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ORIGINAL PAPER

Macular vessels density in diabetic retinopathy: quantitative assessment using optical coherence tomography angiography

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Abstract
Purpose The aim of this study was to evaluate macular perfusion using OCTA automated software algorithms; vessel area density (VD) and non-flow tool to measure FAZ area in treatment-naïve diabetic eyes with moderate or severe NPDR and having macular edema, and correlate these parameters with LogMAR (logarithm of the minimum angle of resolution) visual acuity. Diabetic eyes without macular edema were included, to detect and define differences within the parameters between diabetic eyes with and without macular edema.
Methods Forty-five diabetic eyes with diabetic macular edema, forty diabetic eyes without macular edema, and forty eyes of healthy controls were examined using OCTA (RTVue-XR Avanti; Optovue, Inc, Fremont, CA). The macular vessel area density (VD) and foveal avascular zone (FAZ) area were assessed and statistically compared between the three groups and also correlated with the foveal thickness and visual acuity. Data were entered and analyzed by SPSS 19. Quantitative data were presented as mean and standard deviation, and qualitative data presented as frequency distribution; independent samples t test, Chi square test and Pearson correlation were done.
Results Mean whole image VD was 44.4 ± 3.6 in diabetic eyes with DME, 45.6 ± 4.2 in diabetics without DME, and 49 ± 3.9 in control eyes ($P = 0.001$). Diabetic eyes with DME had significantly lower vessels density values at the level of the deep retinal plexus (in the parafoveal, superior hemi, inferior hemi, temporal, superior, and nasal areas), when compared with diabetic eyes without DME. In diabetic eyes with DME, significant fair negative correlation was found between whole image vessels density at the level of the superficial retinal plexus and LogMAR VA ($r = -0.313$, $P = 0.036$). Also, a significant fair positive correlation was found between FAZ area (at both the superficial and deep retinal plexus) and LogMAR visual acuity, in diabetic eyes with DME, where eyes with larger FAZ area had worse vision ($P = 0.005$ and $P = 0.016$, respectively). Diabetic eyes with DME had significantly larger FAZ area at the level of the superficial capillary plexus (mean superficial FAZ ± SD 0.55 ± 0.25) than diabetic eyes without edema (mean superficial FAZ ± SD 0.41 ± 0.12) and control subjects (mean superficial FAZ ± SD 0.35 ± 0.09).

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Conclusion Using OCTA machine with AngioAnalytics parameters (vessel area density and non-flow area) helped in objective quantification of macular perfusion and accurately measuring the FAZ area in diabetic eyes with macular edema. Both parameters were significantly correlated with visual function in treatment-naïve diabetic eyes with edema. These OCTA biomarkers could be used to predict visual function in such eyes, to monitor response to treatment.

Keywords Macular vessel density · FAZ · Macular perfusion · Macular ischemia · Diabetic retinopathy · OCTA

Abbreviations

OCTA	Optical coherence tomography angiography
FAZ	Foveal avascular zone
LogMAR	Logarithm of the minimum angle of resolution
VD	Vessel area density
SD	Standard deviation
DR	Diabetic retinopathy
DMI	Diabetic macular ischemia
DME	Diabetic macular edema
BCVA	Best corrected visual acuity
D	Diopter
NPDR	Non-proliferative diabetic retinopathy
PDR	Proliferative diabetic retinopathy
SD OCT	Spectral domain optical coherence tomography
A-scan	Amplitude scan
B-scans	Brightness scan
SSADA	Split-spectrum amplitude-decorrelation angiography
SSI	Signal strength index
SPSS	Statistical package for the social sciences
VA	Visual acuity
ILM	Internal limiting membrane
RPE	Retinal pigment epithelium
VLD	Vessel length density
VAD	Vessel area density
Anti-VEGF	Anti-vascular endothelial growth factor
EFI	Extended field imaging

Introduction

The most leading cause of visual loss in the working-age population in developed countries is diabetic retinopathy (DR) [1]. Diabetic macular edema (DME) involves increased retinal thickness at the macular area, due to leakage from hyper-permeable and dilated capillaries and microaneurysms, with subsequent breakdown of the blood–retinal barrier [2]. This edema is considered the most common cause of visual acuity deterioration in patients with DR [3, 4].

Diabetic macular ischemia (DMI) is important in the pathogenesis of diabetic retinopathy (DR) and is characterized by alterations in retinal capillaries including narrowing or occlusion [5]; these vascular changes lead to tissue hypoxia with subsequent increased vascular endothelial growth factor levels, resulting in diabetic macular edema [6]. Diabetic macular ischemia (DMI) results in visual impairment, either associated with DME or not [7].

Several diagnostic tools are used, including fluorescein angiography (FA), optical coherence tomography (OCT), to study different stages of diabetic retinopathy, macular edema, and ischemia. Recently, optical coherence tomography angiography (OCTA) offers a number of advantages over fluorescein angiography as it has allowed for rapid non-invasive acquisition of high-resolution depth-resolved images of the retinal vascular layers. [8]. OCTA has been used to visualize the chorioretinal microcirculation, with high reliability and reproducibility in several diseases (age-related macular degenerations, and retinal vascular diseases, e.g., diabetic retinopathy, retinal vascular occlusion), and in healthy eyes [9–11].

In diabetic eyes, OCTA was able to study vascular retinal layers separately without dye injection and detect retinal vascular abnormalities including microaneurysms, venous beading, erosion of the FAZ, vascular loops, areas of ischemia, and neovascularizations [12].

With the introduction of different automated quantification algorithms, certain angiographic data can be extracted, including foveal avascular zone (FAZ) area, vascular density, areas of non-flow, and vessel length from OCTA scans [13–15]. These algorithms helped in quantification of several angiographic parameters in DR eyes as markers for DMI [9, 13]. In cases with retinal neovascularization, measuring the flow index and vessel area allowed for a quantitative assessment for its activity and extent, which helped to monitor follow-up responses to laser photocoagulation and/or intravitreal injections of anti-vascular endothelial growth factor (VEGF), on a quantitative basis [16].

In this study, we evaluated macular perfusion using OCTA automated software algorithms, evaluated vessel area density (VD), and using non-flow tool measured FAZ area in diabetic eyes with macular edema, correlating them with LogMAR (logarithm of the minimum angle of resolution) visual acuity. Also diabetic eyes without macular edema were included, to detect and define differences within the parameters between diabetic eyes with and without macular edema. Diabetic eyes were selected to be treatment naïve, to obviate any effect of previous treatment on macular perfusion (like intravitreal injection of anti-VEGF or laser therapy). This inclusion criteria made the difference between the current study and some previous studies that had studied FAZ area and vessel area density in diabetic eyes (with or without edema), either received previous treatment or being treatment naïve [5, 12, 13].

Methods

Subjects

This observational case series included 45 eyes from 45 diabetic patients with DME, 40 eyes from diabetic patients without DME, and 40 eyes from 40 age-matched healthy individuals. The study gained approval from the Local Research Ethics Committee, Faculty of Medicine, Minia University Hospital, Egypt. The present study was also conducted in accordance with the Helsinki Declaration. All participants were evaluated at the Ophthalmology Outpatient Clinic of Minia University Hospital between June 1, 2016 and December 31, 2017. Written consent forms were signed by all patients that participated in the study. A complete ophthalmic examination was performed for all participants including history taking, best corrected visual acuity (BCVA) assessment (values were converted into LogMAR VA), anterior segment examination using slit lamp biomicroscopy, fundus examination using a 78D Volk lens, automated refraction using the Nidek autoref/keratometer (LS 900, Haag Streit Diagnostics, Switzerland). Colored fundus photographs were obtained for control and diabetic subjects, and fluorescein angiography photographs were captured for diabetic subjects using a TOPCON TRC-XXX fundus camera (Topcon Corporation, IMAGE net 2000^{TM}, Tokyo, Japan).

All included diabetic eyes fulfilled the following criteria: 1) diagnosed with moderate or severe non-proliferative diabetic retinopathy (NPDR) by fundus examination based on the International Clinical Diabetic Retinopathy and Diabetic Macular Edema Severity Scale [17]; 2) being treatment naïve (no previous history of laser photocoagulation or intravitreal injections); and 3) having or not having macular edema, as diagnosed by spectral domain (SD)-OCT examination. Exclusion criteria included the following: 1) diabetic patients with concomitant retinal disease (e.g., dystrophy, vascular occlusion, or age-related macular degeneration), previous intravitreal injections, laser treatment, media opacity, and/or intraocular surgery; 2) patients with one or more of the following criteria: patients with low signal strength index (SSI; < 50), media opacity obscuring vasculature view, presence of 1 or more blink artifacts, or poor fixation leading to motion artifacts.

Control subjects were defined as having no history of previous ocular trauma, intraocular surgery, or systemic diseases, clear ocular media, with normal ophthalmic examination, and refraction ≤ 2 D.

Only one eye was selected for each participant in both diabetic and control groups, to avoid any false-positive results, if both eyes were enrolled, as there are similar structural and retinal vasculature characteristics in each individual.

Optical coherence tomography angiography image acquisition

The instrument used for OCT angiography images acquisition is the commercial spectral domain OCT system (Avanti RTVue-XR; Optovue, Fremont, CA), which is based on the AngioVue Imaging System to obtain amplitude-decorrelation angiography images. It is a dual-modality OCT-based system, capable of imaging both function and structure of ocular microvasculature. A-scan rate of this machine is 70,000 scans per second, using a light source centered on 840 nm and a bandwidth of 50 nm. The tissue resolution is 5 µm axially, and the beam width is 20 µm. Each OCTA volume contains 304×304 A-scans with two consecutive B-scans captured at each fixed position before proceeding to the next sampling location. Split-spectrum amplitude-decorrelation angiography (SSADA) was used to extract the OCT angiography information. Each OCTA volume is acquired in 3 s, and two orthogonal OCTA volumes were acquired in order to perform motion correction to minimize motion artifacts arising from micro-saccades and fixation changes. Angiography information displayed is the average of the decorrelation values when viewed perpendicularly through the thickness being evaluated. The images were captured with the Angio Retina protocol, with a scan area 6 × 6 mm.

Vessel area density (VD) and foveal avascular zone (FAZ) measurement

AngioAnalytics are quantification tools that enabled measurement of vessel area density, and non-flow area to measure the size of the FAZ. Vessel density map was used to assess and compare the relative density of flow, at the level of the superficial and deep retinal capillaries, as a percentage of the total area. Using the acquired images, the FAZ area was measured in square millimeters (mm^2), using the non-flow function on the OCTA software at the level of the superficial and deep retinal networks. When the observer clicks on the center of the FAZ, the area of FAZ is automatically calculated by the software (RTVue-XR version: 2017.1.0.151). Optical coherence tomography angiography images of the superficial and deep networks were generated using the automated software algorithm. Based on these default settings, the boundaries of superficial network extended from 3 µm below the internal limiting membrane to 15 µm below the inner plexiform layer. A 30-µm-thick layer from the inner plexiform layer was used to visualize the deep retinal plexus, in its entirety. Then the slab section was moved slight posteriorly centered on the outer plexifom layer in order to eliminate projection artifacts from the superficial vascular plexus.

Statistical method

The collected data were coded, tabulated, and statistically analyzed using SPSS program (Statistical Package for Social Sciences) software version 24.

Descriptive statistics were done for parametric quantitative data by mean, standard deviation and minimum and maximum of the range, and for nonparametric quantitative data by median, while they were done for categorical data by number and percentage.

Analyses were done for parametric quantitative data between the three groups using one-way ANOVA followed by post hoc Tukey analysis between each two groups, and for nonparametric quantitative data using Kruskal–Wallis test followed by Mann–Whitney test between the two groups.

Analyses were done for qualitative data using Chi-square test

Correlation between two quantitative variables was done by using Pearson's correlation coefficient. Correlation coefficient ranges from 0 to 1—weak (r = 0–0.24), fair (r = 0.25–0.49), moderate (r = 0.5–0.74), strong (r = 0.75–1).

Analyses were done for parametric quantitative data between the two groups using independent samples t test, and for nonparametric quantitative data using Mann–Whitney test between the two groups. The level of significance was taken at (P value < 0.05).

Results

This prospective observational study included 45 eyes (group I) from 45 diabetic patients with macular edema (17 males and 28 females), 40 eyes (group II) from 40 diabetic patients without macular edema (11 males and 29 females), and 40 eyes (group III) from 40 age-matched healthy individuals (16 males and 24 females). No significant difference was found between both groups, regarding age and sex.

Diabetic eyes with macular edema had significantly larger FAZ area at the level of the superficial capillary plexus (mean superficial FAZ ± SD 0.55 ± 0.25) than diabetic eyes without edema (mean superficial FAZ ± SD 0.41 ± 0.12) and control subjects (mean superficial FAZ ± SD 0.35 ± 0.09). The FAZ area was significantly larger in diabetic eyes (either with or without edema) than the control eyes, at the level of deep capillary plexus.

A significantly worse visual acuity was found in diabetic eyes with macular edema (mean ± SD LogMAR 0.66 ± 0.35), than diabetic eyes without macular edema (0.04 ± 0.05), and controls (Mean ± SD LogMAR 0.05 ± 0.16), as seen in Table 1.

Diabetic eyes with macular edema had significantly lower superficial and deep VD values (whole image, parafoveal, and in all sectors), than control eyes. Also diabetic eyes with macular edema have lower VD values at the level of the deep capillary plexus (in the parafoveal, superior hemi, inferior hemi, temporal and nasal areas) when compared with diabetic eyes without edema. Diabetic eyes without edema had significantly lower whole image VD values (at the level of the superficial and deep capillary plexuses), than control eyes, as seen in Table 2.

Diabetic eyes with macular edema had significantly thicker retina, than diabetic eyes without edema and control group, in all macular regions, with the highest thickness in the superior area, as seen in Table 3.

A significant fair negative correlation was found between the whole image vessels density at the level of the superficial retinal plexus and LogMAR VA in diabetic eyes with macular edema. In diabetic eyes without macular edema, the superficial and deep whole image VDs have strong and moderate negative correlation with LogMAR, respectively (Table 4).

A statistically significant negative correlation was found between the FAZ area and foveal VD at the level of deep retinal plexus, in diabetic eyes with macular

Table 1 Demographic and clinical data of the participants

	Group I DM with edema $N = 45$	Group II DM without edema $N = 40$	Group III Control $N = 40$	*P* value I vs II	I vs III	II vs III
Age[a]						
Range	(22–68)	(22–69)	(23–67)	*0.838*	*0.260*	*0.591*
Mean ± SD	55.2 ± 9.6	54 ± 10	51.8 ± 10.2			
Sex[c]						
Male: *n* (%)	17 (378%)	11 (27.5%)	16 (40%)	*0.314*	*0.834*	*0.237*
Female: *n* (%)	28 (62.2%)	29 (72.5%)	24 (60%)			
VA[b] (Log MAR)						
Range	(0–1.3)	(0–0.18)	(0–1)	***< 0.001****	***< 0.001****	*0.181*
Mean ± SD	0.66 ± 0.35	0.04 ± 0.05	0.05 ± 0.16			
Median	0.6	0	0			
Superficial FAZ[b,¶]						
Range	(0.22–1.23)	(0.25–0.83)	(0.2–0.51)	***0.021****	***< 0.001****	***0.012****
Mean ± SD	0.55 ± 0.25	0.41 ± 0.12	0.35 ± 0.09			
Median	0.47	0.38	0.33			
Deep FAZ[b,¶]						
Range	(0.23–1.84)	(0.3–0.9)	(0.22–0.57)	*0.110*	***< 0.001****	***< 0.001****
Mean ± SD	0.6 ± 0.31	0.47 ± 0.13	0.37 ± 0.1			
Median	0.51	0.44	0.35			

Data are no., no. (%), or mean ± standard deviation

LogMAR logarithm of the minimum angle of resolution, *VA* visual acuity, *FAZ* foveal avascular zone, *SD* standard deviation

[a]One-way ANOVA for parametric quantitative data between the three groups followed by post hoc Tukey analysis between each two groups

[b]Kruskal Wallis test for nonparametric quantitative data between the three groups followed by Mann–Whitney test between each two groups

[c]Chi-square test for qualitative data between groups

¶Significant difference between the three groups ($P < 0.05$)

*Significant difference at P value < 0.05

Table 2 Comparison of the range, mean ± SD of VD values in eyes with diabetic retinopathy (group I with edema, and group II without edema), and healthy controls (group III) at the level of superficial and deep retinal capillaries

VD (%)	Group I Diabetics with edema $N = 45$	Group II Diabetics without edema $N = 40$	Group III Control $N = 40$	*P* value I vs II	I vs III	II vs III
Superficial						
Whole image¶	(38.6–53.9) 44.4 ± 3.6	(38.2–52) 45.6 ± 4.2	(41.4–56.2) 49 ± 3.9	*0.345*	***< 0.001****	***< 0.001****
Foveal area¶	(16.3–58.7) 31.7 ± 8.5	(19.1–39.5) 28.1 ± 4.6	(13.9–34.8) 27.8 ± 5.2	***0.029****	***0.017****	*0.981*
Parafovea¶	(37.4–57.6) 45 ± 4.3	(39.6–53.9) 46.4 ± 4.4	(39.6–57.3) 48.8 ± 5.3	*0.364*	***0.001****	*0.057*
Superior hemi¶	(35.4–58.4) 44.7 ± 4.5	(39.8–55) 46.5 ± 4.6	(33.9–56.8) 48.5 ± 6	*0.246*	***0.003****	*0.193*
Inferior hemi¶	(37.7–56.7) 45.1 ± 4.8	(38.9–54.1) 46.4 ± 4.4	(38.9–58.3) 49 ± 5.2	*0.427*	***0.001****	***0.040****
Temporal¶	(31.3–57.8) 44.9 ± 5.9	(38.6–55) 47.3 ± 4.9	(34.2–57.4) 49.6 ± 6	*0.130*	***0.001****	*0.183*
Superior¶	(35.5–58) 45.2 ± 4.6	(37.4–55.3) 46 ± 4.8	(40.3–58.7) 49.2 ± 5.5	*0.726*	***0.001****	***0.015****
Nasal¶	(32–57.8) 43.8 ± 5.8	(37–56.6) 46.3 ± 4.9	(28.2–55.8) 47.7 ± 6.5	*0.130*	***0.007****	*0.511*
Inferior¶	(32.9–56.8) 45.5 ± 5.7	(35.4–56.2) 46.2 ± 5.2	(40.4–58.5) 50 ± 5.4	*0.826*	***0.001****	***0.006****
Deep						
Whole image¶	(36–51.9) 44.1 ± 4.1	(37.6–53) 46.2 ± 4.7	(42.1–56.5) 49.6 ± 3.9	*0.064*	***< 0.001****	***0.001****
Foveal area¶	(17.6–41.4) 28.7 ± 6.9	(7.3–37) 23.9 ± 7.6	(15.8–34.2) 26.6 ± 5.3	***0.004****	*0.329*	*0.165*
Parafovea¶	(32.9–56) 45.4 ± 5.3	(40–56.3) 48.8 ± 5	(41.2–58.5) 50.4 ± 5.4	***0.010****	***< 0.001****	*0.365*
Superior hemi¶	(32.1–58.1) 45.3 ± 5.7	(39.2–57.2) 49.2 ± 5.3	(35.5–58.7) 49.8 ± 5.7	***0.004****	***0.001****	*0.901*
Inferior hemi¶	(31.4–54.8) 45.5 ± 5.3	(39.2–56) 48.4 ± 4.8	(41.5–58.8) 50.2 ± 5.4	***0.033****	***< 0.001****	*0.285*
Temporal¶	(28.3–60) 46.1 ± 6.6	(29.8–58.7) 49.8 ± 5.9	(35–60) 51.4 ± 5.5	***0.017****	***< 0.001****	*0.465*
Superior	(31.9–60.1) 45.3 ± 6	(37.4–83.6) 49.6 ± 7.9	(40–58.5) 49.4 ± 5.5	***0.008****	***0.012****	*0.990*
Nasal¶	(26.9–57) 44.3 ± 6.7	(38.9–57.8) 49.2 ± 5.7	(35.8–58.7) 50.3 ± 5.8	***0.001****	***< 0.001****	*0.723*
Inferior¶	(34.2–54.3) 45.6 ± 5.2	(36.1–56.8) 47.6 ± 5.7	(38.6–58.3) 50 ± 5.8	*0.228*	***0.001****	*0.124*

One-way ANOVA for parametric quantitative data between the three groups followed by post hoc Tukey analysis between each two groups

VD Vessel density

¶Significant difference between the three groups ($P < 0.05$)

*Significant difference at *P* value < 0.05

edema (group I), as eyes with decreased foveal VD have larger FAZ area. Also LogMAR VA was negatively correlated with foveal VD at the level of the superficial retinal plexus, in diabetic eyes with edema.

In diabetic eyes without macular edema (group II) and control group (group III), the FAZ area was significantly larger in cases with lower foveal vessel density values at the level of both the superficial and deep retinal plexuses, as shown in Table 5.

In diabetic eyes with macular edema (group I), a fair negative correlation was found between the FAZ area and parafoveal VD values, at the level of both the superficial and deep retinal plexuses. While in diabetic eyes without edema (group II), a moderate negative correlation was found between parafoveal VD values (at the level of the superficial and deep retinal plexuses) and LogMAR VA (Table 6).

Diabetic eyes with macular edema (group I), who had larger FAZ areas (either at the level of the superficial or deep retinal plexuses) had worse visual acuity, as a significant fair positive correlation was found between FAZ size (either at the level of the superficial or deep retinal plexuses), and LogMAR VA, as shown in Table 7. While in diabetic eyes without macular edema (group II), larger FAZ areas (either at the level of the superficial or deep retinal plexuses), were associated with decreased foveal thickness, as seen in Table 7.

Table 3 Comparison of the range, mean ± SD of retinal thickness (ILM-RPE) in different macular areas in eyes with diabetic retinopathy (group I with edema, and group II without edema), and healthy controls (group III)

Thickness (μm)	Group I Diabetics with edema $N = 45$	Group II Diabetics without edema $N = 40$	Group III Control $N = 40$	*P* value I vs II	I vs III	II vs III
Foveal area[¶]	(190–1083) 374.9 ± 172.3	(180–270) 227.8 ± 21	(212–278) 241.6 ± 17.2	***< 0.001****	***< 0.001****	*0.826*
Parafovea[¶]	(262–826) 380.8 ± 108.8	(248–343) 306.1 ± 20.2	(297–346) 316.8 ± 13.6	***< 0.001****	***< 0.001****	*0.757*
Superior hemi[¶]	(274–826) 385.8 ± 114.2	(251–346) 305.4 ± 20.3	(293–348) 318.2 ± 14.3	***< 0.001****	***< 0.001****	*0.694*
Inferior hemi[¶]	(249–825) 374.3 ± 111.8	(246–341) 306.9 ± 20.2	(298–344) 316.4 ± 12.6	***< 0.001****	***< 0.001****	*0.809*
Temporal[¶]	(254–814) 378.3 ± 118	(259–339) 299.4 ± 20.4	(283–338) 306.1 ± 15.1	***< 0.001****	***< 0.001****	*0.910*
Superior[¶]	(274–789) 388.3 ± 107.6	(246–350) 309.2 ± 21.2	(297–359) 322.9 ± 14.5	***< 0.001****	***< 0.001****	*0.628*
Nasal[¶]	(264–904) 383.3 ± 120.3	(242–343) 306.5 ± 20.4	(295–353) 317.8 ± 15.8	***< 0.001****	***< 0.001****	*0.769*
Inferior[¶]	(249–796) 371.1 ± 105.5	(246–348) 309.7 ± 20.7	(298–347) 319.3 ± 12.9	***< 0.001****	***0.001****	*0.788*

ILM Internal limiting membrane, *RPE* retinal pigment epithelium

* Significant correlation

Table 4 Correlation between whole image VD (at the level of the superficial and deep retinal plexuses), size of the FAZ (at the level of the superficial and deep retinal plexuses), foveal thickness, LogMAR visual acuity, among eyes with diabetic retinopathy (group I with edema and group II without edema), and healthy control (group III)

	VD whole image	FAZ		Foveal thickness		LogMAR VA	
		R	*P* value	*R*	*P* value	*r*	*P* value
Group I ($n = 45$)	Superficial	*− 0.246*	*0.104*	*0.225*	*0.137*	***− 0.313***	***0.036****
	Deep	*− 0.280*	*0.062*	*0.197*	*0.196*	*0.044*	*0.772*
Group II ($n = 40$)	Superficial	*− 0.086*	*0.599*	*− 0.151*	*0.352*	***− 0.834***	***< 0.001****
	Deep	*− 0.242*	*0.133*	*− 0.159*	*0.326*	***− 0.757***	***< 0.001****
Group III ($n = 40$)	Superficial	*0.036*	*0.825*	*− 0.061*	*0.706*	*− 0.152*	*0.349*
	Deep	*− 0.005*	*0.973*	*− 0.007*	*0.964*	*− 0.150*	*0.355*

Pearson's correlation coefficient

Grades for correlation (r): 0.00 to 0.24 (weak or no association), 0.25 to 0.49 (fair association), 0.50–0.75 (moderate association), and > 0.75 (strong association)

VD Vessel density, *FAZ* foveal avascular zone, *LogMAR* logarithm of the minimum angle of resolution, *VA* visual acuity

* Significant correlation

Although all diabetic eyes in group (I) had macular edema, as diagnosed by SD-OCT examination, no significant correlation was found between VD (either at the level of the superficial or deep retinal plexus) and the corresponding retinal thickness, in all macular regions, as seen in Table 8.

A significant difference was only found between the size of the FAZ area at the level of the deep and the superficial retinal plexuses (deep FAZ area was larger in size) in diabetic eyes without macular edema, also parafoveal vessel density at the level of the deep capillary plexus was significantly higher than parafoveal vessel density at the superficial level, in the same group (group II), Table 9.

Discussion

Optical coherence tomography using split-spectrum amplitude-decorrelation angiography (SSADA) provides a useful non-invasive tool for qualitative imaging and quantitative analysis of the two major layers of the retinal vasculature as well as choriocapillaris without dye injection, aiding in the study of changes in

Table 5 Correlation between foveal VD (at the level of the superficial and deep retinal plexuses), size of the FAZ (at the level of the superficial and deep retinal plexuses), foveal thickness, LogMAR visual acuity, among eyes with diabetic retinopathy (group I with edema, and group II without edema), and healthy control (group III)

	VD foveal	FAZ		Foveal thickness		LogMAR	
		R	*P* value	*R*	*P* value	*R*	*P* value
Group I (*n* = 45)	Superficial	*− 0.267*	*0.076*	*0.127*	*0.407*	***− 0.426***	***0.003****
	Deep	***− 0.340***	***0.022****	*0.220*	*0.147*	*− 0.087*	*0.569*
Group II (*n* = 40)	Superficial	***− 0.596***	***< 0.001****	*0.232*	*0.149*	*− 0.138*	*0.395*
	Deep	***− 0.383***	***0.015****	*0.092*	*0.573*	*0.122*	*0.453*
Group III (*n* = 40)	Superficial	***− 0.450***	***0.004****	***0.327***	***0.039****	*− 0.139*	*0.393*
	Deep	***− 0.684***	***< 0.001****	***0.573***	***< 0.001****	*− 0.093*	*0.568*

Pearson's correlation coefficient

VD Vessel density, *FAZ* foveal avascular zone, *LogMAR* logarithm of the minimum angle of resolution, *VA* visual acuity

* Significant correlation

Table 6 Correlation between parafoveal VD (at the level of the superficial and deep retinal plexuses), size of the FAZ (at the level of the superficial and deep retinal plexuses), parafoveal thickness, LogMAR visual acuity, among eyes with diabetic retinopathy (group I with edema, and group II without edema), and healthy control (group III)

	VD parafoveal	FAZ		Parafoveal thickness		LogMAR VA	
		R	*P* value	*R*	*P* value	*r*	*P* value
Group I (*n* = 45)	Superficial	***− 0.324***	***0.030****	*0.185*	*0.222*	*− 0.324*	*0.030*
	Deep	***− 0.422***	***0.004****	*0.234*	*0.122*	*0.035*	*0.822*
Group II (*n* = 40)	Superficial	*− 0.174*	*0.282*	*− 0.001*	*0.994*	***− 0.616***	***< 0.001****
	Deep	*− 0.311*	*0.051*	*− 0.069*	*0.670*	***− 0.595***	***< 0.001****
Group III (*n* = 40)	Superficial	*− 0.054*	*0.739*	*0.053*	*0.745*	***− 0.365***	***0.021****
	Deep	*− 0.047*	*0.773*	*− 0.030*	*0.852*	***− 0.346***	***0.029****

Pearson's correlation coefficient

VD Vessel density, *FAZ* foveal avascular zone, *LogMAR* logarithm of the minimum angle of resolution, *VA* visual acuity

* Significant correlation

Table 7 Correlation between size of the FAZ (at the level of the superficial and deep retinal plexuses), foveal thickness, LogMAR visual acuity, among eyes with diabetic retinopathy (group I with edema, and group II without edema), and healthy control (group III)

	FAZ	Foveal thickness		LogMAR VA	
		R	*P* value	*r*	*P* value
Group I (*n* = 45)	Superficial	*0.037*	*0.811*	***0.413***	***0.005****
	Deep	*− 0.028*	*0.854*	***0.357***	***0.016****
Group II (*n* = 40)	Superficial	***− 0.502***	***0.001****	*0.197*	*0.222*
	Deep	***− 0.476***	***0.002****	*0.283*	*0.077*
Group III (*n* = 40)	Superficial	*− 0.305*	*0.056*	*0.246*	*0.126*
	Deep	***− 0.332***	***0.036****	*0.230*	*0.154*

Pearson's correlation coefficient

FAZ Foveal avascular zone, *LogMAR* logarithm of the minimum angle of resolution, *VA* visual acuity

* Significant correlation

Table 8 Correlation between vessel density (VD), and retinal thickness (ILM-RPE) in each sector, in eyes with diabetic retinopathy (group I with macular edema, and group II without edema), and healthy controls (group III)

	Thickness (vs) VD	Group I (*n* = 45)		Group II (*n* = 40)		Group III (*n* = 40)	
		R	*P* value	*r*	*P* value	*R*	*P* value
Foveal	Superficial	*0.127*	*0.407*	*0.232*	*0.149*	***0.327***	***0.039****
	Deep	*0.220*	*0.147*	*0.092*	*0.573*	***0.573***	***< 0.001****
Parafoveal	Superficial	*0.185*	*0.222*	*− 0.001*	*0.994*	*0.053*	*0.745*
	Deep	*0.234*	*0.122*	*− 0.069*	*0.670*	*− 0.030*	*0.852*
Superior hemi	Superficial	*0.165*	*0.279*	*− 0.086*	*0.598*	*0.138*	*0.397*
	Deep	*0.182*	*0.232*	*− 0.109*	*0.504*	*0.030*	*0.853*
Inferior hemi	Superficial	*0.177*	*0.245*	*0.072*	*0.658*	*− 0.008*	*0.960*
	Deep	*0.270*	*0.073*	*− 0.003*	*0.987*	*− 0.123*	*0.451*
Temporal	Superficial	*0.144*	*0.345*	*− 0.044*	*0.788*	*− 0.172*	*0.287*
	Deep	*0.189*	*0.214*	*− 0.168*	*0.301*	*− 0.273*	*0.088*
Superior	Superficial	*0.079*	*0.604*	*− 0.098*	*0.547*	*0.167*	*0.304*
	Deep	*0.125*	*0.412*	*0.007*	*0.967*	*0.058*	*0.722*
Nasal	Superficial	*0.143*	*0.349*	*− 0.024*	*0.884*	*− 0.085*	*0.604*
	Deep	*0.188*	*0.217*	*− 0.002*	*0.992*	*− 0.073*	*0.654*
Inferior	Superficial	*0.189*	*0.215*	*0.073*	*0.654*	*0.105*	*0.517*
	Deep	*0.123*	*0.131*	*− 0.029*	*0.858*	*− 0.042*	*0.797*

Pearson's correlation coefficient

* Significant correlation

diabetic eyes at micro-vascular levels and the monitoring of disease progression [8, 12, 16, 18].

In the present study, we evaluated vessel area density and FAZ area in treatment-naïve diabetic eyes with moderate or severe NPDR and macular edema. Previous parameters (VD and FAZ) were compared between the three study groups (diabetics with DME, diabetics without DME, and control). Then, correlations between VD, FAZ area, and LogMAR VA were studied to assess factors affecting visual acuity in eyes with DME.

Vessel area density is an objective tool in certain OCTA machines that is used to detect areas of retinal blood flow and non-perfusion areas for improved diagnosis, monitoring, and management of diabetic retinopathy [19, 20]. Vessel density can also be used as a marker of disease severity, where patients with lower VD values are likely to progress to more severe stages of retinopathy. Notably, Agemy et al. [13] introduced the concept of using the color-coded perfusion density mapping of OCTA to study the quantitative progression of retinal perfusion changes in diabetic eyes.

Through the use of the vessel density (VD) AngioAnalytics™ tool—which measures the percentage of area occupied by flowing vessels (Fig. 1)—our results indicate that diabetic eyes with macular edema exhibited significantly lower macular vessel area density (whole image) values at the level of both the superficial and deep retinal networks in 6 × 6 Angio Retina scans ($P = <0.001^*$) when compared to controls. These results are similar to those of Mastropasqua et al. [20], who studied macular perfusion using OCTA and observed a reduction in superficial and deep capillary vessel density in patients with moderate or severe NPDR compared to controls in their study ($P = 0.012$). However, no significant difference was observed between the two diabetic groups (with DME and without DME) regarding whole image vessel density at either the superficial or deep retinal networks (Table 2).

Our results revealed significant differences in nearly all vessel density values between diabetic eyes with DME and those without DME at the deep retinal level (DRL). Diabetic eyes with DME exhibited significantly lower vessel density values at the level of the deep retinal plexus (in the parafoveal, superior hemi, inferior hemi, temporal, superior, and nasal areas) when compared to diabetic eyes without DME

Table 9 Difference between the size of the FAZ area (at the level of the superficial and deep retinal plexuses), the whole image vessel density (at the level of the superficial and deep retinal plexuses), and parafoveal vessel density (at the level of the superficial and deep retinal plexuses), in each group (group I diabetics with edema, and group II diabetics without edema), and healthy controls (group III)

	Group I Diabetics with edema *N* = 45	Group II Diabetics without edema *N* = 40	Group III Control *N* = 40
FAZ[a]			
Superficial			
Range	(0.22–1.23)	(0.25–0.83)	(0.2–0.51)
Mean ± SD	0.55 ± 0.25	0.41 ± 0.12	0.35 ± 0.09
Median	0.47	0.38	0.33
Deep range	(0.23–1.84)	(0.3–0.9)	(0.22–0.57)
Mean ± SD	0.6 ± 0.31	0.47 ± 0.13	0.37 ± 0.1
Median	0.51	0.44	0.35
P value	*0.420*	***0.011****	*0.196*
Whole image VD[b]			
Superficial			
Range	(38.6–53.9)	(38.2–52)	(41.4–56.2)
Mean ± SD	44.4 ± 3.6	45.6 ± 4.2	49 ± 3.9
Deep range mean ± SD	(36–51.9) 44.1 ± 4.1	(37.6–53) 46.2 ± 4.7	(42.1–56.5) 49.6 ± 3.9
P value	*0.685*	*0.558*	*0.488*
Parafoveal VD[b]			
Superficial			
Range	(37.4–57.6)	(39.6–53.9)	(39.6–57.3)
Mean ± SD	45 ± 4.3	46.4 ± 4.4	48.8 ± 5.3
Deep			
Range	(32.9–56)	(40–56.3)	(41.2–58.5)
Mean ± SD	45.4 ± 5.3	48.8 ± 5	45.4 ± 5.3
P value	*0.703*	***0.026****	*0.193*

[a]Mann Whitney test for nonparametric quantitative data between superficial and deep layers

[b]Independent samples t test for parametric quantitative data between superficial and deep layers

* Significant correlation

(Table 2). Upon comparing VD values at the level of the superficial retinal layer (SRL) between the two diabetic groups (with edema and without edema), no significant difference was observed (Table 2), implying that, in diabetic eyes, the effect of edema on macular perfusion is more significant at the level of the deep retinal plexus rather than the superficial plexus level, so macular ischemia is more profound at the DRL. This could be explained by a previous histological study revealing that DME is largely localized within the DRL layers [21]. Subsequently, the amount of change in retinal vascular parameters is sufficiently large at this level (DRL).

A previous study reported that vascular density in the superficial network decreased as DR worsened, as patients with mild to moderate NPDR were observed to possess higher capillary density and smaller FAZ area when compared to patients at more severe stages of retinopathy [13]. However, in the present study, we were unable to evaluate such correlation, as included diabetic eyes were classified with either moderate or severe NPDR, as we intended to decrease the number of elements that may influence macular perfusion in diabetic eyes (e.g., the stage of diabetic retinopathy or previous treatment).

Notably, Kim et al. used OCTA to evaluate macular perfusion in diabetic eyes at different stages of

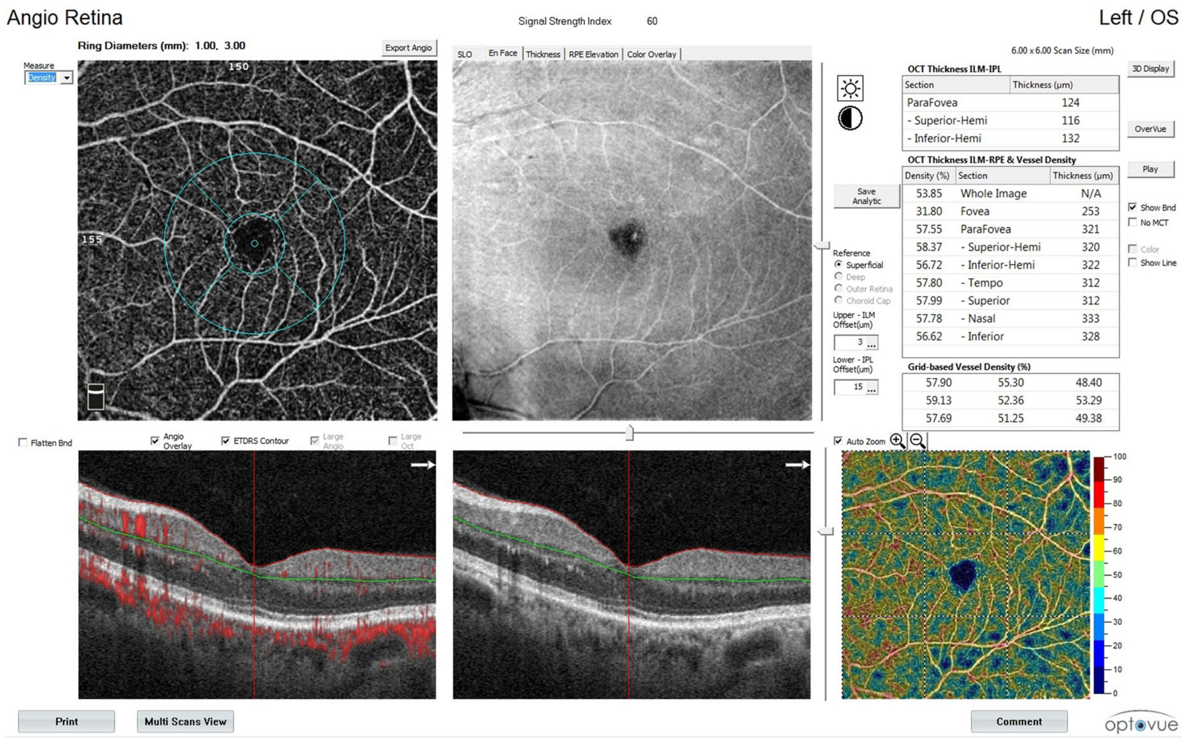

Fig. 1 The 6 × 6-mm OCTA Angio Reina scan is centered on the left macula at the level of the superficial retinal vascular networks (slab set to evaluate the superficial retinal plexus, the red line is 3 μm below ILM, and the green line is 15 μm below the inner plexiform layer). An automated software algorithm integrated into the OCTA system calculated vessel density and is presented on the OCT thickness ILM-RPE and vessel density map (middle right), and on grid-based vessel density (%) (lower right)

diabetic retinopathy, either associated with edema or not, and reported that decreased capillary density and morphology were significantly correlated with diabetic macular edema. The authors referred capillary changes, due to several factors: (1) physical displacement of retinal capillaries by the cystic spaces into adjacent layers; (2) attenuation of decorrelation signal by intra-retinal edema fluid; (3) capillary occlusion and incompetence (leading to edema) in areas with and around intra-retinal fluid; and (4) blood flow prohibition due to mechanical pressure from adjacent cystoid edema. However, the authors could not conclude whether the vascular changes observed in diabetic eyes with edema were due to edema or risk factors for edema [22]. As previously postulated by Arend et al. [6], capillary narrowing or occlusion occurring in retinal ischemia leads to tissue hypoxia with subsequent increase in vascular endothelial growth factor levels that results in diabetic macular edema. In this study, although all diabetic eyes with DME had significantly lower VD values when compared with diabetic eyes without edema at the DRL, we could not judge whether decreased macular perfusion in diabetic eyes is due to edema or an initiator for edema, as we lacked baseline VD values for the studied eyes before developing edema. Therefore, another longitudinal study is required to assess VD values in eyes with diabetic edema and evaluate the correlation between macular thickness changes and VD values over a longer period of time.

In the present study, diabetic eyes with macular edema presented with significantly reduced visual acuity when compared to diabetic eyes without DME as well as control eyes ($P < 0.001$ for both; Table 1).

Samara et al. [5] used the 3 × 3 mm macular scan area (excluding the FAZ area) to study macular vascular density measured by vessel area density and vessel length density at both superficial and deep capillary plexuses in diabetic patients without DME to quantify diabetic macular ischemia. The authors observed a significant moderate negative correlation between vessel density in both vascular networks (superficial and deep) with LogMAR visual acuity, as reduced visual acuity was associated with larger FAZ area in both superficial and deep networks, as well as decreasing vascular density. Our results are congruent with Samara et al. [5], regarding diabetic eyes without DME, as superficial and deep whole image VD has strong and moderate negative correlations with LogMAR visual acuity, respectively (Table 4). Furthermore, we evaluated the correlation between LogMAR VA and parafoveal VD at both the superficial and deep capillary plexuses in diabetic eyes without DME and observed a moderate negative correlation between parafoveal VD values (at the level of the superficial and deep retinal plexuses) and LogMAR VA (Table 6). VA in diabetic eyes with DME was only correlated with the superficial whole image VD, as a significant fair negative correlation was found between whole image vessel density at the level of the superficial retinal plexus and LogMAR VA (Table 4). Diabetic eyes with lower whole image VD values exhibit worse visual acuity. According to this correlation, whole image vessel density at the SRL could be used as a predictive tool for visual acuity in diabetic eyes with DME. Also, in diabetic eyes with DME, a significant fair negative correlation was observed between foveal VD at the SRL and LogMAR VA ($r = -0.426$, $P = 0.003$; Table 5). One explanation for the association between decreased visual acuity and lower VD values at the SRL (signifying retinal ischemia) is the fact that the metabolic supply of the photoreceptors and outer retinal layers is provided by diffusion from choroidal circulation. In cases of retinal hypoxia, with the failure of autoregulation of choroidal circulation, the inner retinal vascular contribution to the metabolic needs of the outer retina becomes more evident [23]. In addition, increased macular thickness in eyes with DME, which is more evident in deep retinal layers, may lead to disrupted retinal anatomic features and altered correlations regarding this layer (DRL).

Alterations in FAZ morphology and size in diabetic eyes have previously been reported by several authors using FA, which has been considered the gold standard technique for detecting areas of capillary dropout used for DMI grading [24]. Enlargement of the FAZ was more significant in the advanced stages of retinopathy compared to no or mild retinopathy [25, 26]. Furthermore, Sim et al. [7] reported increased FAZ size as DMI severity progressed, and this was most evident in PDR. However, FA is an invasive, time-consuming procedure, and its evaluation of FAZ changes in the deep vascular network could not be characterized—with only the superficial vascular network capable of being assessed [27].

Using OCTA as a fast and non-invasive method, several studies have evaluated FAZ area in diabetic eyes [20, 28, 29]. Di et al. [28] investigated FAZ area using OCTA in diabetic eyes without DR and at different stages of DR compared to control and observed a significantly larger FAZ area both in non-DR patients ($P = 0.04$) and DR patients at different stages of DR ($P = 0.00$). Enlargement of the FAZ area in diabetic patients, both in the superficial and deep vascular networks, was also demonstrated by Freiberg et al. and Samara et al. [20, 29]. In the present study, FAZ area was measured using a non-flow AngioAnalytics tool (Fig. 2). In agreement with the aforementioned studies, a significant enlargement of the FAZ area was observed in diabetic eyes with DME at both the SRL and DRL, compared to the control group ($P < 0.001$; Table 1). Moreover, diabetic eyes with DME exhibited significantly larger FAZ area at the SRL when compared to diabetic eyes without DME (Table 1).

A previous report by Balaratnasingam et al. [30] observed a significant correlation between FAZ area and VA in diabetic eyes with macular edema. Furthermore, Samara et al. [5] observed similar results in an evaluation of the correlation between FAZ area and visual function in diabetic eyes without edema, as patients with larger FAZ area exhibited decreased visual acuity. In the present study, FAZ area was significantly correlated with VA in DME eyes only. Our results indicate a significant fair positive correlation between FAZ area (at both the superficial and deep retinal plexus) and LogMAR VA in diabetic eyes with DME, whereas eyes with larger FAZ area exhibited decreased VA ($P = 0.005$ and $P = 0.016$, respectively; Table 7). However, the FAZ size may play a limited role in predicting VA in eyes with diabetic retinopathy, as reported by a previous study that observed significant variation in FAZ area (0.071–0.527 mm^2) among normal healthy individuals with a VA of 20/20 [14]. Also, interindividual differences in FAZ area and morphological characters have been observed [10, 11].

The present study also determined that, upon comparing FAZ area and vessel density values

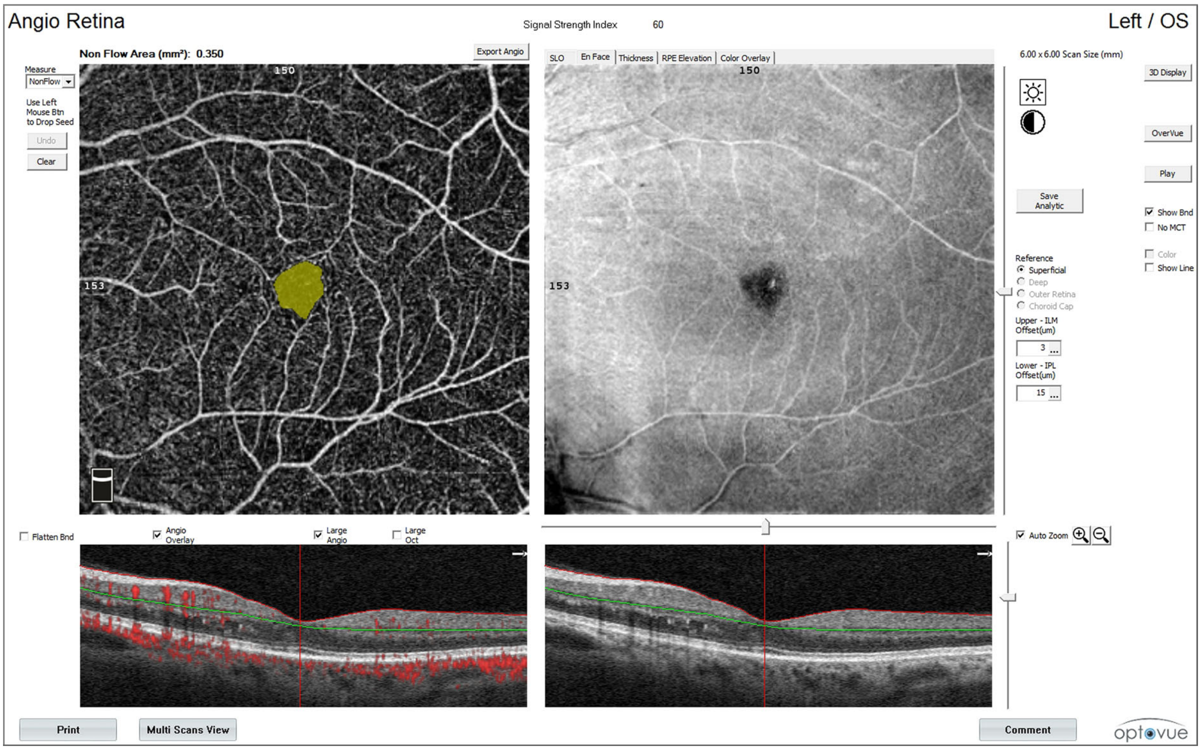

Fig. 2 The same 6 × 6-mm OCTA Angio Reina scan is centered on the left macula at the level of the superficial retinal vascular networks (slab set to evaluate the superficial retinal plexus, the red line is 3 µm below ILM, and the green line is 15 µm below the inner plexiform layer). An automated software algorithm integrated into the OCTA system measured FAZ area (yellow shade), using non-flow measure tool, here the FAZ area = 0.350 mm^2

between the superficial and deep retinal plexuses in each group, a significant difference existed between FAZ area at the level of the deep and superficial retinal plexuses in diabetic eyes without DME, being larger at the deep level. Also, parafoveal vessel density was significantly higher at the deep rather than the superficial level in diabetic eyes without DME (Table 9). While no significant difference was observed between any parameters on the two layers in diabetic eyes with DME, this could be due to the presence of edema, which results in disrupted retinal anatomic features in eyes with edema and may lead to segmentation errors and false vascular density, FAZ measurements, or both [31]. However, this was not a factor in our study since we minimized such errors by excluding eyes with significant artifactual components and used manual segmentation in two eyes with minimal improper automated segmentation algorithms. Moreover, projection artifacts (decorrelation tails)—especially found in deeper layers of the retina—may be another cause for the absence of difference between the two layers in the diabetic group with DME. In the current study, we moved the deep layer slab section slight posteriorly to be centered on the outer plexifom layer in order to eliminate projection artifacts from the superficial vascular plexus. We did not attempt to correct projection tails, based on a previous report from Kim et al. [22] that stated the correction of projection artifacts would have its own effect, which may be confusing or higher than the unidentified magnitude of the projection artifact for any OCTA image [22].

Interestingly, both AngioAnalytics tools (vessel area density and non-flow area: used to assess FAZ area) that were used in this study, were significantly correlated with visual function in diabetic eyes with DME, where eyes with lower vessel area density (lower macular perfusion) at the level of the superficial retinal plexus (whole and foveal) and larger FAZ (at both the superficial and deep retinal plexuses) exhibited reduced vision. In diabetic eyes without DME, VA was correlated with whole image VD and parafoveal VD (at both the superficial and deep retinal plexuses), where eyes with lower macular perfusion exhibited reduced visual acuity. Consequently, these parameters can be used as biomarkers for visual prognosis in diabetic eyes, with more significance for VD values, since FAZ size may have both normal variation and interindividual disparity.

In the present study, control participants were age-matched with diabetic patients to avoid the decrease in vascular density due to aging noted in previous studies that evaluated macular perfusion in healthy eyes using OCTA [15, 32]. Consequently, no significant difference was observed by comparing age between the three study groups (Table 1).

Limitations for the current study include the lack of OCT biomarkers such as outer retinal layers disruption, cystoid changes, and neuro-sensory detachment; however, we plan to include these in a future study. Our study population included diabetics, treatment naïve, with moderate or severe DR having or not DME, to ensure that correlations of visual function with vascular density and FAZ area were not confounded by multiple factors (stage of retinopathy and effect of previous treatment). Further studies are required to aid in the categorization of OCTA biomarkers (particularly vessel area density) according to visual function at different stages of diabetic retinopathy.

Notably, we lacked the baseline VD values and FAZ area measurement for diabetic eyes with DME (before developing edema) to assess whether decreased macular perfusion and enlarged FAZ area are due to increased macular thickness or an initiator for edema. Another longitudinal study is required to assess the correlation between retinal thickness and macular perfusion over a longer period of time.

Conclusions

Using the OCTA machine with AngioAnalytics parameters (vessel area density and non-flow area) aided in the objective quantification of macular perfusion and accurate measurement of the FAZ area in diabetic eyes with macular edema. Both parameters were significantly correlated with visual function in treatment-naïve diabetic eyes with macular edema. These OCTA biomarkers can be used to predict visual function in such eyes for monitoring treatment response.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Research involving human participants and/or animals The study was designed respecting the expected ethical aspects. It was performed according to the Declaration of Helsinki 1975, as revised in 2008, and has gained approval from the Local Research Ethics Committee, Faculty of Medicine, Minia University Hospitals.

Informed consent The study was explained in detail to all participants and written consents were signed by them.

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