Optical Coherence Tomography Angiography Findings in Patients with New-Onset Type 2 Diabetes and No Signs of Diabetic Retinopathy

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SUMMARY

Aim: This study aims to assess and compare by means of optical coherence tomography angiography (OCTA) early modifications in the retinal layer of new-onset type 2 diabetic patients without clinical signs of diabetic retinopathy (NDR).

Materials and Methods: A total of 51 NDR patients and 53 normal individuals' retinal capillary foveal density, superficial and deep capillary plexus, foveal avascular zone area (FAZ) parameters were evaluated with OCTA. Inner retinal layer (IRL) thickness and outer nuclear layer thickness (ONL) at the temporal and nasal margin of FAZ were measured in 26 NDR and 26 healthy subjects, using ImageJ 1.46r software.

Results: Vascular density in the superficial and deep whole image (SWIVD and DWIVD) was significantly lower in patients than in controls for right eyes (RE) and left eyes (LE), (RE SWIVD: $p < 0.001$, LE SWIVD: $p = 0.004$, RE DWIVD: $p < 0.001$, LE DWIVD: $p = 0.028$). There was no significant difference in the values of the FAZ area in the right and left eye between patient and control groups (RE: $p = 0{,}652$; LE: $p = 0.509$). No significant difference was found in the T/N IRL thickness between study and control groups ($p = 0.552$, $p = 0.094$, respectively). The AUC analysis for vascular densities ranged from 0.625 to 0.751.

Conclusion: Our study found that the density of blood vessels decreases before clinical signs of retinopathy appear in newly diagnosed type 2 diabetic patients.

Key words: new-onset type 2 diabetes; foveal avascular zone; optical coherence tomography angiography; inner retinal layer; outer retinal layer

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INTRODUCTION

Diabetes mellitus (DM) is recognized as a global epidemic. In the year 2021, approximately 537 million diabetic patients were reported, and this number will increase to 783 million by 2045 [1].

DM has macroangiopathic and microangiopathic complications. Moreover, diabetic retinopathy (DR) remains the predominant reason for visual impairment in individuals suffering from DM [2]. Chronic hyperglycemia causes increased hypoxia, oxidative stress, and inflammation leading to neovascular changes in the retina [3]. It is known that the earliest signs of diabetic retinopathy are microaneurysms and intraretinal hemorrhages in clinical examinations. In addition, the staging system that relies on biomicroscopic examination and fundus fluorescein angiography is also still in use. However, the onset of microvascular changes begins before the development of clinically significant diabetic retinopathy.

Since the development of optical coherence tomography angiography (OCTA), we can observe the macular capillary network, especially the foveal avascular zone

(FAZ) and superficial, deep capillary plexus in the perifoveal area. Previous studies using this device have reported variable findings in vessel density, FAZ, among patients with type 2 DM without clinical signs of diabetic retinopathy, compared with healthy controls [4-9]. In this study, the vessel densities, FAZ area, inner retinal thickness (IRL), and outer retinal thickness (ONL), assessed by OCTA and image j software, in new-onset type 2 DM patients without clinical signs of DR (NDR) were compared with healthy individuals.

MATERIALS AND METHODS

This study was approved by the institutional Ethics Committee of Adana City Training and Research Hospital (Date: 2018.02.28, No:172). The procedure was carried out in compliance with the ethical principles enumerated in the Helsinki Declaration. This study was conducted as a case-control observational research investigation. Study participants were involved through written informed consent for the conduct of the study, publication of this study, inclusiveness of associated images and their reuse. The inclusion criteria for the diabetic patients were a best-corrected visual acuity of at least 20/20, over 20 years old, recently diagnosed with type 2 diabetes, and no evidence of DR during fundus examination. The inclusion criteria for the healthy controls were normal blood glucose level and no evidence of any retinal pathology during fundus examination. The exclusion criteria were cataract surgery in the last 6 months, glaucoma, uveitis, neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases, corneal opacity change to optic media opacity that obscured the visibility of the fundus, refractive disorders greater than ±3.0 D, measurement quality lower than 7/10, and inability to correctly perform the OCTA examination. The subjects underwent a complete ophthalmic examination, including measurements of refractive error with an autorefractor keratometry (Canon Inc. Ltd., Tokyo, Japan), intraocular pressure with an air puff tonometer (Canon TX-20, Canon Inc. Ltd., Tokyo, Japan), medical background, maximum visual acuity with correction and slit lamp examination. One observer performed OCTA scans, using a spectral domain OCT (RT-Vue-XR Avanti; Optovue; software version 2017.1.0.151).

A total of both eyes of 104 subjects (51 NDR and 53 healthy controls) were prospectively evaluated with OCTA between December 2018 and May 2019. All patients were enrolled in this study within one week of a clinical diagnosis of type 2 diabetes in the Internal Medicine Department. Systemic diseases and HbA1c levels were also evaluated. FAZ, outer retina flow area (FA; selected area: 3.144 mm^2), foveal density (FD, %), central foveal thickness (CFT), and vascular densities (VD) in the superficial capillary plexus (SCP, %) and deep capillary plexus (DCP, %) were assessed with OCTA and automatically calculated by the software. Macular data were acquired over a 3×3 mm^2 area. The FAZ was assessed in the deep vessel plexus, using the FAZ area tool of the software, which automatically delineated the parameters. The software algorithm created superficial and deep retinal vascular networks. FD measurements were determined by the software of the device as VD within a 300 µm ring width surrounding the FAZ. FA detection was performed, using a circle of 1 mm radius manually placed on the intensity image slab of the outer retina. Because of missing data, the number of participants in the control group available for analysis of the FA parameter decreased to 40. The boundaries were as follows for each layer: a slab extending from 3 to 15 µm from the internal limiting membrane (ILM) for detecting SCP; and DCP is determined from a slab found at a depth ranging from 15 to 70 microns below the ILM. Superficial whole image vascular density (SWIVD), deep whole image vascular density (DWIVD), superficial parafoveal vascular density (SPVD), deep parafoveal vascular density (DPVD) were measured in the SCP and DCP by using the "density" tool of the software as a percentage of the area, divided by 1-mm and 3-mm diameter circles centered on the fovea. Each area was divided into temporal, superior, nasal, and inferior sections and examined.

IRL and ONL measurements were performed in 26 NDR and 26 healthy subjects selected from 104 subjects, using the ImageJ program (1.46r, National Institutes of Health, Bethesda, MD). As no flattening procedure was performed on the acquired OCT images, only well-aligned images in the horizontal plane were selected for this analysis.

Methods for measuring the thickness of the inner retinal layer and outer nuclear layer

One of the advantages of using OCTA images is that it is easy to determine the coordinates of points on the FAZ boundary. In this view, we used OCTA images for retinal thickness assessment. Some retinal layers such as the ILM, the border between the ONL and outer plexiform layer, were traced automatically by the device's software. OCTA horizontal B-scan images were loaded into ImageJ software. Thereafter, measurements of IRL and ONL were obtained at the temporal and nasal sides of the FAZ margin (Figure 1).

The grader used the set scale parameter of the software, and CFT was used for the calibration (Figure 2).

IRL thickness, measured at the temporal and nasal FAZ margin, was defined as the distance from the ILM to the inner boundary of the ONL (Figure 3A); while ONL thickness, at the temporal and nasal FAZ margins, was defined as the distance from the inner boundary of the ONL to the external limiting membrane (Figure 3B). In addition, the term CFT refers to the shortest distance between the ILM and the anterior edge of the retinal pigment epithelium.

Statistical analysis

Statistical analyses were conducted using the SPSS 27 software (SPSS Inc, Chicago, IL, USA). Descriptive statistics

were presented as mean ± standard deviation, with normality assessed via the Shapiro-Wilk and Kolmogorov-Smirnov tests. Categorical variables were analyzed, using the independent sample Chi-square test, while the Mann–Whitney U and Independent Student's t-tests determined group significance. Univariate linear regression analyzed the correlation between two variables, with $p < 0.05$ considered statistically significant.

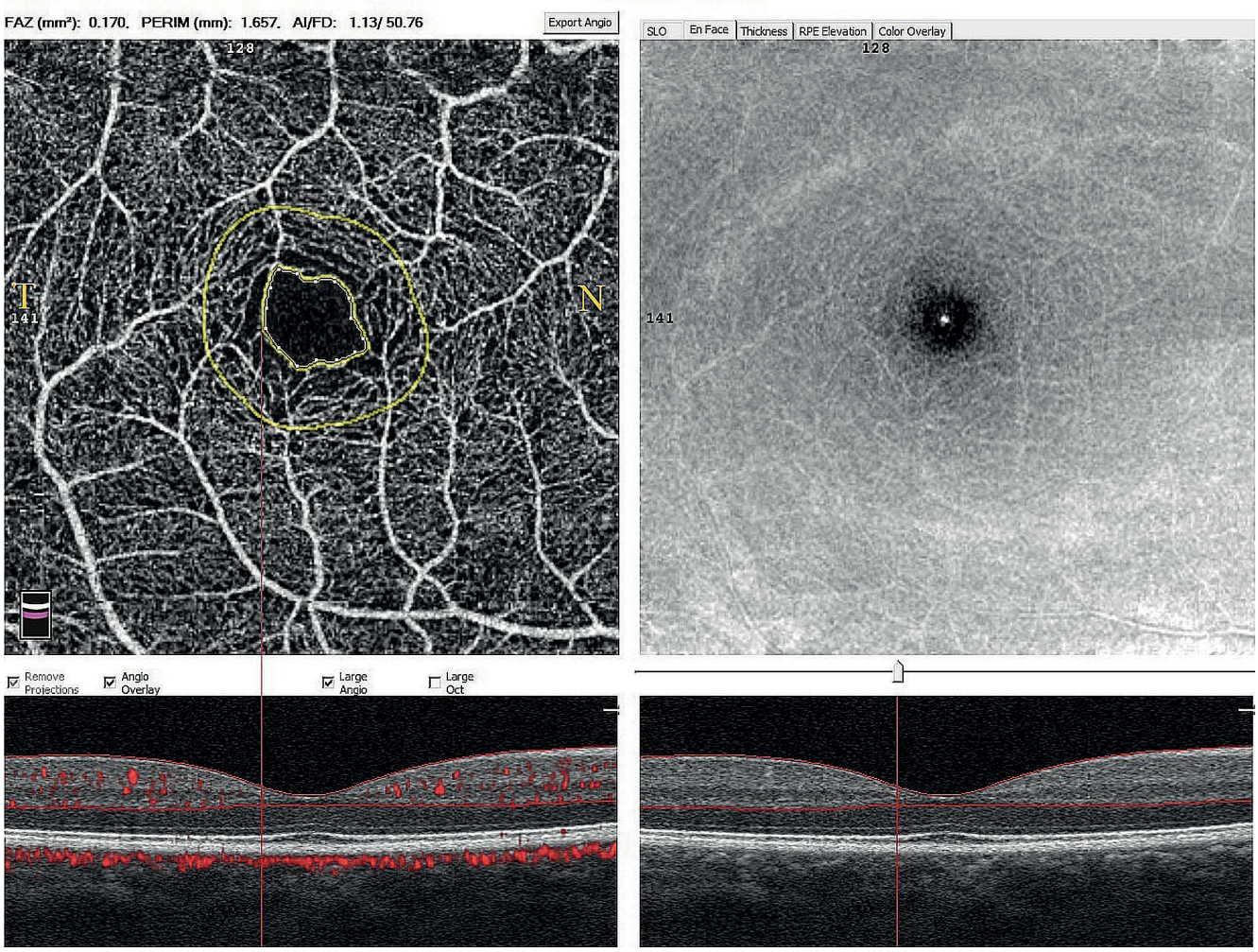


Figure 1. Shows, where a point on the temporal foveal avascular zone margin corresponds to the OCTA horizontal image

FAZ – Foveal avascular zone

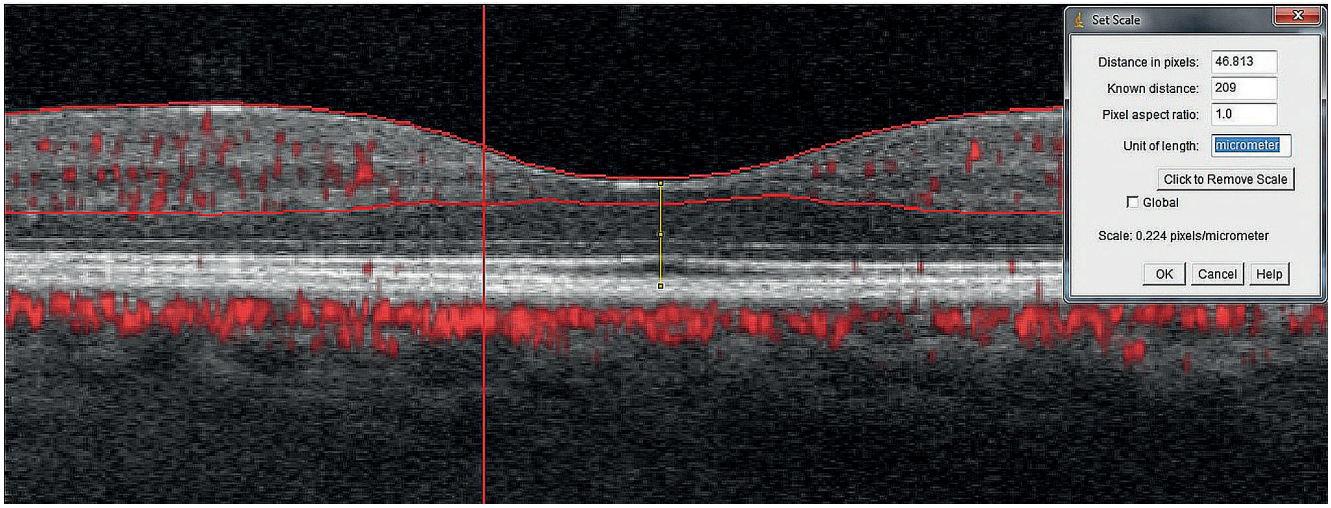


Figure 2. The grader used the set scale parameter of the software and central foveal thickness was used for calibration

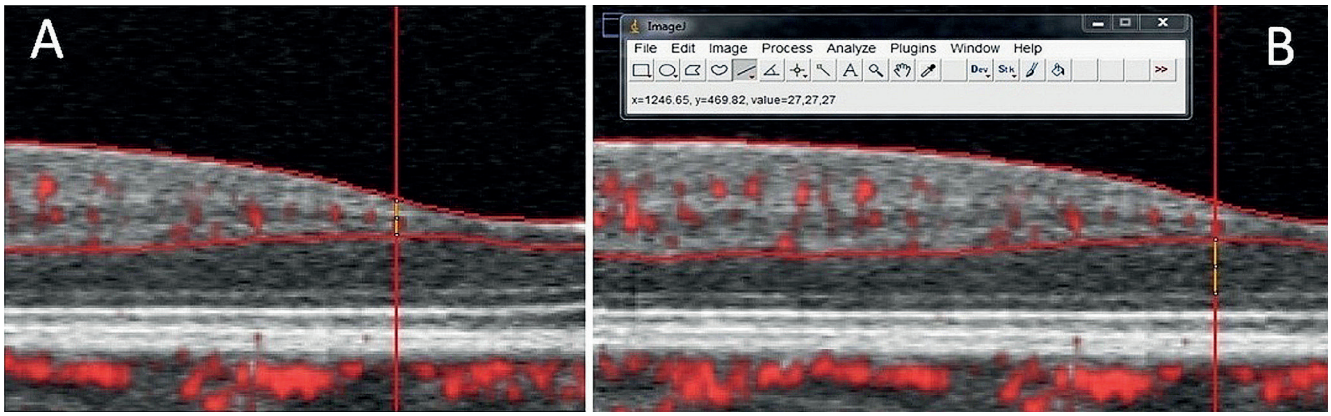


Figure 3. Manual measurements of layers. (**A**) IRL thickness measured at the temporal FAZ margin was defined as the distance between ILM and ONL/OPL. (**B**) ONL thickness at the temporal FAZ margin was defined as the distance between the ONL/OPL and ELM layer

IRL – Inner retinal layer, FAZ – Foveal avascular zone, ILM – Internal limiting membrane, ONL – Outer nuclear layer thickness, OPL – Outer plexiform layer, ELM – External limiting membrane

RESULTS

This study involved 104 subjects: 51 NDR and 53 healthy controls, with mean ages of 50.78 ±8.4 years for the study group and 47.8 ±9.4 years for the control group. 22 of the study group and 26 of the control group were female. The age and sex distributions did not significantly differ between study and control groups (p = 0.058 and p = 0.606). Mean glycosylated hemoglobin level in the study group was 68.67 ±22.97 mmol/mol. There was no significant difference in the values of the FAZ area in the right (RE) and left eye (LE) between patient and control groups. (FAZ RE: df = 102, 95%CI: -0.0507 to 0.032, t = -0.443, p = 0,652, FAZ LE: df = 102, 95%CI: -0.0706 to 0.0128, t = -1,374, p = 0.509). Furthermore, upon comparison of the FA, FD, CFT parameters between patient and healthy controls, we could not find any statistical difference for these parameters in both eyes (FA RE: df = 88, p = 0.539, FA LE: df = 88, p = 0.868, FD RE: df = 102, 95% CI: -0.883 to 2.632, p = 0.326, FD LE: df = 102, 95% CI: -0.865 to 2.112, p = 0.408, CFT RE: df = 102, 95% CI: -2.527to 10.07, p = 0.238, CFT LE: df = 102, 95% CI: -5.009 to 9.076, p = 0.568) (Table 1).

Whole image and parafoveal vascular density were lower in the study eyes than in the controls for both eyes. RE SWIVD: p < 0.001, LE SWIVD p = 0.004, RE DWIVD: p < 0.001, LE DWIVD: p = 0.028, RE SPVD: p < 0.001, LE SPVD: p = 0.009, RE DPVD: p < 0,001, LE DPVD: p = 0.027).

Quantitative measurements of vessel density in SCP and DCP are shown in Table 2.

Correlation analysis showed that the CFT was significantly inversely correlated with the FAZ area in study and control groups. (Study RE: Pearson R = -0.680, p < 0,001, LE: Spearman R = -0.728, p < 0,001, control RE: Pearson R: -0.799, p < 0.001, LE: Spearman R = -0.842, p < 0,001).

Linear regression analysis showed that the FAZ area was significantly negatively affected with the CFT in study and control groups. (Study RE: β = -0.680, t = -6.493, p < 0,001, LE: β = -0.713, t = -7.039, p < 0,001, control RE: β: -0.799, t = -9.39, p < 0.001, LE: β = -0.808, t = -9.811, p < 0,001).

The FAZ area did not show a correlation with HbA1c levels in the study group (RE: Spearman R = -0.095, p = 0.589, LE: Spearman R = -0.055, p = 0.753).

We could not find any correlation between FAZ area and SWIVD in the study group (RE: R = 0.051, p = 0.720,

Table 1. Shows mean values of foveal avascular zone area, flow area, foveal density and central foveal thickness in both groups

	Patient	Control	p
	Mean ±SD	Mean ±SD	
FAZ RE/LE	0.295 ±0.101/0.305 ±0.10 (n = 51)	0.286 ±0.11/0.276 ±0.112 (n = 53)	0.652*/0.509*
FA RE/LE	0.354 ±0.212/0.368 ±0.239 (n = 50)	0.320 ±0.164/0.340 ±0.178 (n = 40)	0.539†/0.868†
FD RE/LE	50.03 ±4.31/50.22 ±3.69 (n = 51)	51.36 ±4.6/51.2 ±3.65 (n = 53)	0.326*/0.408*
CFT RE/LE	215.9 ±16.57/216 ±18.93 (n = 51)	217.8 ±16.28/217.5 ±17.6 (n = 53)	0.238*/0.568*

** – Student-t test, † – Mann-Whitney U test, p < 0.05 – All values are given as mean (SD)*

Table 2. Vascular density results of both groups

PARAMETERS	Study (n = 51)	Control (n = 53)	p
	Mean ±SD	Mean ±SD	
VD (SCP) %	RE/LE	RE/LE	
Whole image	45.8 ±3.48/45.3 ±4.2	47.8 ±4.62/47.4 ±3.51	<0.001†/0.004*
Fovea	14.29 ±5.27/14.6 ±5.3	16.8 ±6.32/16.7 ±6.82	0.035*/0.116*
Parafovea	48.8 ±3.82/48.58 ±4.5	50.86 ±4.79/50.7 ±3.32	<0.001†/0.009†
-Temporal	47.4 ±3.8/47 ±4.48	48.86 ±4.78/48.9 ±3.85	0.003†/0.024†
-Superior	49.5 ±4.64/50 ±5.16	52.2 ±4.45/52 ±4.24	<0.001†/0.035†
-Nasal	48 ±3.94/47.8 ±4.96	50.26 ±5.04/50.28 ±3.61	<0.001†/0.003†
-Inferior	49.9 ±4.02/49.4 ±4.91	52 ±5.76/51.7 ±3.58	<0.001†/0.005*
VD (DCP) %	RE/LE	RE/LE	
Whole image	51.06 ±2.91/52.18 ±4.55	53.4 ±3.89/54.6 ±3.67	<0.001†0.028*
Fovea	32 ±6.36/31.9 ±7.04	33.8 ±7.43/34.9 ±7.75	0.230*/0.067*
Parafovea	53.07 ±3.03/54.4 ±3.81	55.7 ±3.64/55.26 ±3.53	<0.001†/0.027†
-Temporal	53.66 ±3.28/54.5 ±4.37	55.56 ±3.67/56.55 ±3.71	<0.001†/0.022*
-Superior	52.09 ±3.25/54.5 ±4.65	55 ±4.47/55.5 ±4.84	<0.001†/0.226†
-Nasal	54 ±3.09/54.6 ±3.69	55.9 ±3.86/56.8 ±3.39	<0.001†/0.001†
-Inferior	52.6 ±3.99/53.85 ±3.76	55.9 ±4.21/56 ±3.87	<0.001†/0.009*

** – Student-t test, † – Mann-Whitney U test, SCP – Superficial capillary plexus, DCP –* Deep capillary plexus, *VD – vascular density, RE – Right eye, LE – Left eye, p < 0.05 – All values are given as mean (SD)*

Table 3. Receiver operating analysis of several OCTA parameters

	Cut off	AUC	Sig.	95% CI Lower	95% CI Upper
SWIVD RE	46.85	0.717	**<0.001**	0.615	0.819
SWIVD LE	45.35	0.647	**0.006**	0.542	0.752
DWIVD RE	53.25	0.722	**<0.001**	0.620	0.824
DWIVD LE	55.45	0.625	**0.024**	0.517	0.733
SPVD RE	49.95	0.714	**<0.001**	0.611	0.817
SPVD LE	52.55	0.649	**0.005**	0.545	0.754
DPVD RE	54.7	0.751	**<0.001**	0.652	0.849
DPVD LE	56.55	0.626	**0.023**	0.518	0.734
FAZ area RE (mm^2)	0.236	0.525	0.667	0.421	0.637
FAZ area LE (mm^2)	0.291	0.582	0.146	0.471	0.693

SWIVD – Superficial whole image vascular density, DWIVD – Deep whole image vascular density, SPVD – Superficial parafoveal density, DPVD – Deep parafoveal density, FAZ – Foveal avascular zone, RE – Right eye, LE – Left eye

LE: R = -0,201, p = 0.163). In addition, we found a minor relationship between FAZ area and DWIVD of the patients' left eyes in the study group (RE: Pearson R = -0.267, p = 0.058, LE: Spearman R = -0.329, p = 0.020). In addition, univariate linear regression analysis showed that there was no relationship between FAZ area and SWIVD in the study group (RE: β = 0.002, t = 0.360, p = 0.720, LE: β = -0.004, t = -1.317, p = 0.194). We also found a minor relationship between the FAZ area and DWIVD of the patients' left eyes in the study group (RE: β = -0.267, t = -1.941, p = 0.058, LE: β = -0.338, t = -2.486, p = 0.016).

The IRL and ONL measurements were performed in 26 NDR patients and 26 control subjects for right eyes.

The mean temporal and nasal IRL thicknesses were 63.45 ±7.28 µm and 71.9 ±19.4 µm in the study group and 61.83 ±9.87 µm and 64.18 ±11.9 µm in the healthy controls. In addition, the analysis revealed that there was no significant difference in the thickness of the T/N IRL

(intraretinal layer) between the study group and the control group (p = 0.552, p = 0.094, respectively). The mean IRL thickness was slightly positively correlated with the FAZ area in the study group, only for the temporal side, and no correlation was found in the control group (T: Pearson R = 0.538, p = 0.005; N: Pearson R = 0,314, p = 0.118; T: Pearson R = 0.003, p = 0,990; N: Pearson R = 0.213, p = 0.296, respectively).

The mean temporal and nasal ONL thicknesses were 101.8 ±13.45 µm and 104.6 ±12.74 µm in the study group and 99.79 ±11.24 µm 98.83 ±22.06 µm in the healthy controls. There was no statistically significant difference in the T/N ONL thickness between study and control groups (p = 0.735, p = 0.628, respectively).

In addition, receiver operating analysis (ROC) showed that the highest area under curve (AUC) values were derived from the measurements of the SWIVD, DWIVD, SPVD, DPVD (Table 3).

DISCUSSION

Despite significant advancements in the treatment of diabetes, management of microvascular complications, such as retinopathy, neuropathy, and nephropathy, continues to remain a challenge. Clinical retinopathy is one of these challenges. As the condition progresses, there is a notable reduction in vessel density [10,11]. In recent years, numerous OCTA studies have examined the vessel densities of the perifoveal capillary network of NDR patients. However, findings of these studies have varied significantly. Some studies found no significant difference in superficial and deep capillary plexus vascular density between diabetic eyes without DR and controls, while others reported a notable reduction in these densities in NDR compared to healthy individuals [5,12–17]. Our study found that parafoveal SCP and DCP vascular density was significantly lower in NDR patients than in healthy subjects.

Diabetic retinopathy not only affects the vascular structures in the retina, but also causes changes in the FAZ dynamics. Studies examining the FAZ area and vascular structures arrived at different results. Some studies showed that there were no significant differences between eyes of the NDR and the controls in terms of the FAZ area, while other studies reported the FAZ area was found to be larger in NDR patients [12–16,18]. In our study, there were no significant differences for the FAZ parameter between NDR patients and controls. The discrepancies in these findings may be due to the high variability in the FAZ area, or microvascular changes that may be subtle at the beginning of the disease.

Macular thickness plays a crucial role in understanding the progression of diabetic retinopathy and its effects on visual prognosis [19]. Several studies have examined the CFT between normal and NDR patients, but studies have noted mixed results. Some studies found that the CFT was significantly thicker in NDR patients when compared with normal patients [20]. However, others found more thinner CFT in NDR patients [21,22]. However, in our study, we could not find a statistically significant difference between NDR and normal individuals, consistent with a previous study [23].

In this study, we found a negative correlation between the FAZ area and CFT in the study and control groups, consistent with other previous studies which show that the FAZ area narrows as the CFT increases [24,25]. Since a thick fovea is associated with a more compact inner retina, and the continuation of the inner nuclear layer increases the CFT, this causes the FAZ area to become narrower [25].

In addition, in our study we measured the IRL and ONL in patients with NDR and healthy controls. A previous SD-OCT study showed that the mean IRL at the FAZ area margin was significantly thicker in diabetic patients than in controls [26]. In contrast, we found no significant difference in the IRL thickness between the study and control groups. Since the diabetic patients in our study were newly diagnosed and the duration of diabetes was shorter than in the other study, the IRL thickness may not be thicker in diabetic patients compared to the other group. In addition, the same study reported that there was not a significant association between the FAZ area and the mean IRL for diabetics. Our study showed that there was no significant correlation between the IRL and FAZ area in the control group, and a very low correlation between the temporal side IRL and FAZ area, in accordance with a previous report [24]. In addition, Arthur et al. reported that the mean ONL did not differ between the diabetics and the controls, which findings support our results [26]. In our analysis of the AUC results, we found low AUC values for the FAZ area in both eyes [27], while moderate AUC values were observed for the vascular densities [9,27].

There were several limitations of this study. The 3x3 mm OCTA captures only a limited part of the retina. OCTA image projection artifacts may interfere with the visualization of deeper layers. In addition, there were some limitations regarding the duration of diabetes. Although these patients were identified as newly diagnosed with diabetes, the exact disease duration could not be determined, due to delayed diagnoses. The IRL and ONL subgroup analysis had a limited sample size, due to the requirement for properly aligned images for precise thickness measurement.

CONCLUSION

We did not observe a statistically significant difference for the FAZ area in the right and left eyes of NDR patients, compared to healthy controls. Both eyes of the NDR patients showed lower vascular densities compared to the healthy controls. Based on the AUC values, monitoring changes in vascular density can be useful in tracking the progression of the disease over time.

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