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Research Article

# Quantification of Superficial and Deep Capillary Plexus within Foveal Avascular Zone in Non-Proliferative Diabetic Retinopathy with Optical Coherence Tomography Angiography

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**Received:** September 06, 2020; **Published:** September 28, 2020

## Abstract

**Background:** Optical coherence tomography angiography (OCTA) is a novel non-invasive application of OCT to study the dynamic function of retinal vasculature. Diabetic retinopathy is a sequelae of microvascular changes in the retina and OCTA can be used to assess these changes at the level of superficial (SCP) and deep capillary plexus (DCP).

**Methods:** A prospective, comparative observational study of 60 eyes of 49 patients (30 eyes of 19 patients with NPDR and 30 eyes of healthy subjects) attending Ophthalmology outpatient clinic at Research Institute of Ophthalmology, Giza, Egypt between September 2018 to April 2019. All patients underwent spectral domain optical coherence tomography (SD-OCT) and angiography (OCTA) of the macula using Heidelberg Spectralis OCT, Germany.

**Results:** Thirty eyes of 19 patients with NPDR were compared with 30 eyes of healthy subjects as the control group. There was a clinically significant difference noticed in foveal avascular zone (FAZ) surface area, horizontal and vertical diameter and central macular thickness (CMT) in diabetic group compared to the healthy subjects at both SCP and DCP levels.

**Conclusion:** This study suggest that OCTA has a role as a non-invasive tool in early detection of macular microvascular changes in diabetic patients and to study the superficial and deep capillary plexus without the need for Fluorescein angiography (FA).

***Keywords:*** *Optical Coherence Tomography (OCT); Optical Coherence Tomography Angiography (OCTA); Foveal Avascular Zone (FAZ); Superficial Capillary Plexus (SCP); Deep Capillary Plexus (DCP)*

## Introduction

Diabetic retinopathy (DR) is the most common complication of diabetes in the eyes, which initially remains asymptomatic but can progress to cause severe visual impairment [1]. It is the leading cause of visual loss in the world's working adult population [2]. Diabetic retinopathy is generally categorised as non-proliferative diabetic retinopathy (NPDR) and more advance proliferative diabetic retinopathy (PDR) which develops due to retinal ischaemia and neovascularization [3]. Diabetic maculopathy and the complications of PDR; vitreous haemorrhage and retinal detachment are the main sight threatening sequalae of DR [4,5].

Various investigative modalities have been utilised in past decades to monitor the progress of diabetic retinopathy. Digital retinal fundus imaging is widely employed in the diabetic screening programmes world-wide due to high sensitivity and specificity but has shown to have low negative predictive value [6]. Fluorescein angiography (FA) is an invasive technique that has been used in past decades to differentiate and classify proliferative diabetic retinopathy.

Advancement in non-invasive imagining modalities like optical coherence tomography (OCT) utilising low-coherence interferometry to capture cross-sectional two dimensional retinal images has proven revolutionary in the detection and monitoring of diabetic maculopathy [7-9]. A significant advance in the novel use of OCT is optical coherence tomography angiography (OCTA) which utilises the changes in the OCT reflectance signal from blood flow through repeated scans at the same location to visualise retinal and choroidal microvasculature [10,11]. In contrast to fluorescein angiography, OCTA can differentiate in visualising the individual retinal capillary plexuses, like superficial capillary plexus (SCP), deep capillary plexus (DCP) and middle capillary plexus (MCP) more recently [12]. The SCP is composed of horizontal arterioles and venules which are connected by transverse capillaries. While the DCP is organized into capillary vortices which is formed by convergence of radial vessels. Both superficial and deep plexuses are connected by vertical vascular segment [13].OCTA has confirmed the early histopathological vascular changes in diabetics, such as the development of micro aneurysms, happening sooner and are more severe in the DCP than in the SCP [14].

This study explores the SCP and DCP changes in foveal avascular zone in patients with non-proliferative diabetic retinopathy (NPDR) using the OCTA.

## Methods

A prospective, comparative observational study of 60 eyes of 49 patients (30 eyes of 19 patients with NPDR and 30 eyes of healthy subjects) attending Ophthalmology outpatient clinic at Research Institute of Ophthalmology, Giza, Egypt between September 2018 to April 2019.

The study was approved as prospective audit by local institutional review board at Research Institute of Ophthalmology, Giza, Egypt and adhered to the Declaration of Helsinki. All patients provided informed consent for enrolment in the study.

Patients between 30 to 60 years with presence of NPDR in one or both eyes were included in the study and compared with healthy subjects. Any patients with PDR, central macular thickness of more than 300 microns and presence of media opacities preventing reliable retinal imaging and fundus examination were excluded. Patients with other macular pathologies like macular hole, epiretinal membrane, age related macular degeneration, history of macular laser grid or previous vitreoretinal surgery were also excluded.

Data on age, duration of diabetes and previous ocular surgery was collected through history in all subjects. All patients best corrected visual acuity (BCVA) was collected in Snellen's and converted to LogMar units for analysis. Diabetic retinopathy grading was performed on both indirect ophthalmoscopy and direct slit lamp examination.

All patients had spectral domain optical coherence tomography (SD-OCT) and angiography (OCTA) of the macula using Heidelberg engineering, OCT Spectralis, Germany. The imaging protocols used on SD-OCT were Macular radial; to detect the single point central foveal thickness (SPCFT) and Macular thickness map for measurement of central foveal thickness (CFT) among all retinal layers. OCTA of the superficial and deep capillary plexus were captured and were distinctly evaluated using the automatic layer segmentation done by the machine. The protocol consisted of a sequence of 256 sections covering central 10 X 10 recorded in the high-resolution mode (512 A-scans) spaced by 6 μm between individual sections. The SCP was segmented from inner limiting membrane (ILM) to inner nuclear layer (INL), and the DCP was segmented from the INL to outer plexiform layer (OPL). Foveal avascular zone (FAZ) area was measured in both layers

using software "Draw region" tool to outline FAZ area (inner border of the most visible central blood capillaries) manually, the outlined area was then automatically calculated by the software.

Statistical analysis was performed utilising IBM SPSS version 23. The quantitative data were presented as mean, standard deviations and ranges when parametric. Also, qualitative variables were presented as number and percentages. The comparison between groups regarding qualitative data was done by using Chi-square test. The comparison between two independent groups regarding quantitative data with parametric distribution was done by using Independent t-test. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. The confidence interval was set to 95% and the margin of error accepted was set to 5%. A p value of < 0.05 was considered statistically significant.

## Results

Thirty eyes of 19 patients with NPDR were compared with 30 eyes of healthy subjects as control group between September 2018 to April 2019 at Research Institute of Ophthalmology, Giza, Egypt. The mean age of the patients in diabetic group was 56 years ± 2.73 (range 51 - 60 years) and healthy control group was 54.40 years ± 4.48 (range 45 - 59 years) with no clinically significant difference between the two groups (paired t test: -1.669, p = 0.10). The mean duration of diabetes was 13.77 years ± 5.23 (range 7 - 26 years).

Table 1 shows the rate of detection of peri foveolar microaneurysms, capillary drop outs, shape and regularity of FAZ by OCTA in SCP and DCP. Microaneurysms in both SCP and DCP appears as focally dilated capillaries while capillary drop outs appear as retinal capillary loss outside FAZ.

| | SCP | DCP |
|---|---|---|
| Microaneurysms detection | 60% | 86.70% |
| Regularity of FAZ | 70% irregular, 30% regular | 76.7% irregular, 23.3% regular |
| Shape of FAZ | Fusiform/triangular 70%, circular/oval 30% | Diagonal/fusiform 76.7%, circular 23.3% |
| Areas of capillary drop out | 83.30% | 83.30% |

***Table 1:*** *Rate of microaneurysm detection, regularity and shape of foveal avascular zone (FAZ) and areas of capillary drop out detection in superficial (SCP) and deep capillary plexus (DCP) respectively.*

Table 2 shows the comparison of FAZ parameters (surface area, vertical and horizontal diameter) between diabetic and control group in SCP and DCP respectively.

| **A: Comparison between control group and diabetic patients at superficial capillary plexus (SCP)** | | | | | |
|---|---|---|---|---|---|
| **FAZ Parameters** | | **Control group** | **Diabetic patients** | **Test value** | **P-value** |
| **No. = 30** | | **No. = 30** | | | |
| FAZ superficial (mm) | Mean ± SD<br>Range | 0.21 ± 0.04<br>0.15 - 0.28 | 0.44 ± 0.12<br>0.21 - 0.76 | -9.810• | 0.000 |
| Vertical diameter (µm) | Mean ± SD<br>Range | 477.27 ± 57.29<br>400 - 560 | 693.33 ± 114.09<br>454 - 1044 | -9.270• | 0.000 |
| Horizontal diameter (µm) | Mean ± SD<br>Range | 493.40 ± 60.80<br>403 - 595 | 754.97 ± 137.96<br>456 - 986 | -9.503• | 0.000 |
| **B: Comparison between control group and diabetic patients at deep capillary plexus (DCP)** | | | | | |
| **FAZ Parameters** | | **Control group** | **Diabetic patients** | **Test value** | **P-value** |
| **No. = 30** | | **No. = 30** | | | |
| FAZ superficial (mm) | Mean ± SD<br>Range | 0.13 ± 0.03<br>0.08 - 0.18 | 0.32 ± 0.12<br>0.1 - 0.54 | -8.248• | 0.000 |
| Vertical diameter (µm) | Mean ± SD<br>Range | 364.40 ± 55.71<br>230 - 440 | 594.77 ± 143.92<br>325 - 910 | -8.176• | 0.000 |
| Horizontal diameter (µm) | Mean ± SD<br>Range | 387.00 ± 61.38<br>260 - 483 | 624.13 ± 156.40<br>325 - 923 | -7.730• | 0.000 |

***Table 2:*** *Comparison between control group and diabetic patients at superficial capillary plexus (SCP) (A) and deep capillary plexus (DCP) (B).*

Table 3 shows the correlation between the risk factors like age, duration of diabetes with FAZ parameters and functional and structural changes like best corrected visual acuity and central macular thickness with FAZ parameters. There was a clinically significant difference noticed between CMT and FAZ vertical diameter at both SCP and DCP levels which is elaborated further in figure 1. No clinical significance was noticed between FAZ parameters and the above mentioned associated factors.

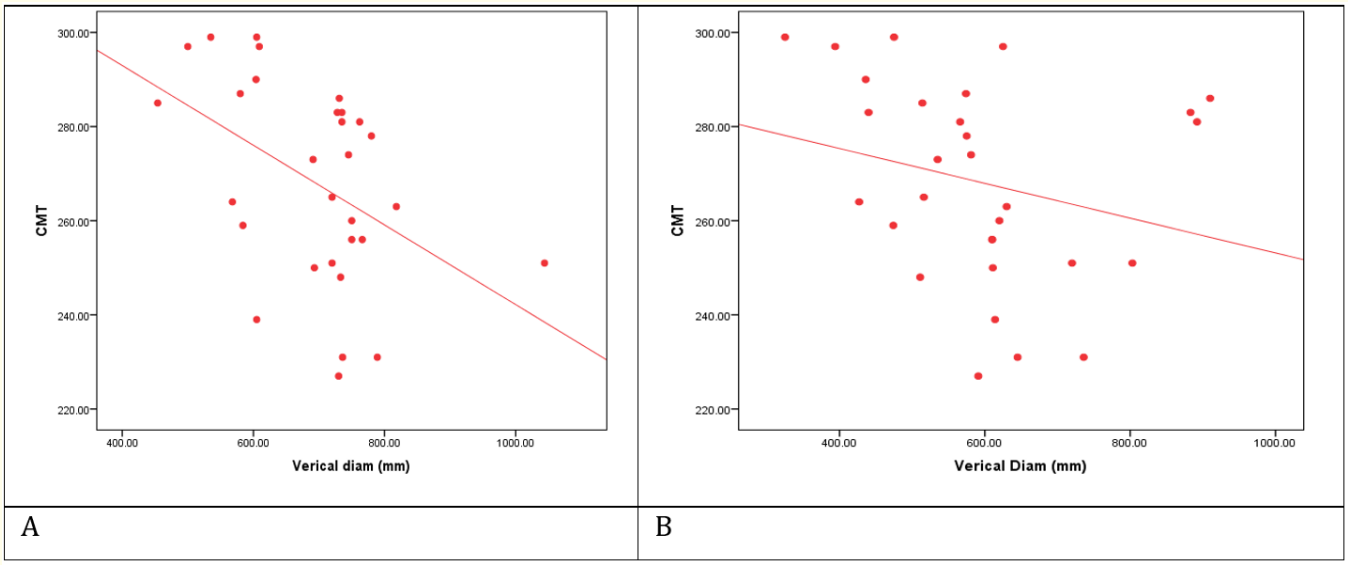
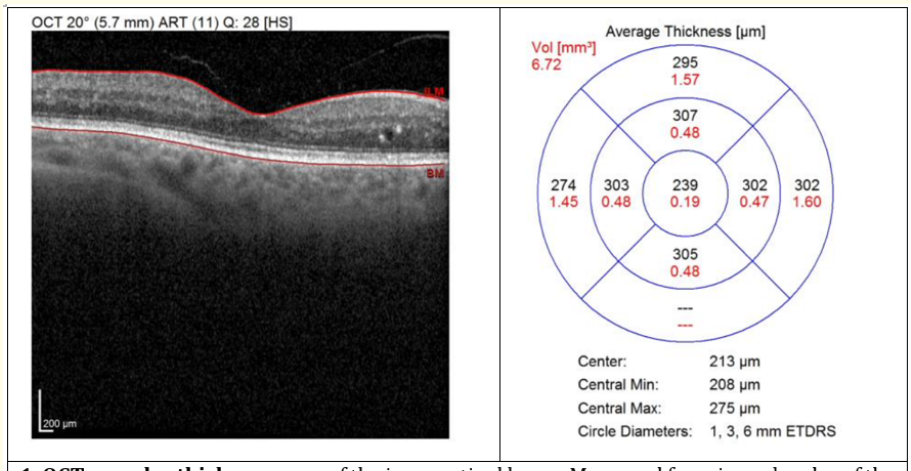
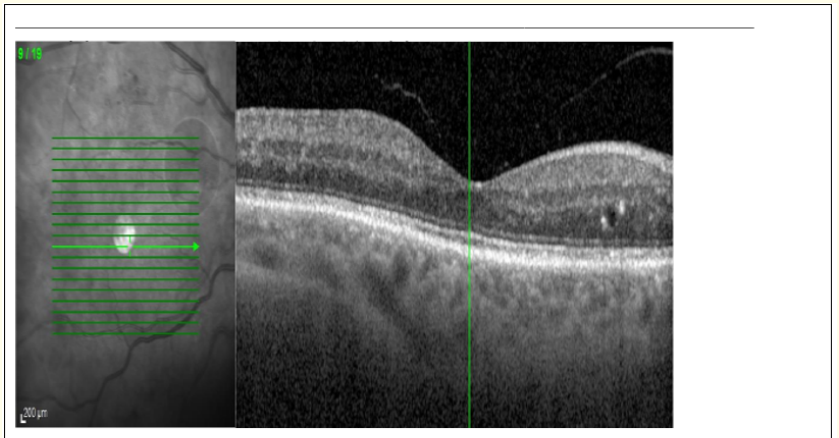


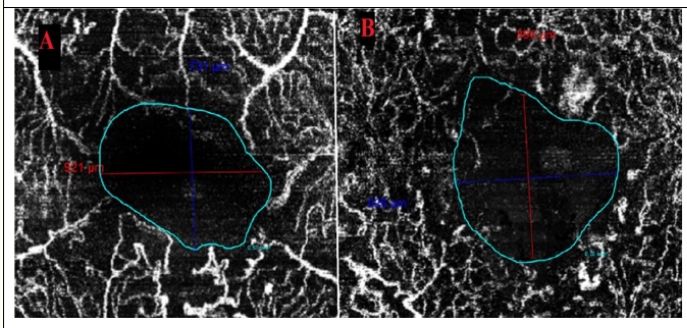

***Figure 1:*** *Correlation between CMT and vertical diameter in Superficial capillary plexus (SCP) (A) and deep capillary plexus (DCP) (B).*




**1. OCT macular thickness map** of the inner retinal layers Measured from inner border of the ILM (inner limiting membrane) to outer border of ELM (external limiting membrane)



2. OCT Macula radial scans




3. OCTA (A)Superficial capillary plexus SCP (B) Deep capillary plexus DCP

***Figure 2:*** *(1) OCT Macular thickness and (2) macular radial maps and corresponding (3) OCTA of superficial and deep capillary plexus (SCP) and (DCP) of single patient.*

| FAZ Parameters | Age | Duration of diabetes | BCVA | CMT |
|---|---|---|---|---|
| | R (P value) | R (P value) | R (P value) | R (P value) |
| FAZ surface area in SCP ($mm^2$) | 0.029 (0.878) | -0.085 (0.655) | -0.034 (0.859) | -0.318 (0.086) |
| FAZ vertical diameter SCP (μm) | -0.007 (0.969) | -0.022 (0.907) | 0.152 (0.422) | -0.463 (0.010) |
| FAZ horizontal diameter SCP (μm) | 0.211 (0.262) | -0.035 (0.856) | -0.267 (0.153) | -0.065 (0.732) |
| FAZ surface area DCP ($mm^2$) | 0.048 (0.801) | 0.046 (0.808) | -0.157 (0.407) | -0.305 (0.101) |
| FAZ vertical Diameter DCP (μm) | 0.176 (0.353) | 0.206 (0.274) | -0.229 (0.223) | -0.382 (0.037) |
| FAZ horizontal diameter DCP (μm) | 0.139 (0.463) | 0.061 (0.750) | -0.234 (0.213) | -0.198 (0.295) |

***Table 3:*** *Foveal avascular zone (FAZ) parameters and studies associations (Age, duration of diabetes, best corrected visual acuity BCVA, central macular thickness (CMT). (R- Correlation coefficient, P < 0.05 clinically significant).*

Figure 2 shows the a single patient OCT (macular thickness map and macula radial maps) and OCTA showing FAZ parameters at both SCP and DCP levels.

## Discussion

Fluorescein angiography (FA) has been accepted as a gold standard investigation for the assessment of diabetic macular ischaemia (DMI) in past decades. Fundus photography and FA has been used as diagnostic investigations to classify diabetic retinopathy into (mild, moderate and severe) NPDR and PDR by the early treatment DR study [15]. It has been shown in studies employing widefield FA that early DR is characterised by peripheral non perfusion which then progress towards macula as the disease progresses [16,17]. Despite the diagnostic accuracy, FA is an invasive procedure which can trigger frequent untoward symptoms like nausea and rarely severe reactions like anaphylactic shock [18]. Furthermore, FAZ visualisation can be inaccurate and retinal vascularization analysis can be unreliable in the presence of retinal pigment epithelium atrophy with FA [19].

In this study, OCTA was used to detect early microvascular changes in the SCP and DCP in FAZ of patients with NPDR and compared with healthy controls. When evaluating patients with DM all quantitative and qualitative parameters detected on OCTA were altered in both SCP and DCP compared with healthy controls. The FAZ was larger and irregular both in the SCP and DCP in NPDR patients compared to healthy controls. The FAZ in SCP and DCP was (0.44 $mm^2$ ± 0.12) and (0.32 $mm^2$ ± 0.12) respectively while in healthy controls it was (0.21 $mm^2$ ± 0.04 $mm^2$) and (0.13 $mm^2$ ± 0.03) respectively (p value < 0.01).

The morphological changes in the superficial and deep capillary plexuses were studied and more pronounced changes were observed in DCP than SCP in FAZ irregularity and presence of microaneurysms. These finding are aligned with study by Akihiro., *et al*. demonstrating that majority of microaneurysms are located in DCP [20]. Frieberg., *et al*. in contrast, demonstrated that the mean FAZ diameter on OCTA is larger in diabetic eyes compared to healthy subjects in superficial vascular layer [21].

This study confirmed no difference in capillary drop out area in SCP and DCP, while Akihiro and colleagues measured the non-perfusion area using RTVue XR Avanti (Optovue Inc, Fremont, California, USA) and demonstrated that the average area of retinal non perfusion was larger for SCP compared to DCP [20]. These findings can be explained through the histopathological evidence on the difference in the extent of retinal non perfusion in superficial and deep plexus, where the microvascular network is well developed in the inner and outer border of inner nuclear layers rendering a protective effect from micro thrombosis in deep plexus [22-25].

OCTA has limitations from several artefacts which can only partially overcome by modern algorithms. Poor fixation caused by reduced visual acuity in diabetic patients can result in motion artefacts [26-28]. Errors in slab segmentation due to macular oedema or thinning of retinal layers can cause erroneous visualization of en face OCTA images [29]. We observed increasing difficulties in obtaining quality scans due to artefacts in patients with media opacities like corneal scarring and cataracts. Hagag., *et al*. explained such artefacts are caused due to back scattering and defocusing of light from the media opacities [30].

Currently, FA is recognised as gold standard for detection of the FAZ and leakage from microaneurysms, but unable to differentiate between superficial and deep retinal vasculature [31]. Limitations of the study include absence of comparative FA images with OCTA. Thus, future studies may be necessary to compare the sensitivity and specificity of OCTA versus FA findings.

## Conclusion

This study suggest that OCTA has a role as a non-invasive tool in early detection of macular microvascular changes in diabetic patient and to study the superficial and deep capillary plexus without the need for FA. The study confirmed a progressive increase in FAZ diameter in NPDR in comparison with controlled group. Validation studies using larger cohorts of the patients on the severity of NPDR would be useful and can facilitate in future clinical application of this investigation as screening and referring tool in DR.

## Ethics Approval and Informed Consent

The study was approved as prospective audit by local institutional review board at Research Institute of Ophthalmology, Giza, Egypt and adhered to the Declaration of Helsinki. All participants provided informed consent for participation in the study.

## Funding

None.

## Conflict of Interest

No financial and non-financial conflicting interests exists for any author.

## Authors Contribution

All authors meet conditions 1-5 of IMCJE authorship guidelines.

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