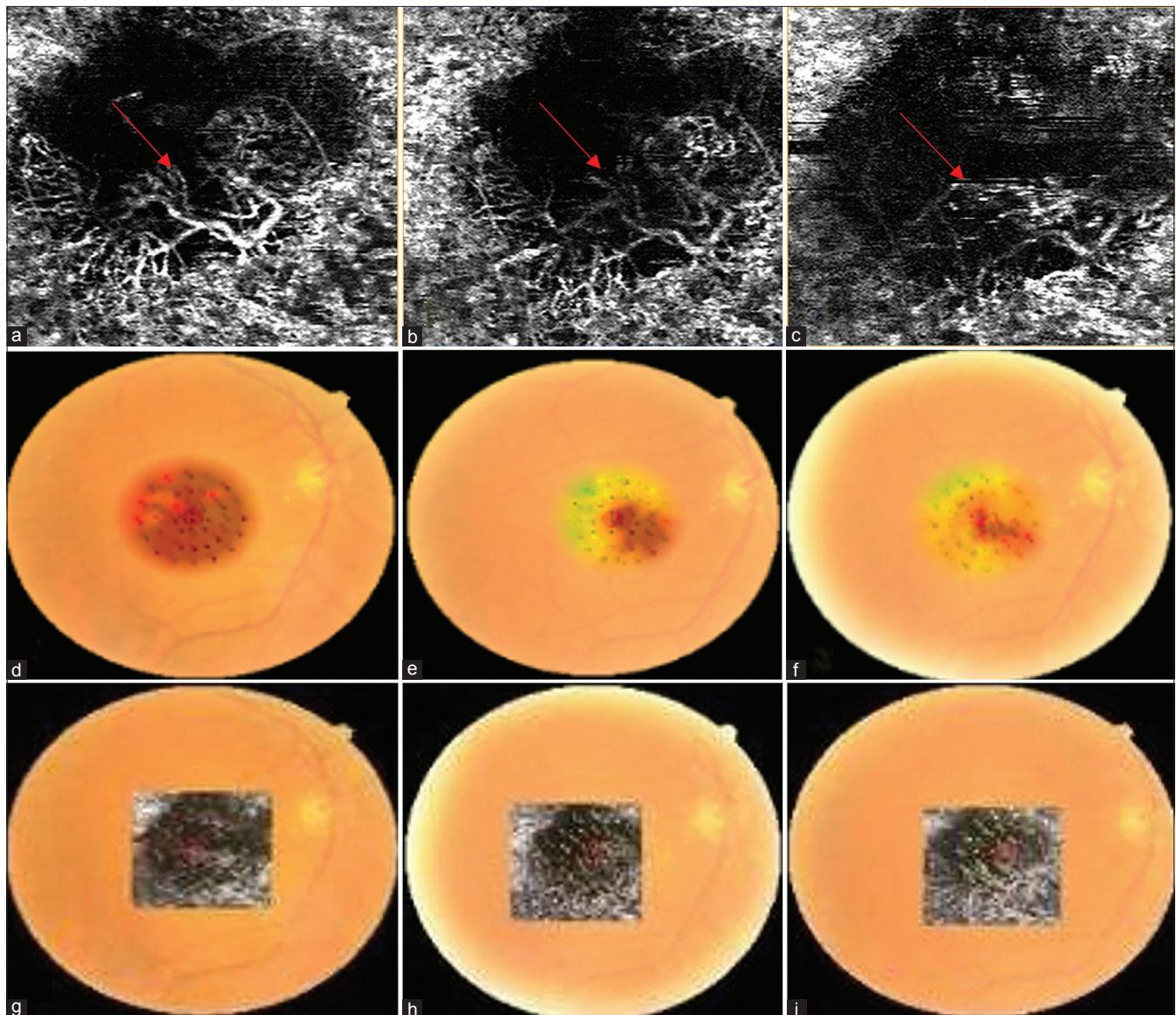

Figure 1: First, second, and third columns show images at baseline, first month, and fourth month respectively post-treatment with anti-VEGF. (a–c) OCTA at the choroidal level shows reduction in size of branching neovascular network and peripheral anastomosis (arrow) from baseline to follow-up. (d–f) Microperimetry (MP3) shows increasing retinal sensitivity at the same topographical location of neovascular network corresponding to OCTA. (g–i) Overlay of OCTA on MP3

Figure 2: First, second, and third columns show images at baseline, first month, and fourth month respectively post-treatment with anti-VEGF. (a–c) OCTA at the choroidal level shows branching neovascular network (red arrow) shrinking in size from baseline to follow-up. (d–f) Microperimetry (MP3) shows increasing retinal sensitivity at the same topographical location of neovascular network corresponding to OCTA. (g–i) Overlay of OCTA on MP3
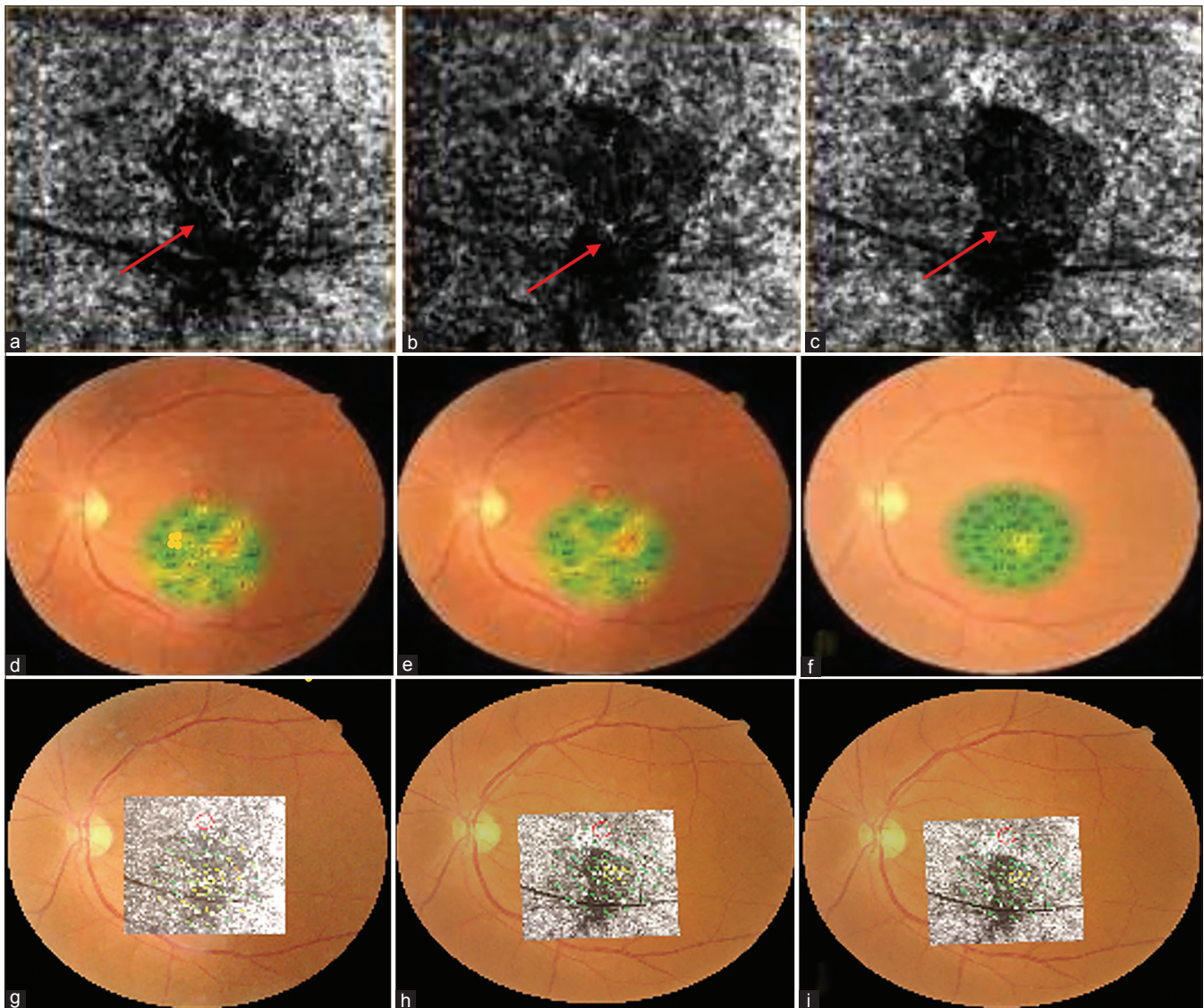
to familiarize themselves with the testing procedure and the finger pressure required to trigger a response when a stimulus was observed. Using the MP3 microperimeter software, retinal image of the same eye, acquired with OCTA technique, were imported and overlapped onto the sensitivity map. The procedure was performed in a darkened and quiet room without any dilation of the pupil. Dark or light adaptation was not used prior to testing due to the mesopic testing conditions. The lights were turned off after the nonstudy eye was patched, and the patient comfortably positioned at the device with chin on the chin rest and the finger ready to press the response trigger. MP3 has a software by which it calculates and compensates for the refractive error of the patients.

The following testing parameters were used for MP3: a 37-stimuli grid overlying the central 12°; Goldmann III stimulus with a duration of 200 ms; 4–2 threshold strategy; and red circle fixation target for MP3. The standardized stimulus grid consisted of a single central foveal response and four concentric rings of retinal loci at 1°, 2°, 4°, and 6° from the center point.[6] Recordings were considered reliable when false positive responses remained under 25%.

Image analysis

For all cases, images acquired at presentation and subsequent follow-up examination were examined by two independent masked examiners MN and NM. OCTA images were analyzed using the AngioScan software (RS-3000Advance). Images of the choroidal vasculature generated on OCTA were compared, correlated, and analyzed with MP3 at presentation and at each subsequent follow-up visits. Red, yellow, and green colors on MP3 show low, medium, and high retinal sensitivity, respectively. Images of FA and ICGA were also correlated with OCTA images. This helped to assess whether OCTA scan can noninvasively help in the detection of similar features as provided by invasive ICGA and FA.
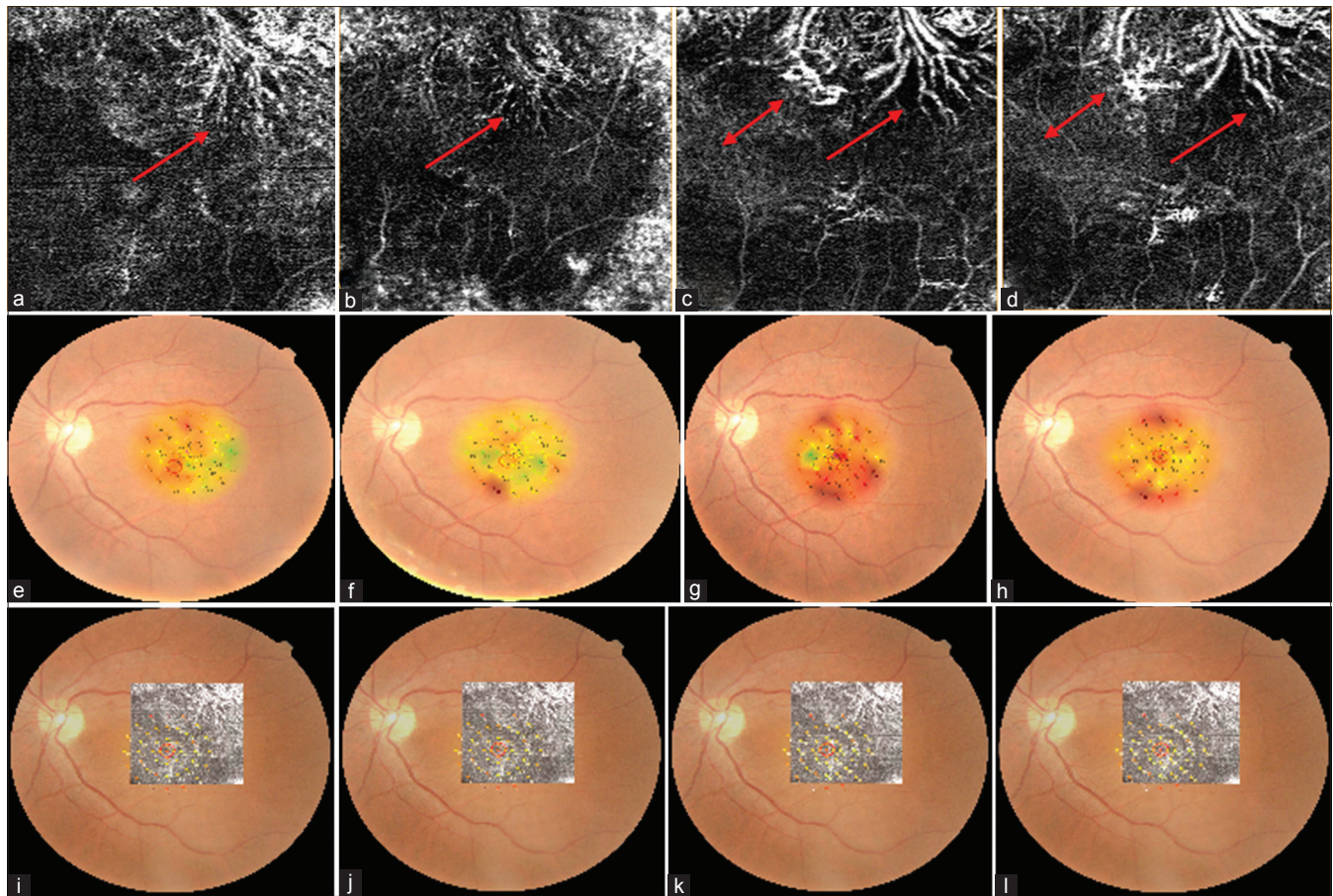
Figure 3: First, second, third, and fourth columns show images of CNVM treated with anti-VEGF at baseline, fourth, sixth, and seventh month, respectively. Anti-VEGF given at baseline till fourth month (1 injection/month) and repeated at sixth month. (a and b) OCTA at the choroidal level shows classic branching network (arrow) decreasing in size from baseline to 1 month. (e–h) Microperimetry (MP3) shows increasing and decreasing retinal sensitivity corresponding to the network size on OCTA. (c and d) A new neovascular network (double-headed arrow) near the regressing old network. (i–l) Overlay of OCTA on MP3

Statistical analysis

Descriptive observational analysis and student's paired t-test was used to compare the baseline and post treatment retinal sensitivity. The data were analyzed using SPSS statistics software and $P < 0.05$ was considered statistically significant. Descriptive observational analysis was used to compare the appearance of the neovascular network on OCTA.

Results

Seventy-two eyes of 49 patients were studied. Of these 56 eyes, 44 patients with WAMD who met our inclusion criteria were included in the study. The mean age at presentation was 67.54 ± 2.3 years (range 55–85). The gender distribution comprised 34 eyes of 24 males (10 males with bilateral and 14 with unilateral presentation) and 22 eyes of 20 females (2 females with bilateral and 18 with unilateral presentation). All subjects included in the study were Asian Indians. The demographic details of patients are listed in Table 1. OCTA in the pretreatment phase revealed CNVM as an abnormal vascular network like loss of peripheral arcades and anastomosis [Fig. 1a], classic branching pattern [Figs. 2a and 3a] arising from the choroid, and invading the subretinal space. On MP3, decreased retinal sensitivity was observed corresponding to the area of large CNVM [Figs. 1d, 2d, and 3e]. Post-treatment, OCTA revealed reduction

in abnormal vascular network in 51 (91.07%) eyes that correlated with increased retinal sensitivity [Figs. 1b,c,e, 2b,c and 3a,b] at the corresponding area in MP3 [Figs. 1f, 2e,f, and 3f] for all 51 eyes. In 1 patient [Fig. 3] there was a resurgence of new neovascular network near the old network at 6 months follow up from baseline [Fig. 3c and g]. we repeated anti-VEGF resulting in shrinkage of the new network [Fig. 3d and h]. Correlation of OCTA with MP3 features in WAMD patients are listed in Table 2. Paired *t*-test was used and mean retinal sensitivity at the site of CNVM at baseline, 1 month, and 3 months postinjection follow-up was 320.07 ± 177.97, 521.53 ± 164.52, and 730.20 ± 215.21 dB ($P < 0.0001$), respectively. Final visual outcomes were better in patients with good retinal sensitivity at baseline [Table 3].

Discussion

In this study, we have made an attempt to correlate the structural data provided by OCTA with the functional information obtained by MP3 using a novel "overlay" software [Figs. 1g-i, 2g-i and 3i-l]. There was a significant correlation observed between the neovascular network on OCTA with decreased retinal sensitivity on MP3. Post-treatment with resolution of neovascular network on OCTA, a significant improvement in retinal sensitivity was documented on MP3.

Various studies in the literature have made an attempt to correlate the structural and functional information in cases of age-related CNVM by using SD-OCT and MP-1.[7-10]

For decades, BCVA has been the main indicator of visual function in retinal diseases; however, as it is based on responses from the fovea cones, it does not represent a comprehensive assessment of macular function. This is particularly true for AMD patients whose entire visual function depends on the amount and location of the neovascular network and subretinal fluid. Functional testing in and around the areas of CNVM may provide additional useful information regarding the severity and extent of disease in the macula, and the potential impact of new therapeutic agents. More recently, microperimetry has effectively provided fundus-correlated functional testing, test–retest reliability for the same test point, precise assessment of the location and stability of fixation, and compensation for ocular movements.[8] By incorporating these features, microperimetry has become the clinical investigation of choice to assess residual visual function and functional vision beyond the fovea in macular diseases. Retinal sensitivity assessed by fundus-related microperimetry may provide more reliable insight into macular function deficiencies than by distance vision or reading performance. Accurate measurement of functional deficiency and determination of the responsible morphological correlate enable a better understanding of AMD by detecting the underlying cause of vision loss, which allows a precise determination of the functional stage and may improve the chance of treatment success. The retinal sensitivity measurements generated by microperimetry are now being considered as potentially useful endpoints in ophthalmic clinical trials.

The 12-megapixel fundus camera in the MP3 acquires high resolution images of retinal pathology and allows easy image acquisition. The MP3 measures local retinal sensitivity for functional assessment of the retina.[7] The results can be displayed over a color fundus image, correlating retinal anatomy to retinal function. Autotracking and autoalignment functions provide more accurate measurements increasing patient and operator comfort and efficiency. These functions allow easy follow-up and reduce variations between examiners, resulting in well-aligned follow-up examinations. MP3 allows for automated assessment of macular function as well as automated correction for eye movements, as unstable fixation.[10,11] It also gives an idea about the fixation pattern and the preferred retinal loci (PRL) of patients. These PRL are mapped in relation to fixed anatomical landmarks, so they can be re-examined in subsequent examinations, a point which allows for future comparisons. This feature allows evaluation of disease progression or assessment of pre- and post-treatment outcomes. Even in cases of good visual acuity with early juxtafoveal CNVM, MP3 detects the presence of decreased retinal sensitivity. OCTA on the other hand is the only noninvasive tool that provides three-dimensional analysis of CNVM and provides structural information of neovascular network. Overlaying the details of OCTA and MP3 with the help of novel software provided by Nidek provide us a new insight into the understanding of disease process that was never explored previously.

Our study has few limitations as small number of cohorts, lack of further long-term follow-up beyond 6 months, and require further studies in future for validation.

Conclusion

The novel "overlay" software is a promising imaging tool. Combining the findings of OCTA and MP3 using "overlay" features give us precise information of structure–function correlation at presentation and also in response to treatment. More importantly, it helps to improve patient's compliance, confidence to treatment, and their understanding of the disease process as well.[12-14]

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Conflicts of interest
There are no conflicts of interest.

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