Irish Journal of Medical Science (1971 -)
https://doi.org/10.1007/s11845-022-02979-y

ORIGINAL ARTICLE

Retinal ultrastructural, electrophysiological, and microvascular morphological outcomes in diabetic macular edema treated with intravitreal bevacizumab

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Received: 17 February 2022 / Accepted: 13 March 2022

Abstract

Background Investigation of retinal ultrastructural, electrophysiological, and microvascular morphological changes, as well as correlations between these changes and visual outcome in naïve diabetic macular edema (DME) patients after intravitreal bevacizumab therapy (IVBT).

Methods This prospective interventional study enrolled 31 DME patients' eyes treated with monthly IVBT for three months. Best-corrected visual acuity (BCVA) and intraocular pressure (IOP) were measured, and fundus fluorescein angiography, optical coherence tomography (OCT), microperimetry, as well as optical coherence tomography angiography (OCTA) were performed before and after IVBT. Patients were grouped based on BCVA improvement after three consecutive IVBT: group 1: > 10 letters, group 2: ≤ 5 letters, and group 3: between 6 and 10 letters.

Results Mean BCVA increased significantly from 34.2 to 39.9 letters ($p < 0.001$). Central macular thickness decreased significantly from 335.1 to 276.4 µm ($p < 0.001$). Fixation stability, retinal sensitivity, and local deficit all improved significantly ($p < 0.001$ for all). There was no statistically significant change in IOP ($p = 0.665$). Although OCTA parameters did not change significantly, lower foveal avascular zone (FAZ) area, higher foveal vessel density 300 µm area around FAZ and deep plexus vascular density were associated with highly improved BCVA, retinal sensitivity, and local deficit. Also, there were no significant intergroup differences in gender, age, baseline BCVA, HbA1c, IOP, phakic/pseudophakic lens ratio, concomitant hypertension, and superficial capillary plexus vascular density.

Conclusions IVBT was associated with significantly improved BCVA, retinal ultrastructural integrity, and electrophysiological patterns in naive DME patients. Improvements in retinal electrophysiology correlated with ultrastructural improvements, which could be predicted using OCTA.

Keywords Diabetic macular edema · Intravitreal bevacizumab · Microperimetry · Optic coherence tomography angiography · Visual acuity

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Published online: 23 March 2022

Background

Diabetic macular edema (DME) can cause significant visual dysfunction and, in the worst-case scenario, blindness. In many parts of the world, this could impose a significant social burden on people of working age and the elderly [1–3]. Prevalence of DME increases with disease duration, reaching 60% after 20 years [1], and it has been reported to affect 26% of the patients with diabetic retinopathy [4].

In DME, vascular endothelial growth factors (VEGF) are presumed to be more powerful in modifying vascular permeability [3], resulting in retinal edema from blood-retina barrier breakdown and subsequent vascular hyperpermeability. Besides, long-term exposure to these factors may eventually result in neovascularization as well as endothelial cell proliferation [5]. Early treatment of eyes at DME risk has been revealed to be beneficial in controlling visual dysfunction symptoms [6], and in this regard, intravitreal anti-VEGF therapy in DME may improve visual outcomes [7, 8].

Optical coherence tomography angiography (OCTA) is capable of providing depth-resolved details in a non-invasive manner that conventional dye-based angiography cannot. It also enables separate assessment of the retinal microvascular morphological changes in superficial capillary plexus (SCP), intermediate capillary plexus, and deep capillary plexus (DCP) [9]. Changes in OCTA parameters may occur in diabetic patients without clinical diabetic retinopathy manifestations. These parameters could be potential DME biomarkers since they indicate early microvascular morphological changes in respective capillary plexuses [10–12]. Nonetheless, as a significant proportion of existing studies is cross-sectional, very little is understood about the predictive significance of OCTA parameters on DME after therapy.

Aside from central visual acuity, the neuroretinal function has a significant impact on patients' quality of life. Microperimetry enables a comparative analysis of retinal pathology with psychophysical data as well as an objective analysis of fixation patterns, providing a better understanding of treatment outcomes [13, 14]. There is a significant association among retinal ultrastructural integrity, neuroretinal electrophysiological functions, microvascular morphology, and visual outcomes in DME. Vision-based evaluation is frequently inadequate for determining the severity and location of retinal damage [15]. Understanding changes in all of these parameters would help with DME management in real time.

This study was therefore designed to investigate retinal ultrastructural, electrophysiological, and microvascular morphological changes, as well as correlations between these changes and visual outcome in naïve DME patients after intravitreal bevacizumab therapy (IVBT) (1.25 mg/0.05 mL, Altuzan®, Roche) using OCT, microperimetry, and OCTA.

Materials and methods

Study design and participant selection

Thirty-one eyes of 31 patients with visual complaints who were diagnosed and followed-up with naive DME were included in this prospective interventional single-centered study conducted at Afyonkarahisar Health Sciences University Faculty of Medicine Ophthalmology Department Retina Unit. The study procedure adhered to the Helsinki Declaration's ethical standards and was approved by the University Clinical Research Ethics Committee in its entirety. All patients were given detailed information about the applications and tests that would be conducted. The ethics committee-approved consent document was read aloud to the participants, who were informed about the study's exclusion criteria and gave their consent.

Diabetic macular edema diagnosis was made using binocular stereoscopic fundus examination, OCT (Spectralis HRA OCT, Heidelberg Engineering, Heidelberg, Germany), and fundus fluorescein angiography (Zeiss, Visucam 5000, Germany) features. All patients were given information about the disease and treatment procedures before the study. Those found to be compliant with the recommended anti-VEGF therapy were eventually included in the study, in which therapy was initiated if there was ≥ 240 µm CMT as revealed by OCT with intraretinal or subretinal fluid, or > 5 letter decrement in best-corrected visual acuity (BCVA) relative to a preceding examination level.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (a) newly diagnosed DME, (b) absence of prior DME therapy such as laser photocoagulation due to diabetic retinopathy, (c) absence of lenticulo-corneal opacity that would impede OCT, OCTA, and microperimetry acquisition, (d) absence of retinal vascular diseases other than DME such as age-related macular degeneration, hypertensive retinopathy, and retinal vascular occlusion, (e) absence of a history of posterior segment surgery, and (f) absence of a history of uveitis or other inflammatory ocular pathology.

Naive DME patients who did not adhere to the follow-up schedule did not cooperate with microperimetry and OCTA acquisition, declared therapy discontinuation, had any intravitreal injection-related ocular complications, such as endophthalmitis, retinal detachment, vitreous hemorrhage,

and so on, and had any intravitreal agent-related systemic adverse effects were excluded from the study.

Baseline examination

Each patient was subjected to baseline examination prior to inclusion in the study. Systemic investigations included blood investigations and ophthalmological examination, such as measurements of auto-refraction (Canon R-F10m; Canon Inc., Tokyo, Japan), BCVA using an Early Treatment Diabetic Retinopathy Study chart, and intraocular pressure (IOP) (Goldmann; Haag-Streit AG, Köniz, Switzerland), as well as anterior and posterior segment slit-lamp biomicroscopy before and after artificial mydriasis. Baseline color fundus photography and fundus fluorescein angiography images were also taken and used to determine angiographic features and lesion classification. Patients were instructed to have their blood sugar, blood lipids, and blood pressure closely monitored by an endocrinologist during the study period.

Optical coherence tomography scanning

All OCT scanning procedures were conducted by the same technician using the same device. This device detected and recorded central macular thickness (CMT), macular volume (MV), as well as defects at the vitreoretinal interface. If any segmentation errors were detected while measuring, they were manually corrected by placing reference lines on the internal limiting membrane and Bruch's membrane.

Microperimetry

The same technician took all microperimetry-1 (Nidek Inc., Padova, Italy) shots. Microperimetry-1 offers a 45° view of the fundus while automatically correcting for ocular movements. Each patient received pretest training after pupil dilatation and dark adaptation. A contralateral eye was kept closed during the procedure. A 4–2 ladder strategy was used in conjunction with the Goldmann III white stimulus. The central retina was subjected to a grid of 68 randomly presented stimuli over a 20° span. A 2° diameter red-cross was chosen as a fixation target. The background brightness of the instrument was 1.27 cd/m^2, with a maximum stimulus intensity of 127 cd/m^2. The stimulus was presented on a white background for 200 ms (ms) with intensities ranging from 0 to 20 decibels (dB) using a 4–2 ladder strategy. Each point stimulated by the device had its final threshold value determined.

Mean retinal sensitivity was calculated by taking into account the sensitivity of all stimulated retinal points. The device mapped the sensitivity difference of each stimulated point according to the normative data in the device memory as a local deficit. The mean local deficit was then calculated by averaging the local deficit values of all stimulated points.

During the procedure, fundus movements were monitored while the patient-focused on the fixation target to assess fixation. An automatic tracking system calculated horizontal and vertical shifts relative to a reference frame and mapped the patient's ocular movements. Recorded fixation points were divided into three groups for fixation stability analysis, that is, stable fixation (S1), relatively unstable fixation (S2), and unstable fixation (S3). S1 was characterized by the presence of $> 75\%$ of the fixation points in a 2° circle centered on the center of all fixation points. S2 was characterized by $< 75\%$ of fixation points in a 2° circle centered on the center of all fixation points and $> 75\%$ in a 4° diameter circle. S3 was characterized by the presence of $< 75\%$ of the fixation points located within a 4° diameter circle.

Optical coherence tomography angiography acquisition

All OCTA (Optovue, Inc., Fremont, California, USA) scanning procedures were also carried out by the same technician using the same device. The Angio Retina mode was used during the B-scanning procedure on 304 evenly spaced sections along the *x*-axis, followed by the *y*-axis in the 3×3 mm retinal area. These sections were then combined to create 3×3 mm OCTA images. The patient's axial length, refraction correction, and image polarization were automatically adjusted during image acquisition by using the "Auto Adjust" mode. The scan quality indicator (SQ), which is the score (range: 1–10) given to OCTA images by the device at the end of each acquisition by evaluating signal strength, ocular movements, and image focusing together, was also used. Images with low SQ ($SQ \leq 6$) were removed from the study, and the shots were repeated until the recording quality was acceptable. Motion artifacts were minimized using an eye-tracking system of the device, and they were eliminated using a projection artifact removal system.

Foveal vascular density (VD) value in the central 1 mm diameter ring determined by Early Treatment Diabetic Retinopathy Study, and mean VDs of superficial and deep capillary plexuses in the superior, nasal, inferior, and temporal quadrants of the outer ring, defined as the parafoveal area extending from 1 to 3 mm, were recorded. Foveal avascular zone (FAZ) area and foveal VD 300 µm area around FAZ (FD-300) were also recorded.

Intravitreal bevacizumab therapy

A standard protocol was followed for all intravitreal injections and drug preparations that were performed in the operating room. Initially, the conjunctiva was instilled with 0.5% proparacaine and 5% povidone-iodine drops, and the eyelid skin and lashes were cleaned with 10% povidone-iodine. A sterile wire lid speculum was placed, and the patient's

lashes were directed away from the eye. The IVBT was performed in the superior temporal quadrant, 3.5 mm posterior to the limbus, under sterile conditions and topical anesthesia. The light perception was tested after the injection, and paracentesis was performed as needed. This was followed by the administration of one drop of moxifloxacin (Vigamox; Alcon Laboratories Inc, Fort Worth [TX], USA). All patients were then given prophylactic topical moxifloxacin drops four times per day for a week. For the first three consecutive months, all patients received IVBT on a monthly basis.

Patients were then scheduled for monthly follow-up appointments and control exams, which included routine ophthalmological exams as well as OCT scanning. Microperimetry and OCTA procedures were repeated during the 3rd-month control after the first intravitreal injection.

The outcome measures

The primary outcome measures of the study were changes in OCTA and microperimetry parameters following therapy, as well as their correlations. Secondary outcome measures included changes in BCVA and IOP, as well as OCT parameters and their correlations. As a result, data for BCVA, IOP, OCT, microperimetry, and OCTA parameters obtained three months after IVBT were compared to baseline. Correlations between baseline microperimetry and OCTA parameters, as well as baseline OCT and BCVA, were also analyzed. Furthermore, patients were classified based on BCVA improvement. Those with improved BCVA of > 10 letters were classified as group 1, ≤ 5 letters as group 2, and between 6 and 10 letters as group 3. The OCT, OCTA, and microperimetry data were then compared among groups.

Data analysis

Collected data were coded and entered into a computer program, and the Statistical Package for Social Science (SPSS, 18.0 Worldwide Headquarters SPSS Inc.) program was used for statistical analysis. The Chi-square test, paired sample *t*-test, and Wilcoxon test were used to compare baseline to the 3rd-month data in terms of BCVA, IOP, as well as OCT, OCTA, and microperimetry parameters. The Kruskal–Wallis and ANOVA tests were used for intergroup comparison of the classified groups based on BCVA improvements. The Pearson bivariate correlation analysis was used to analyze data correlations. Statistical significance was defined as a $p < 0.05$.

Results

One patient was removed from the study due to myocardial infarction, another due to vitreous hemorrhage, and two for a variety of reasons, including delaying control exams and inability to cooperate with microperimetry procedure. The remaining 31 naive DME patients (31 eyes) with moderate non-proliferative diabetic retinopathy were then studied (left eyes: 58.1%). Male patients made up 54.8% of the total study participants. Patients ranged in age from 45 to 71 years old. Sixty-seven percent of the patients were phakic. There were no complications such as retinal tears, retinal detachment, or endophthalmitis during the course of the study. The age ($p = 0.261$), gender ($p = 0.518$), lens condition ($p = 0.344$),

Table 1 Demographic characteristics of the patients overall and by group

Parameters	Overall	Group 1	Group 2	Group 3
Age (years)	57.7	56.3	60.4	56.6
Gender (male/female)	17/14	5/3	4/6	8/5
Lens (pseudophakic/phakic)	10/21	2/6	5/5	3/10
Laterality (right/left)	13/18	3/5	3/7	7/6
HbA1c	9.9	9.61	10.1	9.91
Concomitant hypertension (presence/absent)	11/20	3/5	5/5	3/10
Intraocular pressure (mmHg)	15.6	15.5	16.3	14.9
Baseline BCVA (letters)	34.2	33.2	31.9	36.6
BCVA change (letters)	5.8	12.63	−1.2	6.85

group 1: patients with improved BCVA of > 10 letters; group 2: patients with improved BCVA of ≤ 5 letters; group 3: patients with improved BCVA between 6 and 10 letters

HbA1c glycohemoglobin A1c, *mmHg* millimeter of mercury, *BCVA* best-corrected visual acuity

Table 2 The OCT, microperimetry and OCTA results of the respective groups

Parameters	Group 1	Group 2	Group 3
Mean baseline CMT (μm)	363.8	364.1	295.3
CMT change (μm)	−125	−6.7	−58.1
Mean baseline MV (mm^3)	10.15	11.1	9.56
MV change (mm^3)	−1.03	−0.37	−0.47
Mean retinal sensitivity change (dB)	5.48	1.4	2.84
Mean local deficit change (dB)	5	0.9	2.9
Mean FAZ area (mm^2)	0.28	0.4	0.34
Mean FD-300 (%)	50.97	42.24	46.55

group 1: patients with improved BCVA of > 10 letters; group 2: patients with improved BCVA of ≤ 5 letters; group 3: patients with improved BCVA between 6 and 10 letters

OCT optical coherence tomography, *OCTA* OCT angiography, *CMT* central macular thickness, *MV* macular volume, *FAZ* Foveal avascular zone, *FD-300* foveal vessel density 300 μ area around FAZ, *μm* micrometer, mm^3 millimeter cube, *dB* decibel, mm^2 millimeter square, % percent

Table 3 Changes in BCVA, CMT, and MV during therapy

Duration	Parameters/ values	95% CI		*P*-value
		Lower limit	Upper limit	
BCVA (letters)				
Baseline	34.22 ± 8.09			
3**rd** month	39.96 ± 10.22	31.25	44.34	< 0.001
CMT (µm)				
Baseline	335.2 ± 85.3			
3**rd** month	276.4 ± 79.7	220.8	320.7	< 0.001
MV (mm^3)				
Baseline	10.21 ± 1.25			
3**rd** month	9.63 ± 1.18	8.7	10.5	< 0.001

CI confidence interval, *CMT* central macular thickness, *MV* macular volume, *µm* micrometer, mm^3 millimeter cube, mm^2 millimeter square

and HbA1C ($p = 0.879$) did not differ statistically significantly among groups 1, 2, and 3. Table 1 summarizes the demographic characteristics of the patients overall and by the group. And the OCT, microperimetry, and OCTA results for each group are shown in Table 2.

Best-corrected visual acuity analysis

The highest and lowest BCVA increased from 43 and 16 letters at baseline to 54 and 20 letters, respectively, three months after IVBT. The overall mean BCVA improved statistically significantly after three IVBT, increasing from 34.2 letters at baseline to 39.9 letters ($p < 0.001$) (Table 3, Fig. 1(a)). No statistically significant difference in baseline BCVA was found when groups 1, 2, and 3 were compared ($p = 0.244$) (Fig. 1(b)).

Intraocular pressure analysis

The overall mean IOP before and after IVBT was 15.51 and 15.67 mmHg, respectively ($p = 0.665$). The IOP intergroup analysis revealed no statistically significant differences as well ($p > 0.392$).

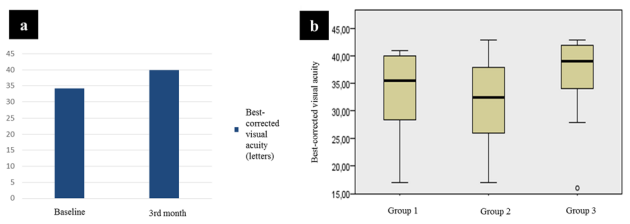

Fig. 1 Graphical representation of changes in best-corrected visual acuity (in letters) at baseline and after three consecutive months of intravitreal bevacizumab therapy in overall (**a**) and by group (**b**)

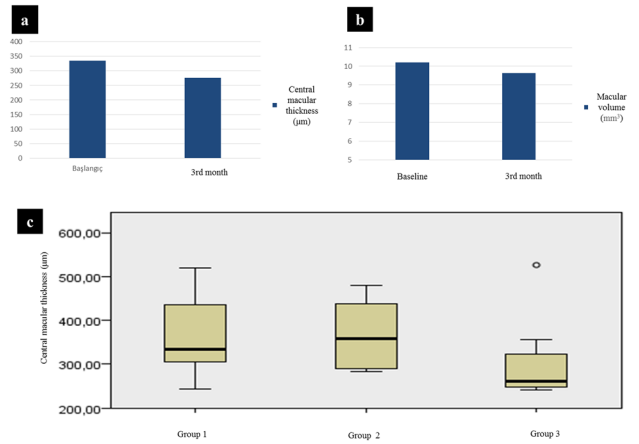

Fig. 2 Graphical representation of changes in mean central macular thickness (**a**) and macular volume (**b**) as measured by optical coherence tomography at baseline and after 3 months of intravitreal bevacizumab therapy. (**c**) Baseline central macular thickness comparison among between groups

Optical coherence tomography analysis

The mean CMT decreased statistically significantly from 335.2 µm (242–526) to 276.4 µm (164–455) after IVBT (Table 3, Fig. 2(a)), as did mean MV, which decreased from 10.21 mm^3 (8.7–13) to 9.63 mm^3 (7.6–12.2) ($p < 0.001$ for both) (Table 3, Fig. 2(b)). Intergroup analysis of mean baseline CMT ($p = 0.48$) and MV ($p = 0.007$) revealed significantly lower values in group 3 compared to group 2. However, no statistically significant differences were revealed between groups 1 and 2, or groups 1 and 3 ($p > 0.05$ for all) (Fig. 2(c) for CMT results).

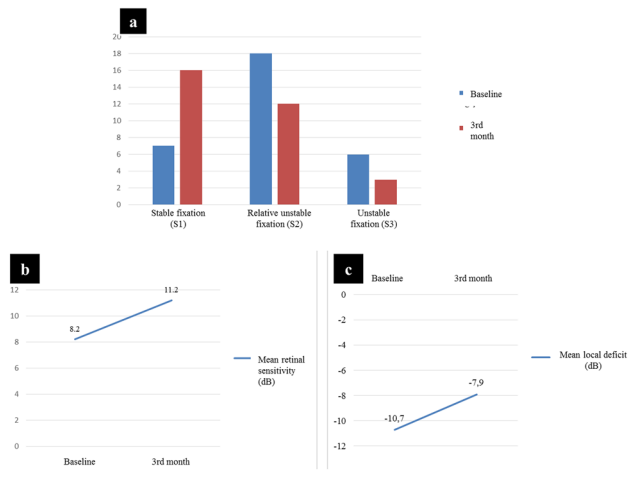

Fig. 3 Microperimetry analysis: Graphical representation of the fixation stability analysis (**a**), changes in mean retinal sensitivity (**b**), and local deficit (**c**) at baseline and after 3 months of intravitreal bevacizumab therapy

Microperimetry analysis

Fixation stability Analysis using chi-square test at baseline and after IVBT revealed significant improvements in all fixation subsets, with stable fixation showing the most significant improvement from 22.5 to 51.6% ($p=0.013$) (Fig. 3(a)).

Retinal sensitivity The mean retinal sensitivity (Fig. 3(b)) and local deficit (Fig. 3(c)) improved significantly after IVBT relative to baseline ($p<0.001$ for both) (Table 4).

Optical coherence tomography angiography analysis

Foveal avascular zone area The overall mean FAZ area and FD-300 decreased from 0.35 to 0.34 mm^2 ($p=0.730$) and 46.3 to 45.75% ($p=0.310$), respectively. The mean FAZ area in group 2 was significantly larger than that in group 1 ($p=0.006$). However, the differences between groups 1 and 3 ($p=0.090$), as well as groups 2 and 3 ($p=0.700$) were not statistically significant. Group 1 had significantly higher FD-300 than groups 2 ($p<0.001$) and 3 ($p=0.016$), as did group 3 relative to group 2 ($p=0.012$).

Superficial capillary plexus vessel density There were no significant differences in overall SCP VDs between baseline and after therapy in foveal ($p=0.921$), as well as parafoveal temporal ($p=0.869$), superior ($p=0.789$), nasal ($p=0.923$), or inferior ($p=0.941$) quadrants. Also, no significant differences were revealed among groups in SCP VDs in both foveal and parafoveal areas ($p>0.05$ for all).

Deep capillary plexus vessel density There were no significant differences in overall DCP VDs between baseline and after IVBT in foveal ($p=0.555$), as well as parafoveal temporal ($p=0.408$), superior ($p=0.536$), nasal ($p=0.339$), and inferior ($p=0.354$) quadrants. While foveal DCP VDs in group 1 were significantly higher than in group 2 ($p=0.004$), no statistically significant differences existed between groups 1 and 3 ($p=0.053$), nor between groups 2 and 3 ($p=0.530$). Furthermore, DCP VDs in parafoveal temporal, nasal, and inferior quadrants were significantly higher in group 1 than in groups 2 and 3 ($p=0.001$ for all), as were VDs in group 3 compared to group 2 ($p=0.001$, 0.002, and 0.078, respectively). Despite the fact that DCP VDs in the parafoveal superior quadrant were significantly higher in group 1 than in groups 2 ($p=0.001$) and 3 ($p=0.019$), there was no significant difference between groups 2 and 3 ($p=0.095$).

Correlation analyses

Optical coherence tomography versus best-corrected visual acuity results The mean BCVA improvement correlated significantly with CMT decrease ($r=0.743$; $p<0.001$) (Fig. 4(a)) but not with MV decrease ($r=0.287$; $p=0.117$).

Microperimetry versus best-corrected visual acuity results The mean BCVA improvement correlated significantly with improvements in both retinal sensitivity ($r=0.623$; $p<0.001$), as well as the local deficit ($r=0.731$; $p<0.001$) (Fig. 4(b, c)).

Optical coherence tomography angiography versus best-corrected visual acuity results None of the OCTA parameters, including FAZ, FD-300, and SCP VDs, were correlated with mean BCVA improvement ($p>0.05$).

Microperimetry versus optical coherence tomography and optical coherence tomography angiography results While mean retinal sensitivity improvement was found to be significantly correlated with decreases in CMT ($r=0.501$; $p=0.004$) and MV ($r=0.498$; $p=0.004$), no significant

Table 4 Microperimetry analyses

Fixation stability				
Duration	Stable (S1)	Relative unstable (S2)		Unstable (S3)
Baseline	7 (22.5%)	18 (58%)		6 (19.3%)
3**rd** month	12 (38.7%)	12 (38.7%)		3 (9.6%)
Retinal sensitivity and local deficit				
Duration	**Retinal sensitivity (dB)**	**95% CI**		***P*-value**
		Lower limit	**Upper limit**	
Baseline	8.2 ± 2.5			
3**rd** month	11.2 ± 2.9	9.4	13.1	< 0.001
Local deficit (dB)				
Baseline	−10.7 ± 2.5			
3**rd** month	−7.9 ± 3.2	−5.1	−8.9	< 0.001

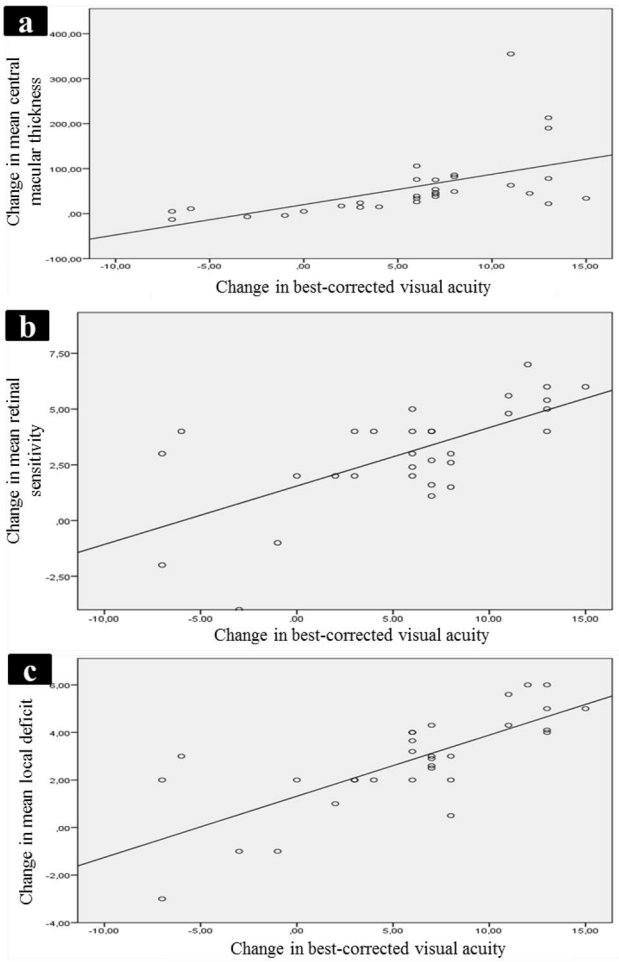

Fig. 4 Graphical representation of the correlation analyses between improved in best-corrected visual acuity and changes in mean central macular thickness (**a**), mean retinal sensitivity (**b**), and mean local deficit (**c**) after 3 months of intravitreal bevacizumab therapy

correlation was found between changes in microperimetry and OCTA parameters.

Discussion

Diabetic macular edema, the most commonly diabetic retinopathy microvascular complication, is one of the main reasons diabetic patients visit an ophthalmologist, as it causes decreased visual acuity. There are approximately 21 million DME patients worldwide, a figure that is expected to rise gradually as diabetes mellitus prevalence rises and patients live longer [16]. As a consequence, the significance and efficacy of DME treatment have grown. Visual acuity alone does not accurately reflect the visual function of DME patients. Scotoma extent and depth may also cause additional visual symptoms in these patients. This was the primary motivation for designing this study, which sought to investigate naive DME patients in terms of changes in various ocular microvascular, anatomical, and functional parameters from different diagnostic perspectives, including OCT, microperimetry, and OCTA after IVBT. A thorough investigation of the correlations between these changes and visual outcomes was also carried out.

Anti-VEGF agents have emerged as the first step in DME treatment, after the discovery of the VEGF molecules' role in DME pathogenesis and anti-VEGF agent development. Bevacizumab, an off-label recombinant monoclonal IgG1 antibody, is the most commonly used intravitreal anti-VEGF agent today, alongside ranibizumab and aflibercept [17, 18]. It has been used to treat DME, retinal vein occlusions, age-related macular degeneration, and choroidal neovascularization. Its effectiveness lasts 4–6 weeks, with a dose of 1.25 mg being the most commonly used. Because of its structural and functional similarity to ranibizumab, as well as its low cost, it is widely used all over the world. A prospective randomized comparison of macular laser and IVBT in DME treatment, involving 103 eyes of 97 patients divided into 1.25 mg bevacizumab, 1.25 mg bevacizumab plus 2 mg triamcinolone combined, and laser groups, revealed significantly higher visual acuity in the first two groups compared to the last [19]. Likewise, a 12-month BOLT study [20] involving 80 eyes of 80 DME patients randomly assigned to receive intravitreal 1.25 mg bevacizumab at baseline and at 6 and 12 weeks or macular laser treatment at baseline and then assessed every 4 months revealed that the former group gained an average of 5.6 letters while the latter group lost an average of 4.6 letters. Furthermore, in the former group, the proportion of patients with more than 10 letter gains was 5.1 times higher than in the latter.

Generally, visual acuity evaluation, color fundus photographs, and OCT are routinely used in diabetic retinopathy monitoring. Aside from nonquantitative data such as photoreceptor inner segment/outer segment band, exudations, serous macular detachment, retinal inner layer disorganization, and vitreoretinal interface disorders, OCT provides quantitative data such as CMT and MV that can be used for longitudinal patient monitoring as well as clinical study comparison [21]. These OCT data have been used to distinguish clinically significant macular edema from clinically nonsignificant macular edema despite their nonsignificant differences [22]. Furthermore, a significant correlation has been reported between decreased CMT and improved visual acuity following macular laser photocoagulation [23]. Evaluation of initial CMT effects on IVBT revealed a group that benefited most from treatment to be the one with CMT > 350 µm [24]. Similarly, initial CMT was found to be higher in responders than in non-responders after intravitreal ranibizumab treatment in DME patients, leading the authors to conclude that initial CMT was the most powerful predictor of treatment response [25]. Another 12-month IVBT study involving 175 eyes from 142 DME patients, grouping

patients based on CMT, that is, those with CMT < 400 µm and those with CMT > 400 µm, revealed that the latter group had more injection needs than the former. However, there was no statistically significant difference in letter gain between them [26]. In the current study, both CMT and MV also decreased significantly after IVBT, which correlated significantly with visual improvement. Nonetheless, baseline CMT and MV had no effect on letter gain following IVBT.

Microperimetry provides varying-intensity stimuli to predetermined points on the macula, revealing the functional status of relevant anatomical points and allowing evaluation of retinal sensitivities at these points. It can thus contribute to diabetic retinopathy patient monitoring by combining anatomical and functional evaluation of the retina in this context. In a cross-sectional study of 61 eyes from 32 diabetic patients divided into three groups, without macular edema, clinically nonsignificant macular edema, and clinically significant macular edema, there was a gradual increase in CMT and a gradual decrease in central macular sensitivity from the first to the third group, with a significant correlation between them ($r = -0.37$, $p < 0.0001$) [27]. A correlation between retinal sensitivity and visual acuity in DME patients has also been reported, as has a proportional decrease in retinal sensitivity and visual acuity as CMT increases [28]. Also, the mean retinal sensitivity and local deficit in DME patients have also been reported to be significantly lower than in healthy individuals ($p = 0.001$) [29]. Similarly, significantly increased BCVA and mean retinal sensitivity, as well as significantly decreased CMT, have been reported after a single intravitreal triamcinolone acetonide [30]. Malagola et al. [31] reported significantly improved visual acuity ($p = 0.012$) and mean retinal sensitivity ($p = 0.025$), as well as significantly decreased CMT ($p = 0.003$) in 76.9% of 26 DME patients in a 4-week interval 1 mg IVBT study. In the same study, retinal sensitivity increased from 8.29 to 14.26 dB before and after treatment, respectively. Before treatment, 23.07% of the patients had relatively stable fixation, while the other 52% had unstable fixation. However, 38.46% of patients had relatively stable fixation after treatment, while 69.57% had unstable fixation. Moreover, mean retinal sensitivity in capillary non-perfused areas on fundus fluorescein angiography in diabetic retinopathy patients has been reported to range from 0 to 1.7 dB, with nearly all of them being 0 dB (completely unresponsive). When compared to capillary perfused areas, retinal sensitivity of the points closest to capillary non-perfused areas decreased significantly ($p < 0.01$) [32]. In the current study, microperimetry analyses revealed significant improvements in fixation stability, mean retinal sensitivity, and mean local deficit in naive DME patients after three IVBT sessions. Furthermore, these neuroretinal functional improvements, particularly mean retinal sensitivity and local deficit, were significantly correlated with improved BCVA.

Alonso-Plasenca et al. [33] published a study comparing diabetic retinopathy patients to healthy individuals with BCVAs of 20/25 and 20/20, respectively, and found that the former group had significantly lower mean retinal sensitivity in the entire central macular area and significantly lower VDs in seven out of nine central macular areas than the latter. Only one area was associated with a moderate correlation between VDs and retinal sensitivity in diabetic retinopathy patients ($r = 0.501$, $p = 0.01$). Pereira et al.'s study [34] of five ischemic DME patients who received unspecified dose IVBT for 6 months revealed that after IVBT, BCVAs increased from 20/180 to 20/74 ($p = 0.01$), and mean retinal sensitivity increased from 11.66 to 16.26 dB ($p < 0.007$). One patient had stable fixation, three had relatively unstable fixation, and one had unstable fixation prior to IVBT. Following IVBT, two patients had stable fixations, while three had relatively unstable fixations. The same study also found that posttreatment microperimetry changes were more closely related to retinal thickness changes than BCVA and macular ischemia and that patients had enlarged FAZ areas measured with OCTA after IVBT ($p = 0.02$). Likewise, microperimetry changes such as mean retinal sensitivity and local deficit were found to be correlated with changes in BCVA, CMT, and MV in the current study. There was, however, no statistically significant change in the FAZ area after IVBT.

Capillary microcirculation disorder develops in early DM stages, particularly in diabetic retinopathy patients, and this disorder, which may be observed on OCTA but not directly revealed on fundus fluorescein angiography, is more common in DCP [35]. Compared to anti-VEGF responders, non-responders had significantly lower DCP VDs, which were associated with more microaneurysms and larger FAZ areas in one DME study. However, there were no significant differences in SCP VDs, FAZ area, or the number of microaneurysms between them [36]. A comparison of 29 eyes of 29 diabetic patients without diabetic retinopathy versus 33 eyes of 33 healthy individuals also revealed significantly lower VDs in SCP and DCP, as well as a significantly larger FAZ area of SCP in the former group [12]. Furthermore, SCP and DCP VDs have been reported to be lower in 102 eyes of 102 diabetic retinopathy patients compared to 60 eyes of 60 healthy individuals, with a negative correlation between both capillary plexus VDs and diabetic retinopathy severity, as well as a positive correlation between DCP FAZ area and diabetic retinopathy severity. These findings indicate that DCP could be primarily affected earlier in diabetic retinopathy [37]. Likewise, a comparison among 45 DME patients, 40 non-DME patients, and 40 healthy individuals revealed significantly lower DCP VDs in the first group, which was accompanied by a significant negative correlation between SCP VDs and BCVA, as well as a significant positive correlation between

FAZ areas of both capillary plexuses and BCVA [38]. In contrast to the preceding reports, Sorour et al. [39] found no significant changes in SCP and DCP VDs after treatment in 55 eyes (46 DME and 9 proliferative diabetic retinopathy) of 35 patients who were treated and followed up with intravitreal bevacizumab, aflibercept, and ranibizumab. They also found no evidence of any correlation between treatment and response.

The current study also found no significant changes in OCTA parameters after IVBT. However, intergroup comparisons revealed significant differences in FAZ area between groups 1 and 2, but not between groups 1 and 3 or 2 and 3. The mean FD-300 in group 1 was also significantly higher than in groups 2 and 3. There were no significant differences in SCP VDs among groups. Despite the fact that group 1 had significantly higher foveal, as well as superior and inferior parafoveal DCP VDs than group 2, no significant differences existed between groups 1 and 3 or 2 and 3. The temporal and nasal parafoveal DCP VDs also increased significantly more in group 1 than in group 2, and significantly more in group 3 than in group 2. In general, these findings indicate that patients with significantly smaller FAZ areas, as well as significantly increased FD-300 and DCP VDs, could benefit more from intravitreal anti-VEGF therapy, in this case, bevacizumab.

Limitations of the current study were primarily its relatively short follow-up period, small study population, as well as the lack of a control group. A suitable control group, on the other hand, maybe hard to obtain because the majority of DME patients are presently receiving anti-VEGF therapy at least initially. Nonetheless, given the current study outcomes, a sham control group in a later study appears to be somewhat relevant for at least a limited time. Besides, many imaging artifacts are reportedly connected to OCTA imaging, which could have affected the study outcomes. In the current study, however, multiple strategies were adopted to reduce the impact of these artifacts on the outcomes. This entailed removing patients with lenticulo-corneal opacity that would impede OCT, OCTA, and microperimetry acquisition, persistent major artifacts, or images with an $SQ \leq 6$ on OCTA, as well as repeating imaging so that there were no significantly visible artifacts. Furthermore, minor segmentation errors were manually corrected, full-thickness retinal slabs were used, which are minimally influenced by both segmentation errors and edema, motion artifacts were minimized using the device's eye-tracking system, and any remaining artifacts were removed using a projection artifact removal system. Large-scale anti-VEGF studies with longer follow-up periods, including not only naive DME but also other stages of DME, would be worthwhile to investigate correlations of retinal microvascular status with ultrastructural and functional improvements.

Conclusions

A smaller FAZ area, as well as higher FD-300 and DCP VDs in OCTA analysis, appeared to be associated with improved functional recovery after IVBT in naïve DME patients. Significant improvements in both OCT findings demonstrating anatomical and ultrastructural integrity and microperimetry findings demonstrating improved neuroretinal function correlated with each other and could thus be predicted by OCTA findings.

Author contribution FFG: Project administration, conceptualization, methodology, and software. HHG: Methodology, data curation, and writing-reviewing and editing. SI: Project administration, visualization, investigation, and methodology. MCS: Formal analysis, visualization, investigation, and writing-original draft preparation. AA: Formal analysis, visualization, investigation, writing-original draft preparation. FFG = Furkan Fatih GULYESIL; HHG = Hamidu Hamisi GOBEKA; SI = Sibel INAN; MCS = Mehmet Cem SABANER; AA = Anar ALIZADA.

Availability of data and materials Data are available from the corresponding author on reasonable request.

Declarations

Conference presentation disclosure This original study has not been submitted or is not under consideration for publication in another journal. The abstract of the study, however, was presented orally at the 19TH BLACK SEA OPHTHALMOLOGICAL SOCIETY CONGRESS September 24–26, 2021 Chisinau, Republic of Moldova, ONLINE.

Ethics approval The study procedure abided by the ethical standards of the Helsinki Declaration and obtained full approval from the Institutional Review Boards of the Afyonkarahisar Health Sciences University Ethics Committee. Prior to the study, all participants issued informed written consent.

Consent to participate My colleagues and I conducted the research and co-authored the manuscript. We have all approved the manuscript for submission and publication in your journal, and I am the corresponding author.

Consent for publication My colleagues and I conducted the research and co-authored the manuscript. We have all approved the manuscript for submission and publication in your journal, and I am the corresponding author.

Conflict of interest The authors declare no competing interests.

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