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Relation between quantitative measurement of the foveal avascular zone, outer retinal layers disruptions, and visual acuity in diabetic macular ischemia

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Abstract
Background: With the potential to offer more in-depth images of the macula than foveal avascular (FA) imaging, optical coherence tomography angiography (OCTA) is emerging as a novel imaging tool for the detection of diabetic macular ischemia (DMI). The study's goal was to determine the relationship between visual acuity (VA) in DMI, foveal avascular zone (FAZ) and retinal vessel density (VD) as measured by OCTA, and the integrity of the outer retinal layers as measured by optical coherence tomography angiography (OCT).
Methods: This a cross sectional research enrolled 25 eyes of 25 diabetic cases diagnosed with DMI using FFA, and 25 normal eyes as a control group. All cases underwent ocular exam including VA testing, anterior segment exam, intra ocular pressure, fundus exam, and fundus fluorescein angiography (FFA).
Results: Reduction of vision was noticed in ischemic cases, the mean BCVA for the cases was 0.88±0.27 (LogMAR), while for the control was 0.03±0.04. A statistically prominent positive relation was detected between best corrected visual acuity (BCVA) (LogMAR) and (FAZ) area (rs=0.530, P=0.006), a statistically prominent negative relation was detected between BCVA (LoMAR) and both vessel area density (VAD) parameters (SCP and DCP) (VADSCP: rs= -0.415, P=0.039) (VADDCP: rs = -0.525, P =0.007).
Conclusions: OCTA allows for the quick, automated, and non-invasive quantification of vascular density and FAZ in DMI.

Keywords: Foveal avascular zone, outer retinal layers disruptions, visual acuity, diabetic macular ischemia

Introduction
Diabetic retinopathy (DR) is an ocular microvascular complication of diabetes mellitus (DM). The most common causes of vision impairment are proliferative DR and macular edema [1]. Both microvascular leakage and microvascualr blockage have been linked to DR. Retinal hypoxia then causes changes to the blood retina barrier, leading to fluid buildup, micro-architecture disturbance, and vascular permeation [2].
DR is characterized by structural and functional retinal vasculature changes which can be recently analyzed because of development in digital imaging technology. It has been hypothesized that proliferative DR is associated with a reduction in capillary blood flow and velocity and an increase in the foveal avascular zone (FAZ) [3]. It has been presumed that FAZ and the retinal vessel density (RVD) are correlated with visual acuity (VA) and can give an idea about the activity of a retinal disease [4].
Fluorescein angiography (FA) has been the gold standard for diagnosis and grading of diabetic macular ischemia. It can detect the changes of the FAZ, capillary drop out and the changes at the level of superficial capillary plexus. But it is an invasive, technique, time consuming and dye involving [5]. Optical coherence tomography (OCT) provides a cross-section retinal imaging and has been clinically adopted to observe structural changes of retina [6]. Optical coherence tomography angiography (OCTA) is a non-invasive technique for detection of retinal and choroidal vascular changes, providing 3D mapping of both choroidal and retinal microvasculature [7]. It can image not only the superficial capillary plexus but also the deep capillary plexus and allows detection of retinal vessel density through motion contrast imaging and high-speed scanning [8].
The purpose of this work was to investigate the relation between the integrity of the outer retinal layers by OCT, the FAZ and RVD by OCTA and VA in diabetic macular ischemia (DMI).

Patients and Methods

Twenty five eyes of 25 diabetic cases and 25 control eyes of healthy individuals were subjected to this cross-sectional research, with clinical criteria of DM and ischemic maculopathy with or without diabetic macular edema as seen by fundus fluorescein angiography (FFA). At the opthalmology clinic of the Ophthalmology Dep., University of Tanta, Egypt from October 2019 to October 2020.

The Ethical Committee of Tanta University Hospitals in Tanta, Egypt, gave their blessing to the study. All participants provided signed consent after receiving necessary information.

Exclusion criteria were other cases with media opacity or narrow pupil that interfere with the reliability of imaging techniques, history of vitreoretinal surgery and cases who received any kind of treatment.

All subjects underwent ocular examination including: history taking, VA testing using Snellen chart, slit-lamp of the anterior segment, intraocular pressure measurement with tonometer, and 20-diopter indirect ophthalmoscopy of the fundus and slit lamp using 90 diobpter lens, FFA using Visucam 500, spectral-domain OCT using the Zeiss Cirrus 5000 HD-OCT (Carl Zeiss Meditec, AG) and OCTA. Tropicamide 1% was used to dilate the pupils to facilitate imaging.

FFA

The proliferative and non-proliferative were differentiated by the existence of leaky vessels either at or near the optic disc neovascularization of the optic disc (NVDs) or elsewhere in the retina (NVEs) in cases of PDR. OCT and OCTA images were obtained (The Zeiss Cirrus 5000 HD-OCT), It features a scanning depth of 2 mm and an axial resolution of 5 Mm at a scanning speed of 27,000 scans per second (A-scan). Light with an 840 nm wavelength is used in the apparatus. Fast Track, an eye tracking system, was used to track eye movements and reduce motion artifacts. Only scans with signal strength equal to 6 or higher were used.

OCTA imaging

Scanning regions of 3 by 3 millimeters, centered on the fovea, were used for OCTA imaging. The macular region's retina and choroid were divided into four sections: The region between the internal limiting membrane (ILM) and the internal limiting layer (IPL) is known as the superficial capillary plexus (SCP). The region between the superficial capillary plexus (SCP) and the deep capillary plexus (DCP), the outer retina is typically avascular and stretches from the outer nuclear layer to the retinal pigment epithelium and the choriocapillaris: a 20 –µm thick slab starting 10 µm below the RPE-Brush's membrane (BM) complex. FAZ (by outlining the FAZ in SCP images) in mm² and Vessel area density % (Image J program calculates the fraction of the segmented region that is taken up by blood vessels) were utilized to put a value on the OCTA findings at macula.

OCT imaging

Macular edema was characterized as the presence of increased macular thickness or by morphological changes (the presence of intra retinal cystic changes or the presence of intraretinal or subretinal fluid). Central foveal thickness was measured using caliber. breakage the ELM or the inner-outer segment (IS/OS) junction breakage was considered an example of an outer retinal alteration. Macular scans revealed thinning and disorder of the inner retinal layers (DRIL), an area where the borders of the ganglion cell-inner plexiform, inner nuclear, and outer plexiform layers were not clearly delineated.

Statistical analysis: IBM's statistical software, SPSS, version 20.0, was used to examine the data provided into the computer. IBM Corp., Armonk, New York. To ensure a normally distributed sample, the Shapiro-Wilk test was performed. Quantitative and percentage descriptions of qualitative data were provided. The minimum and maximum values, as well as the mean, standard deviation, median, and interquartile range (IQR), were used to characterize the quantitative data. We compared categorical variables between groups using the Chi-square test, nonparametric quantitative variables between groups using the Mann Whitney test, and nonparametric quantitative variable relationships using the spearman coefficient. The obtained results were deemed significant at the 5% level.

Results

The demographic data was comparable between groups. CFT, FAZ, and BCVA (LogMAR) were prominently higher in cases compared to controls. VAD was prominently higher in controls compared to cases (p<0.001). Table 1

Table 1: Comparison between the two studied groups according to demographic data, CFT, FAZ, VAD (mm^2) and BCVA (LogMAR)

		Cases (n = 25)	Control (n = 25)	p-value
Age (years)		56.44 ± 12.10	53.92 ± 12.32	0.469
Sex	Male	11 (44.0)	10 (40.0)	0.774
	Female	14 (56.0)	15 (60.0)	
CFT		283.64 ± 80.18	243.96 ± 19.34	0.023*
FAZ (mm^2)		0.48 (0.40 – 0.65)	0.20 (0.18 – 0.24)	<0.001*
VAD	Scp	57.05 ± 3.92	63.33 ± 1.19	<0.001*
	Dcp	61.88 ± 3.78	67.02 ± 2.48	
BCVA (Log MAR)		0.99 (0.60 – 1.10)	0.0 (0.0 – 0.08)	<0.001*

Data are presented as Mean ± SD or frequency (%), or medina (IQR). CFT: complement fixation test, FAZ: foveal avascular zone, VAD: vessel area density, BCVA: best corrected visual acuity *: Statistically prominent at p ≤ 0.05.

The mean duration of DM for the cases was 16.28 years, none of the cases was known to have kidney disease, and DME could be detected in 64% of the cases (44% in the form of cystoid macular edema and 20% in the form of focal oedema). Table 2

Table 2: Distribution of the studied cases according to clinical data and different parameters in cases' group (n = 25)

Parameters		
DM therapy	Insulin	14 (56.0)
	OHG	11 (44.0)
Type of DM	1	3 (12.0)
	2	22 (88.0)
DM duration (years).		16.28 ± 3.86
Diseases	No	16 (64.0)
	HTN	9 (36.0)
Grading	PDR	13 (52.0)
	Severe NPDR	8 (32.0)
	Moderate NPDR	4 (16.0)
Edema (oct)	No	9 (36.0)
	CME	11 (44.0)
	Focal edema	5 (20.0)
IOP		17.04 ± 2.01
Outer retina	Intact	16 (64.0)
	Interrupted	9 (36.0)
Inner retina	Normal	18 (72.0)
	DRIL	7 (28.0)

Data are presented as frequency (%) or Mean ± SD. DM: diabetes mellites.

A statistically prominent positive relation was detected between the BCVA (LogMAR) and FAZ (rs=0.530, P=0.006). There was a statistically prominent negative relation between BCVA (LoMAR) and both VAD parameters (SCP and DCP) (VAD_{SCP}: rs= -0.415, P=0.039) (VAD_{DCP}: rs = -0.525, P =0.007). A statistically prominent negative relation was detected between the DM duration and VAD Scp, VAD DCp (rs=-0.399, P=0.048) (rs=-0.547, P=0.005). A statistically prominent positive relation was detected between the DM duration and BCVA (log MAR) (rs=0.739, P=0.001). Table 3

Table 3: Relation between BCVA (Log MAR) with FAZ (mm2) and VAD in cases' group (n= 25), and between DM duration and FAZ (mm2), VAD and BCVA (log MAR) in cases' group (n= 25)

		r_s	p
BCVA (Log MAR)	FAZ (mm^2)	0.530	0.006*
	VAD Scp	- 0.415	0.039*
	VAD Dcp	- 0.525	0.007*
DM. duration	FAZ (mm^2)	0.442	0.041*
	VAD Scp	- 0.399	0.048*
	VAD Dcp	- 0.547	0.005*
	BCVA (Log MAR)	0.739	0.001*

rs: Spearman coefficient. BCVA: best corrected visual acuity, DM: diabetes mellites, FAZ: foveal avascular zone, VAD: vessel area density. p: p value for comparing between the studied groups. *: Statistically prominent at $p \leq 0.05$.

There is not a prominent relation between outer retinal integrity and FAZ, VAD Scp and VAD Dcp, (P= 0.846, 0.300, 0.563), however there is a prominent relation between outer retinal integrity and BCVA (Log MAR), (P=0.007). Also, shows relations between inner retinal changes (DRIL) and different parameters in cases' group, there is a prominent relation between inner retinal changes and FAZ, VAD Scp, BCVA (Log MAR), (P= 0.002, 0.011, 0.006), however there is not a prominent relation between inner retinal changes and VAD Dcp, (P=0.658). Table 4

Table 4: Relation between outer retina and inner retinal changes (DRIL) with different parameters in cases' group (n= 25)

		Intact (n= 16)	Interrupted (n= 9)	p
Outer retina	FAZ (mm^2)	0.47	0.48	0.846
	VAD Scp	57.67 ± 3.71	55.94 ± 4.27 (51.73-62.41)	0.300
	VAD Dcp	62.21 ± 3.68	(61.28 ± 4.10)	0.563
	BCVA (Log MAR)	0.78 ± 0.27	1.05 ± 0.14 (0.78-1.30)	0.007*
		Normal (n = 18)	DRIL (n = 7)	
Inner retina	FAZ (mm^2)	0.45	0.71	0.002*
	VAD Scp	58.24 ± 3.38	53.97 ± 3.72	0.011*
	VAD Dcp	61.66 ± 3.89	62.43 ± 3.71	0.658
	BCVA (Log MAR)	0.79 ± 0.22	1.12 ± 0.23	0.006*

Data are presented as median or mean ± SD. SD: Standard deviation. P: p value for comparing between the studied groups. BCVA: best corrected visual acuity, FAZ: foveal avascular zone, VAD: vessel area density *: Statistically prominent at $p \leq 0.05$

In our research we found that disorganization of retinal inner layers (DRIL) was the most independent factor that affect the vision. Table 5

Table 5: Multiple logistic regression analysis

	OR (95% CI)	P value
FAZ	0.519 (0.319 – 0.759)	0.037*
VAD Scp	1.725 (0.367 – 2.926)	0.095
VAD Dcp	2.625 (1.237 – 7.805)	0.037*
DM. duration	0.552 (0.142 – 0.751)	0.024*
Inner retina (DRIL)	0.658 (0.316 – 0.857)	0.009*
Outer retina (interrupted)	0.558 (0.397 – 0.732)	0.014*

DM: diabetes mellites, FAZ: foveal avascular zone, VAD: vessel area density *: Statistically prominent at $p \leq 0.05$

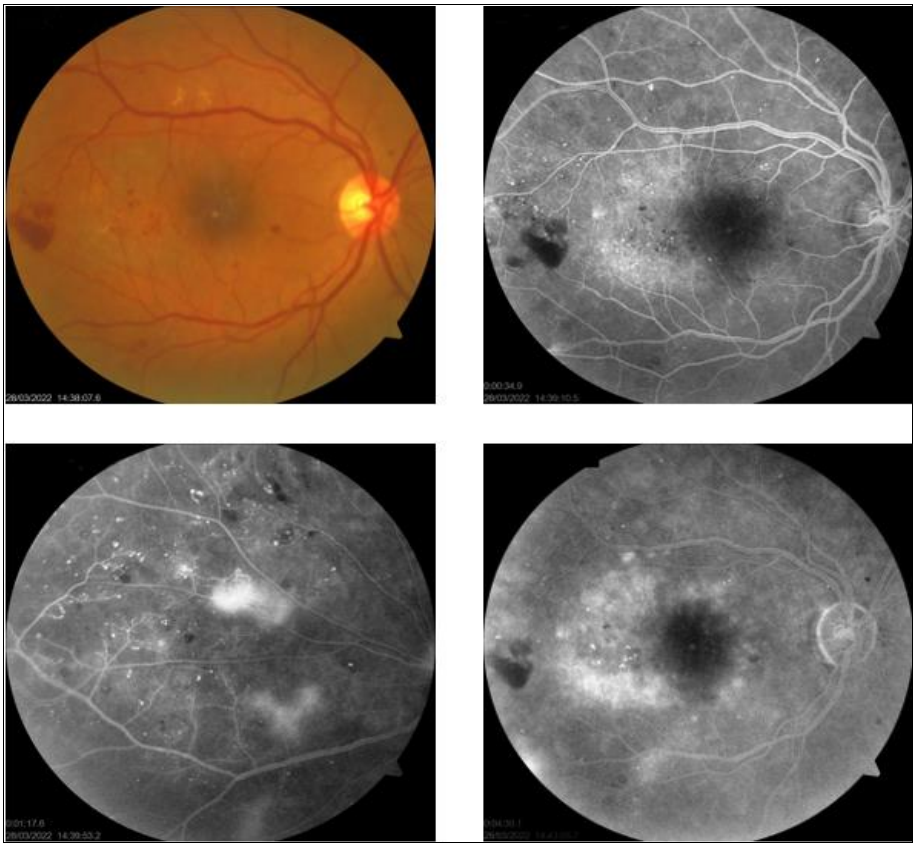

Fig 1: FFA of case 1 shows PDR with macular ischemia

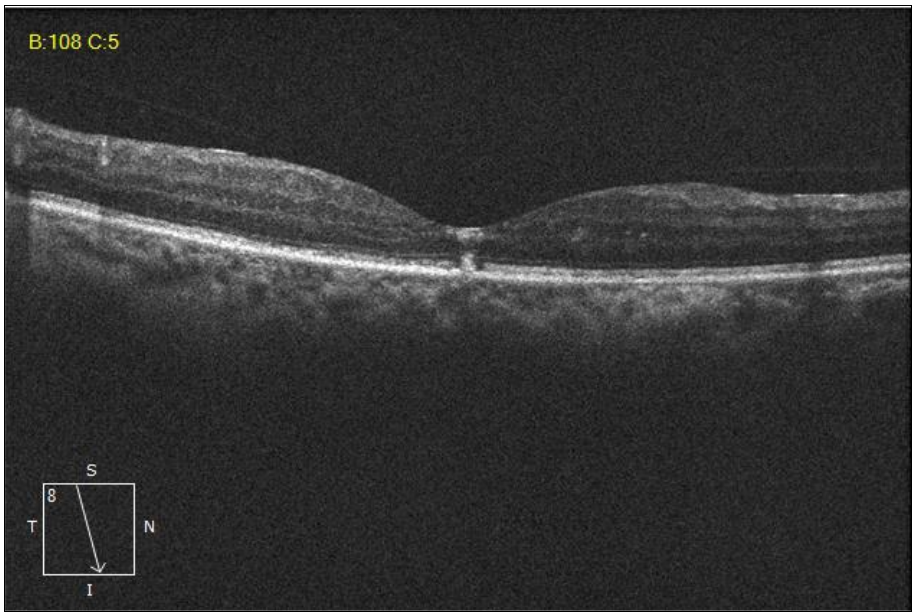

Fig 2: OCT of case 1, (yellow arrow) shows breakages of outer retinal layers

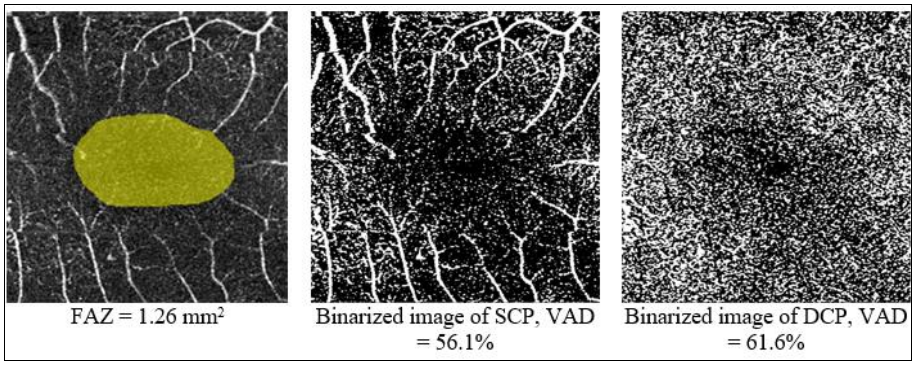

Fig 3: OCTA of case 1

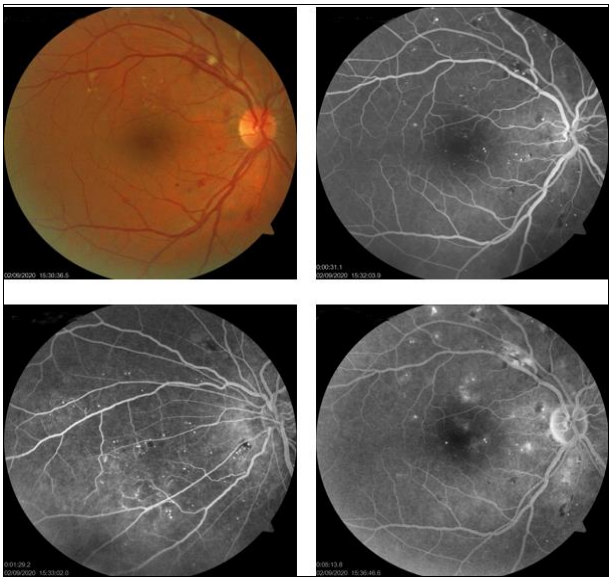

Fig 4: FFA of case 2 shows severe NPDR with macular ischemia

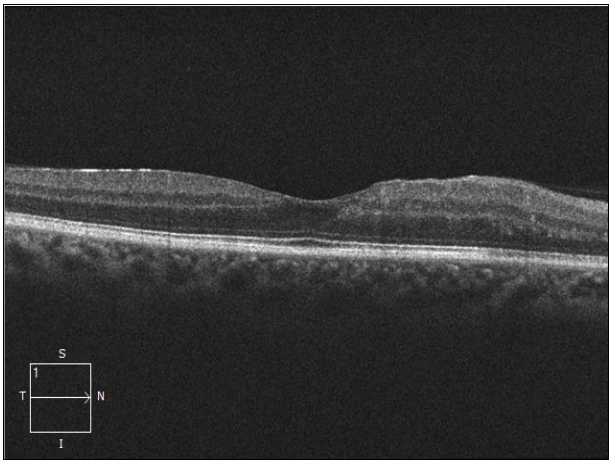

Fig 5: OCT of case 2 shows intact both inner and outer retina

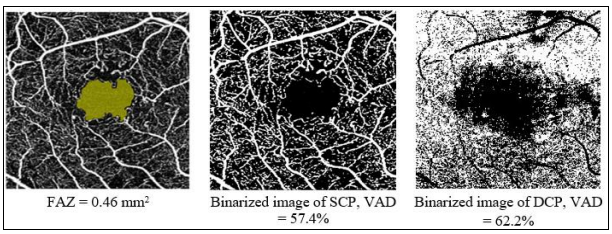

Fig 6: OCTA of case 2

Discussion

A major subtype of DR, diabetic macular ischemia (DMI) is characterized by a thickening and disturbance of the FAZ. Retinal capillary occlusion and atrophy accompany precapillary arteriole constriction or obliteration characterize the macula in this condition Sim *et al*., [6].

In the current research, the mean duration of DM for the cases was 16.28 years, none of the cases was known to have kidney disease, and diabetic macular edema (DME) could be detected in 64% of the cases (44% in the form of cystoid macular edema and 20% in the form of focal oedema). This agrees with Rabiolo *et al*., [9] who established the presence of linkbetween peripheral and macular perfusion that provides evidence in favor of the theory that the underlying cause of capillary nonperfusion in both illnesses is a shared pathogenic mechanism. In contrast, Sim *et al*., [6] couldn't observe any linkbetween the severity of DR or maculopathy grades and macular ischemia progression.

In the current research, OCT results showed that mean CFT among cases was 283.6 microns and in the controls was 243.9 microns (p=0.023). There was aprominent difference between the two groups. Scarinci *et al*. [10] reported that cases mean thickness of the fovea ranged from 230 to 342 µm (mean ± SD 275 ± 29.2 µm). Hsiao *et al*. [11] stated that the mean CFT was 359 ± 114 µm (range: 161–784 µm), while it was 282.5 µm in DaCosta *et al*. [12] research. In contrast to Samara *et al*. [13] who found that central macular thickness was similar between all groups (257±31µm) in diabetic cases and (259±20 µm) in control group (P = 0.20).

We found that, breakage of the outer layers of the retina was detected in 36% of cases in the cases' group. Both ELM and IS/OS continuity were affected in all cases (central 1mm diameter). This coincides with the results of Murakami *et al*. [14] and Lee *et al*. [15] They found that OS shortening and disruption of the IS/OS junction in photoreceptors may be related with DMI in the context of DME.

Regarding the DRIL we found that 28% of cases have DRIL which coincide with Scarinci *et al*. [10] who found that 28.5% of cases displayed a retinal contour irregularity at the level of the deepest retinal layers.

Regarding the FAZ, there was a statistically prominent enlargement of FAZ in ischemic group compared to control group. In agreement with Sim *et al*. [6], Di *et al*. [16], Freiberg *et al*. [17], Hwang *et al*. [18], Samara *et al*. [13] and Moein *et al*. [19] research, in which the mean FAZ was prominently larger in cases group versus controls.

In the current research, substantially greater variation was seen at the SCP level (mean SCP VAD in cases vs. normal eye=57.05±3.92 vs.63.33 ±1.19, p<0.001 mean DCP VAD in cases vs. controls =61.88±3.78 vs. 67.02±2.48, p<0.001). Mean VAD was lower in DM eyes versus controls. In line with Samara *et al*. [13] research, in which controls had higher VD in both networks versus the DM eyes, as the mean SCP in diabetic cases (49.44±3.837) was statistically prominently lower than control group (55.09±2.584).

Our research showed that the mean BCVA for the cases was 0.88 and the median was 0.99 (range 0.50-1.50) (LogMAR). It indicates that the vision becomes worsen. This result is coincided with Hsiao *et al*. [11] that found the mean logMAR of BCVA was 0.65 ± 0.39 (range: 0.05–1.52).

Our result showed, a statistically prominent positive relation was detected between the BCVA (LogMAR) and FAZ (rs=0.530, P=0.006). This result indicates that enlargement of the FAZ correlated with worsening of the vision. Like our research, Samara *et al*. [13] reported that the FAZ had a somewhat positive cprrelation with logMAR VA at the deep network, but only a weakly positive relation at the surface level.

This agree with Sim *et al*. [6] who found that DMI is connected to worsened VA in eyes scoring moderate to severe ETDRS-DMI ischemia.

We further explored the relation of VD with VA and found a statistically prominent negative relation was detected between BCVA (LoMAR) and both VAD parameters (SCP and DCP) (VADSCP: rs= -0.415, P=0.039) (VADDCP: rs = -0.525, P =0.007). This result showed lowering of VAD in both superficial and deep networks correlated with reduction of VA. Similarly to our research, Samara *et al*. [13] reported that the VD (VAD and VLD) measurements were based on the entire 3*3-mm scan density excluding the FAZ, ensuring that these relations are FAZ independent. VD was found to have a moderate negative relation with logMAR VA at both the superficial (VAD, r = -0.52;; P 0.001) and deep (VAD, r = -0.50;; P 0.001) levels. Reduced artery density and an

enlarged FAZ have both been linked to impaired vision in DR without DME. Also, Dupas *et al*. [20] found that, in cases with functional problems, indicated as a decrease in VA, were related to the degree of capillary loss in the deep capillary complex in people with type 1 DMwithout retinal oedema and bilateral severe non-proliferative or proliferative DR.

We found that, there is not a prominent relation between outer retinal integrity and FAZ or VAD Scp and VAD Dcp, (P= 0.846, 0.300, 0.563), which is in line with Benitez-Herreros *et al*. (2015) research We did not locate any significant correlations between FAZ and the average area of the IS/OS interface that was broken or the ELM. Simonett *et al*. [21] and Scarinci *et al*. [10] Studies DR, both at the SCP and DCP levels, is associated with vascular anomalies in the foveal and perifoveal region.

However, our research results have revealed that there is a prominent relation between outer retinal integrity and BCVA (Log MAR), (P=0.007). In line with Sayed *et al*. [22] research, in which a decline in BCVA is strongly linked to disturbances in the IS and OS. Studies nearly all came to the same conclusion. Multiple researchers have found a correlation between the health of the photoreceptor layer and BCVA: Scarinci *et al*. [10], Nesper *et al*. [23], Hareedy *et al*. [24], and Abd Elhamid [25]. The current state of IS/OS disruption thus appears to be a potentially useful predictor of VA Chung *et al*.[26].

In the current research, there is a prominent relation between inner retinal changes and FAZ, VAD Scp, BCVA (Log MAR), (P= 0.002, 0.011, 0.006), however there is not a prominent relation between inner retinal changes and VAD Dcp, (P=0.658). Similar to Sun *et al*. and Radwan *et al*. [27] studies demonstrated that low VA in instances with DME is linked to impaired inner retinal architecture on OCT even after the DME has resolved. Similarly, Yeung *et al*. [28] and Sun *et al*. [27] FFA findings of DMI areas have been reported to be substantially linked with the presence of inner retinal layer disorder or loss.

In our research, a statistically prominent positive relation was detected between the DM duration and FAZ, (rs=0.442, P=0.041). In contrast to Takase *et al*. [29] and Durbin *et al* [30], Niestrata-Ortiz *et al*. [31] studies that found no relation was detected between the FAZ parameters and the DM duration.

A statistically prominent negative relation was detected between the DM duration and VAD Scp, VAD DCp (rs=-0.399, P=0.048) (rs=-0.547, P=0.005). In disagreement with Karim *et al*. [32] who found that as regards the duration of DM, there is inprominent negative relation with VAD of SCP, VAD of DCP. A statistically prominent positive relation was detected between the DM duration and BCVA (log MAR) (rs=0.739, P=0.001). In agreement with Sayed [22] research, in which there is a strong link between decreased BCVA and long duration of DM.

Limitations: the small number of cases and the usage of FFA as a method for cases' enrollment which limited the studied cases for cases with advanced disease

Conclusions

Therefore, OCT angiography may be preferable than FA for the early diagnosis of DR and for the evaluation of the FAZ in DR since it allows for the quantification of VD and FAZ in DMI in a rapid, automated, and non-invasive manner.

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