Original Article

Quantitative analysis of retinal microvascular changes in prediabetic and diabetic patients

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Purpose: To evaluate and correlate retinal microvascular changes in prediabetic and diabetic patients with functional and systemic parameters. **Methods:** Optical coherence tomography angiography (OCTA) was performed on all subjects after medical evaluation and laboratory investigations for blood sugar, glycosylated hemoglobin, and others. Automated quantification of vascular indices of the superficial plexus were analyzed. **Results:** Hundred and eleven persons (222 eyes) were grouped into prediabetic (PDM) (60 eyes), diabetic without retinopathy (NDR) (56 eyes), diabetic with retinopathy (DR) (66 eyes), and healthy controls (CTR) (40 eyes). The superficial retinal capillary plexus showed no significant changes in the prediabetic and NDR groups; however, central foveal thickness (CFT) was significantly reduced in PDM (P = 0.04). The circularity of the foveal avascular zone (FAZ) (P = 0.03) and the vessel density (VD) (P = 0.01) showed significant reduction from PDM to NDR. All vascular parameters were significantly reduced in DR and correlated with disease severity. The CFT correlated significantly with FAZ area. The VD and perfusion density were seen to correlate significantly with HbA1c and contrast sensitivity. The visual acuity was significantly correlated with the FAZ. Logistic regression revealed VD [OR 20.42 (7.9–53)] and FAZ perimeter [OR 9.8 (4.2–23.2)] as the strongest predictors of DR. **Conclusion:** The changes in OCTA can help predict onset of DR. FAZ changes are seen in early stages and are correlated well with systemic parameters, making it an easy target to monitor and screen for severity of DR. Significant reduction in the CFT in PDM suggests that neuronal damage precedes vascular changes.

Key words: Central foveal thickness, diabetic retinopathy, optical coherence tomography angiography, prediabetes, retinal microvascular changes, vessel density

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Diabetes is a major health problem which has reached epic proportions. As per the International Diabetes Federation estimation, globally there are nearly half a billion people with diabetes at present.[1] A 51% increase in the incidence of diabetes has been predicted by the year 2045.[1] Thus, diabetes is likely to be the single most common cause of visual impairment. Preceding the onset of diabetes, a state of prediabetes is increasingly being recognized which carries a high chance of developing into diabetes. In prediabetes, the blood glucose levels are above normal but below diabetes thresholds due to impaired glucose tolerance (postload plasma glucose of 140–199 mg/dL, 2 h after oral glucose).[2] The global prevalence of impaired glucose tolerance is estimated to be 7.5% (374 million) in 2019 and projected to reach 8.0% (454 million) by 2030 and 8.6% (548 million) by 2045.[3]

In order to understand disease progression, it is imperative to unravel pathological processes and changes associated with asymptomatic or preclinical stages of the disease. Even this small elevation of blood glucose may have damaging influence on endothelial cells and small capillaries of the retina. There is a lacuna in the literature with respect to the earliest retinal vascular changes in the prediabetic stage. Optical coherence tomography angiography (OCTA) has rapidly proved itself to be a reliable, fast and noninvasive method for screening of changes in the retinal vasculature.[4,5] Additionally, it can quantify the changes making it easy to compare between patients.

This study was undertaken to evaluate microvascular changes in the retina in prediabetic and diabetic patients and to study their correlations across systemic factors as well as functional parameters.

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Methods

In a prospective study conducted at our institutes between December 2017 and October 2018, 111 persons, including prediabetic, type 2 diabetic patients and controls, were recruited. The study was approved by the institutional review boards and followed the tenets of the Declaration of Helsinki. An informed consent was obtained from every person prior to enrolment in the study. A detailed medical and ocular history was taken for all subjects. The duration of diabetes was recorded. All subjects also underwent estimation of fasting blood sugar levels (FBS) (110–125 mg/dL), post prandial blood sugar, glycosylated haemoglobin (HbA1C), routine urine examination, blood pressure, and body mass index measurements. The best-corrected visual acuity (BCVA) was measured using the logarithmic minimum angle of resolution (logMAR) charts. Contrast sensitivity was measured using the Pelli Robson charts at 1 m. The following criteria were used to define various categories. Any subject with a FBS of 100–125 mg/dL and/or HbA1C of 5.7–6.4% was labeled as prediabetic. An oral glucose tolerance test (OGTT) was performed to further confirm the diabetic or prediabetic state. A value of 140–199 mg/dL at 2 h suggested impaired glucose tolerance and was labeled as prediabetes. Type 2 diabetes was defined as FBS ≥ 125 mg/dL, HbA1C ≥ 6.5%, and OGTT ≥ 200 mg/dL at 2 h. The diabetic retinopathy was defined according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) criteria. Patients were grouped into controls (CTR), prediabetic patients (PDM), diabetic patients with no diabetic retinopathy (NDR), and diabetic patients with diabetic retinopathy (DR). The controls were recruited from among the staff and their relatives who underwent blood tests and were found to be normal without diabetes or prediabetes and had no significant ocular problems apart from mild refractive errors or mild age-related cataract.

The exclusion criteria included the presence of dyslipidemia, chronic renal disorder, uncontrolled hypertension, ischemic heart disease, tobacco chewing or smoking, and pregnancy or any other systemic disorder. Also, patients with media opacities, refractive error more than ± 6 diopters, intraocular pressure >25 mm Hg, ocular pathology other than DR, history of intravitreal injection, laser, or major ocular surgery in the past 4 months were excluded from the study.

Data acquisition

All subjects underwent OCTA after dilation with tropicamide eye drops, with the Zeiss Angioplex OCTA 5000 (Carl Zeiss Meditec, Inc., Dublin, CA, USA). All the measurements were taken by a single operator (DD). A 3 × 3 and 6 × 6 mm square cube angio scans were taken centered on the fovea. Vascular indices and FAZ measurements for the superficial retinal plexus are provided automatically for the 3 × 3 and 6 × 6 angio scans by the built-in software. The vascular indices included vessel density (VD) which is defined as the total length of perfused vasculature per unit area in a region of measurement and perfusion density (PD), which is defined as the total area of perfused vasculature per unit area in a region of measurement. The regions of the tissue were subdivided according to ETDRS subfields. Measurements provided in both tabular form and as density maps (ETDRS grid) through the angioplex metrics tool box were used for analysis. Scans with poor signal strength (less than 5) and motion artifacts were excluded for analysis.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) Version 20.0 software (IBM Corp, Armonk, NY) and Microsoft Excel 2013 (Microsoft Corp, Redmond, WA). To compare difference between independent groups, Student *t*-test for parametric data and Mann–Whitney *U* test for nonparametric data were done. Spearman correlation for nonparametric and Pearson correlation for parametric data was performed to find the strength and direction of association between two parameters. As we used both eyes of the subjects, a repeated measures analysis was done to correct for within subject variation. A P value of < 0.05 was considered as statistically significant. Logistic regression was done in SPSS to find the strongest predictors for diabetic retinopathy.

Results

The study included 60 eyes (30 patients) in the prediabetic group (PDM), 56 eyes (28 patients) of diabetic patients without retinopathy (NDR), 66 eyes (33 patients) with diabetic retinopathy (DR) and controls (CTR) (40 eyes of 20 patients). The demographic details are given in Table 1. The mean duration of diabetes in the DR group (209.11 ± 95.06 months) was higher compared to the diabetic patients with NDR (92.46 ± 82.98 months) ($P < 0.001$). The mean HbA1c values increased from control to the diabetic retinopathy group and were significantly different ($P < 0.001$) across all the groups. The mean log MAR BCVA reduced in the patients with diabetic retinopathy ($P < 0.001$) as well as patients with NDR ($P = 0.02$). There was no significant change in the BCVA while comparing control with prediabetic group ($P = 0.09$). Contrast sensitivity reduced and was significantly different

Table 1: Patient details including demography, mean HbA1c, mean vision, and contrast sensitivity

	Normal	Prediabetic	No DR	DR	*P*
Age (mean + SD)	47.70±6.94	51.92±8.43	54.23±7.47	59.32±8.80	
Total no of patients	20	30	28	33	
Male (*n*, %)	11 (55)	18 (60)	17 (60.7)	22 (66.7)	
Female (*n*, %)	9 (45)	12 (40)	11 (39.3)	11 (33.3)	
Duration of diabetes in months (mean + SD)	-	-	92.46 + 82.98	209.11 + 95.06	<0.001
HbA1c (mean + SD)	5.36±0.30	6.01±0.20	8.32±2.03	9.73±2.28	<0.001
BCVA (mean + SD)	-0.04±0.08	-0.01±0.09	0.02±0.13	0.25±0.26	<0.001
Contrast sensitivity (mean + SD)	1.69±0.04	1.68±0.05	1.63±0.12	1.31±0.35	<0.001

SD: Standard deviation; HbA1c: Hemoglobin A1c; BCVA: Best-corrected visual acuity; DR: Diabetic retinopathy

Table 2: Optical coherence tomography angiography parameters across the groups in 3 × 3 mm and 6 × 6 mm scans

Parameter	Area	Control		Prediabetic		DM with no DR		DR		*P*					
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	CTR Vs PDM	CTR Vs NDR	CTR Vs DR	PDM Vs NDR	PDM Vs DR	NDR Vs DR
	CFT	185.43	13.63	177.64	17.08	183.88	26.05	284.19	182.48	0.04	0.13	0.00	0.15	0.00	0.01
	SFCT	322.80	44.15	310.02	58.18	299.54	56.91	300.58	64.05	0.23	0.02	0.04	0.34	0.42	0.93
FAZ 3 × 3 scan	AREA	0.37	0.10	0.41	0.15	0.37	0.12	0.53	0.32	0.36	0.68	0.00	0.17	0.01	0.00
	PERIMETER	2.66	0.45	2.81	0.56	2.74	0.53	3.69	1.03	0.16	0.45	0.00	0.48	0.00	0.00
	CIRCULARITY	0.66	0.08	0.65	0.07	0.61	0.11	0.49	0.12	0.39	0.01	0.00	0.03	0.00	0.00
VESSEL DENSITY 3 × 3 scan	INFERIOR	20.43	2.58	20.82	2.13	20.08	2.37	16.76	2.50	0.58	0.49	0.00	0.08	0.00	0.00
	SUPERIOR	20.26	2.33	20.98	2.00	19.61	2.53	16.77	2.22	0.11	0.20	0.00	0.00	0.00	0.00
	NASAL	20.65	1.97	21.02	2.07	20.00	2.34	16.84	2.45	0.20	0.16	0.00	0.02	0.00	0.00
	TEMPORAL	20.30	2.54	20.92	1.69	20.30	2.11	16.84	2.42	0.35	1.00	0.00	0.09	0.00	0.00
AVERAGE VESSEL DENSITY 3 × 3 scan	CENTER	8.22	2.47	7.38	3.15	7.99	2.94	6.34	3.30	0.15	0.70	0.00	0.29	0.08	0.01
	INNER	20.42	2.05	20.93	1.71	20.00	2.12	16.81	1.91	0.18	0.34	0.00	0.01	0.00	0.00
	FULL	19.05	1.99	19.40	1.72	18.64	2.10	15.63	1.80	0.44	0.34	0.00	0.04	0.00	0.00
PERFUSION DENSITY 3 × 3 scan	INFERIOR	0.37	0.05	0.38	0.04	0.37	0.04	0.33	0.05	0.83	0.67	0.00	0.19	0.00	0.00
	SUPERIOR	0.37	0.04	0.38	0.04	0.36	0.04	0.33	0.04	0.12	0.27	0.00	0.01	0.00	0.00
	NASAL	0.38	0.04	0.38	0.04	0.37	0.04	0.33	0.05	0.72	0.25	0.00	0.04	0.00	0.00
	TEMPORAL	0.38	0.05	0.39	0.03	0.38	0.04	0.33	0.05	0.63	1.00	0.00	0.40	0.00	0.00
AVERAGE PERFUSION DENSITY 3 × 3 scan	CENTER	0.15	0.05	0.13	0.06	0.14	0.06	0.12	0.06	0.10	0.70	0.00	0.22	0.15	0.01
	INNER	0.38	0.03	0.38	0.03	0.37	0.04	0.33	0.04	0.23	0.47	0.00	0.03	0.00	0.00
	FULL	0.35	0.03	0.35	0.03	0.34	0.04	0.31	0.03	0.46	0.45	0.00	0.10	0.00	0.00
VESSEL DENSITY 6 × 6 scan	INFERIOR	17.08	2.41	17.59	1.98	17.49	1.93	13.66	4.01	0.26	0.51	0.00	0.49	0.00	0.00
	SUPERIOR	17.08	1.91	17.54	1.68	17.11	2.65	14.28	4.06	0.25	0.30	0.00	0.97	0.00	0.00
	NASAL	16.73	2.45	17.61	2.15	18.86	10.78	13.98	4.34	0.04	0.17	0.00	0.32	0.00	0.00
	TEMPORAL OUT	17.07	2.36	17.47	1.70	17.53	1.93	14.44	4.03	0.33	0.34	0.00	0.69	0.00	0.00
	INFERIOR (OUT)	17.31	2.44	17.51	1.97	17.53	1.70	14.20	3.79	0.66	0.60	0.00	0.95	0.00	0.00
	SUPERIOR (OUT)	17.21	2.14	17.65	1.45	19.25	13.61	14.48	3.90	0.39	0.38	0.00	0.89	0.00	0.00
	NASAL (OUT)	18.68	1.86	19.11	1.46	18.75	2.06	15.80	4.43	0.20	0.39	0.00	0.50	0.00	0.00
	TEMPORAL (OUT)	16.23	2.59	16.18	2.46	16.66	2.17	13.75	3.80	0.92	0.38	0.00	0.27	0.00	0.00
AVERAGE VESSEL DENSITY 6 × 6 scan	CENTER	7.63	3.39	7.24	3.13	7.93	2.86	6.74	4.27	0.55	0.42	0.27	0.29	0.47	0.02
	INNER	17.00	2.06	17.56	1.69	17.37	2.01	14.10	3.81	0.15	0.32	0.00	0.89	0.00	0.00
	OUTER	17.37	2.04	17.62	1.51	17.60	1.68	14.56	3.55	0.57	0.45	0.00	0.98	0.00	0.00
	FULL	17.01	2.00	17.32	1.48	17.29	1.71	14.24	3.50	0.37	0.35	0.00	0.94	0.00	0.00

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Table 2: Optical coherence tomography angiography parameters across the groups in 3 × 3 mm and 6 × 6 mm scans

Parameter	Area	Control		Prediabetic		DM with no DR		DR		*P*					
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	CTR Vs PDM	CTR Vs NDR	CTR Vs DR	PDM Vs NDR	PDM Vs DR	NDR Vs DR
PERFUSION DENSITY 6 × 6 scan	INFERIOR	0.41	0.06	0.43	0.05	0.43	0.05	0.35	0.10	0.26	0.22	0.00	0.87	0.00	0.00
	SUPERIOR	0.41	0.05	0.42	0.04	0.42	0.07	0.36	0.09	0.28	0.27	0.00	0.76	0.00	0.00
	NASAL	0.40	0.06	0.42	0.06	0.42	0.05	0.35	0.10	0.08	0.32	0.01	0.44	0.00	0.00
	TEMPORAL OUT	0.41	0.06	0.42	0.04	0.43	0.05	0.36	0.09	0.42	0.09	0.01	0.30	0.00	0.00
	INFERIOR (OUT)	0.44	0.06	0.44	0.05	0.44	0.04	0.37	0.09	0.36	0.66	0.00	0.69	0.00	0.00
	SUPERIOR (OUT)	0.43	0.06	0.44	0.04	0.44	0.05	0.37	0.09	0.45	0.31	0.00	0.86	0.00	0.00
	NASAL (OUT)	0.46	0.05	0.47	0.04	0.46	0.05	0.40	0.10	0.11	0.36	0.00	0.53	0.00	0.00
	TEMPORAL (OUT)	0.40	0.07	0.40	0.06	0.41	0.05	0.35	0.09	0.85	0.19	0.01	0.19	0.00	0.00
AVERAGE PERFUSION DENSITY 6 × 6 scan	CENTER	0.17	0.08	0.16	0.07	0.18	0.07	0.16	0.10	0.50	0.63	0.43	0.17	0.77	0.16
	INNER	0.41	0.06	0.42	0.04	0.42	0.05	0.35	0.09	0.19	0.13	0.00	0.66	0.00	0.00
	OUTER	0.43	0.05	0.44	0.04	0.44	0.04	0.37	0.08	0.59	0.33	0.00	0.78	0.00	0.00
	FULL	0.42	0.05	0.43	0.04	0.43	0.04	0.36	0.08	0.53	0.25	0.00	0.72	0.00	0.00

FAZ: foveal avascular zone, CFT: central foveal thickness, SFCT: subfoveal choroidal thickness, DM: diabetes mellitus, DR: diabetic retinopathy, NDR: no diabetic retinopathy, CTR: control, PDM: prediabetic patients, SD: standard deviation

across all the groups ($P < 0.001$) except between CTR and PDM groups. Table 2 shows the comparison of the OCTA parameters across the groups. All the parameters were similar across control and prediabetic patients with no significant difference except central foveal thickness (CFT). The CFT was significantly reduced in the PDM group ($P = 0.04$). The subfoveal choroidal thickness ($P = 0.02$) and FAZ circularity ($P = 0.01$) varied significantly between CTR and NDR groups, while the rest of the parameters showed no significant difference. Although the average and quadrant wise VD increased in the prediabetic patients compared to control, it decreased from prediabetic to diabetic retinopathy patients. All the vascular parameters showed significant variation in the DR group. On comparing PDM with NDR, significant differences were noted in the VD and PD of the superior ($P < 0.001$, $P = 0.01$) and nasal quadrants ($P = 0.02$, $P = 0.04$). Along with this, the average VD and PD of the inner rings also varied significantly ($P = 0.01$, $P = 0.03$). These changes were observed only in the 3 × 3 mm scan but not in the 6 × 6 mm scan. Table 3 shows the comparison of OCTA parameters across different DR stages. All the parameters were similar in mild and moderate nonproliferative diabetic retinopathy (NPDR). However, the VD in the 3 × 3 mm scan differed significantly between mild NPDR and proliferative diabetic retinopathy (PDR) ($P < 0.001$). The PD of the inferior ($P < 0.001$) area showed significant difference among mild NPDR and PDR. The nasal outer quadrant in the 6 × 6 mm scan alone showed significantly different VD ($P = 0.02$). Between moderate NPDR and PDR only the inferior and the temporal VD differed significantly ($P = 0.01$, $P = 0.02$). Table 4 shows the correlation of the systemic parameters, functional parameters of vision and vascular indices. Age showed poor negative correlation with VD ($R = 0.26$, $P = 0.05$) and PD ($R = 0.28$, $P = 0.03$) in DR. HbA1c was also negatively correlated with VD ($R = 0.35$, $P = 0.01$) and PD ($R = 0.36$, $P = 0.01$).

The logMAR BCVA showed a positive correlation with FAZ area in PDM ($R = 0.44$, $P < 0.001$) and NDR group ($R = 0.41$, $P < 0.001$). The CFT was seen to be negatively correlated with FAZ area in prediabetic ($R = 0.55$, $P < 0.001$) and diabetic groups ($R = 0.37$, $P < 0.001$). The significant correlations are highlighted in Table 5.

Binary logistic regression was done to find the risk of developing DR. Table 6 lists the odds ratio for OCTA parameters. The strongest predictors were VD and FAZ perimeter. If the VD is < 17.25 mm, there is 20.42 times the risk of developing DR. If the FAZ perimeter is > 3.91 mm, the risk of DR is 9.8 times higher.

Discussion

The OCTA is a convenient, noninvasive method of assessing the earliest structural and microvascular changes in the retina in diabetes. It is now widely believed that a state of hyperglycemia termed as prediabetes precedes the onset of actual diabetes nearly by 5–13 years.[2] There is accumulating evidence that retinal neurodegenerative changes occur at this stage much before the onset of microvascular changes.[6] In this study, the CFT was significantly reduced in prediabetic group indicating the presence of early neurodegenerative changes. Hyperglycemia leads to a cascade of events involving glycosylation, release of reactive oxygen species, and advanced glycation end products. These lead to alteration in the blood

Table 3: Optical coherence tomography angiography (OCTA) parameters according to DR severity in 3 × 3 mm and 6 × 6 mm scans

Parameter	Area	Mild NPDR (14 eyes)		Moderate NPDR (19 eyes)		PDR (15 eyes)		*P*		
		Mean	SD	Mean	SD	Mean	SD	Mild Vs Mod NPDR	Mild Vs PDR	Mod NPDR Vs PDR
CFT		202.86	94.46	284.05	148.98	326.37	218.56	0.07	0.02	0.65
SFCT		274.57	46.72	291.68	72.35	322.50	60.01	0.45	0.01	0.12
FAZ 3 × 3 scan	AREA	0.52	0.21	0.46	0.16	0.55	0.44	0.31	0.88	0.70
	PERIMETER	3.57	0.90	3.54	0.86	3.77	1.21	0.93	0.58	0.47
	CIRCULARITY	0.52	0.11	0.46	0.11	0.48	0.13	0.29	0.28	0.76
VESSEL DENSITY 3 × 3 scan	INFERIOR	18.39	1.69	17.41	1.83	15.49	2.63	0.13	0.00	0.01
	SUPERIOR	17.29	2.85	17.03	2.26	16.30	1.82	0.77	0.18	0.24
	NASAL	17.96	1.94	16.72	2.66	16.35	2.48	0.15	0.04	0.64
	TEMPORAL	17.70	2.20	17.60	1.98	15.94	2.45	0.89	0.03	0.02
AVERAGE VESSEL DENSITY 3 × 3 scan	CENTER	5.50	2.26	7.43	5.00	6.20	2.20	0.19	0.34	0.53
	INNER	17.84	1.72	17.21	1.62	16.03	1.93	0.29	0.01	0.28
	FULL	16.46	1.58	16.09	1.69	14.91	1.80	0.54	0.01	0.26
PERFUSION DENSITY 3 × 3 scan	INFERIOR	0.36	0.03	0.34	0.05	0.31	0.05	0.17	0.00	0.37
	SUPERIOR	0.33	0.05	0.33	0.04	0.33	0.03	0.99	0.93	0.64
	NASAL	0.35	0.05	0.32	0.05	0.33	0.05	0.10	0.07	0.67
	TEMPORAL	0.34	0.05	0.34	0.05	0.32	0.05	0.94	0.22	0.15
AVERAGE PERFUSION DENSITY 3 × 3 scan	CENTER	0.10	0.04	0.13	0.09	0.12	0.04	0.24	0.20	0.22
	INNER	0.35	0.03	0.33	0.03	0.32	0.04	0.30	0.05	0.58
	FULL	0.32	0.03	0.31	0.03	0.30	0.04	0.51	0.09	0.72
VESSEL DENSITY 6 × 6 scan	INFERIOR	14.76	3.31	13.89	3.52	13.81	3.83	0.49	0.44	0.95
	SUPERIOR	15.62	2.64	14.89	3.28	13.98	4.11	0.51	0.18	0.44
	NASAL	15.04	3.56	13.81	4.30	14.30	3.89	0.48	0.57	0.69
	TEMPORAL OUT	15.69	2.58	15.29	2.63	14.04	4.25	0.68	0.19	0.28
	INFERIOR (OUT)	15.85	2.94	13.98	3.00	14.26	3.48	0.09	0.15	0.52
	SUPERIOR (OUT)	16.03	2.28	14.72	3.13	14.36	3.90	0.20	0.15	0.75
	NASAL (OUT)	17.67	2.54	15.11	5.35	15.98	3.10	0.16	0.02	0.85
	TEMPORAL (OUT)	15.01	2.68	14.21	2.64	13.64	3.94	0.41	0.25	0.60
AVERAGE VESSEL DENSITY 6 × 6 scan	CENTER	5.85	2.97	7.62	5.09	7.38	4.31	0.26	0.24	0.87
	INNER	15.28	2.63	14.48	3.03	14.06	3.78	0.44	0.29	0.70
	OUTER	16.14	2.18	14.51	2.87	14.56	3.25	0.10	0.11	0.96
	FULL	15.66	2.16	14.30	2.76	14.26	3.31	0.14	0.16	0.97
PERFUSION DENSITY 6 × 6 scan	INFERIOR	0.36	0.08	0.35	0.09	0.34	0.10	0.62	0.52	0.88
	SUPERIOR	0.38	0.07	0.37	0.08	0.35	0.10	0.74	0.28	0.55
	NASAL	0.36	0.09	0.34	0.11	0.35	0.10	0.71	0.72	0.74
	TEMPORAL OUT	0.38	0.06	0.38	0.07	0.35	0.11	0.78	0.52	0.58
	INFERIOR (OUT)	0.40	0.07	0.36	0.08	0.36	0.10	0.13	0.20	0.81
	SUPERIOR (OUT)	0.40	0.06	0.37	0.08	0.36	0.11	0.16	0.22	0.76
	NASAL (OUT)	0.44	0.07	0.38	0.14	0.40	0.09	0.15	0.13	0.93
	TEMPORAL (OUT)	0.38	0.07	0.36	0.07	0.34	0.11	0.94	0.31	0.38
AVERAGE PERFUSION DENSITY 6 × 6 scan	CENTER	0.13	0.07	0.17	0.12	0.17	0.10	0.63	0.23	0.80
	INNER	0.37	0.07	0.36	0.08	0.35	0.10	0.48	0.38	0.68
	OUTER	0.40	0.06	0.37	0.08	0.37	0.09	0.15	0.16	0.99
	FULL	0.39	0.06	0.36	0.07	0.36	0.09	0.18	0.22	0.92

FAZ: foveal avascular zone, CFT: central foveal thickness, SFCT: subfoveal choroidal thickness, DR: diabetic retinopathy, NPDR: nonproliferative diabetic retinopathy, PDR: proliferative diabetic retinopathy, SD: standard deviation

retinal barrier along with proinflammatory changes causing accelerated death of ganglion cells, bipolar, amacrine cells, and photoreceptors.[7,8] In experimental animals, apoptosis of neuroretinal cells was seen as early as 1 month after inducing hyperglycemia.[9] Retinal ganglion cells and amacrine cells are the first to die due to hyperglycemia.[10] Thus, thinning of

Table 4: Correlation of systemic factors with functional parameters of vision and optical coherence tomography angiography parameters

Parameter	Parameter	Control		Prediabetic		No DR		DR	
		R	*P*	*R*	*P*	*R*	*P*	*R*	*P*
Age	HbA1c	-0.58	0.00	0.11	0.39	-0.10	0.47	-0.01	0.92
	BCVA	0.53	0.00	0.56	0.00	0.42	0.00	-0.06	0.63
	CS	0.03	0.88	-0.27	0.04	-0.29	0.03	0.23	0.09
	CFT	0.30	0.06	0.16	0.27	-0.11	0.42	-0.07	0.61
	SFCT	-0.42	0.00	-0.54	0.00	-0.42	0.00	-0.14	0.30
	FAZ AREA	-0.20	0.22	0.24	0.07	0.13	0.34	-0.19	0.14
	Average vessel density	0.08	0.61	-0.15	0.26	-0.16	0.26	-0.26	0.05
	Average perfusion density	0.08	0.64	-0.15	0.24	-0.15	0.27	-0.28	0.03
HbA1c	BCVA	-0.11	0.50	0.19	0.14	0.30	0.03	-0.10	0.44
	CS	-0.07	0.65	-0.37	0.00	-0.15	0.27	0.13	0.33
	CFT	-0.13	0.43	-0.04	0.79	-0.24	0.08	-0.55	0.00
	SFCT	0.34	0.03	-0.15	0.28	0.02	0.87	-0.41	0.00
	FAZ AREA	-0.12	0.45	0.06	0.67	0.07	0.61	0.19	0.15
	Average vessel density	-0.04	0.82	-0.03	0.80	-0.35	0.01	0.10	0.45
	Average perfusion density	-0.02	0.91	-0.06	0.67	-0.36	0.01	0.07	0.59
BCVA	CS	-0.32	0.04	-0.21	0.11	-0.22	0.11	-0.74	0.00
	CFT	-0.02	0.91	-0.07	0.60	-0.17	0.22	0.35	0.01
	SFCT	-0.63	0.00	-0.36	0.01	-0.25	0.07	0.28	0.03
	FAZ AREA	-0.02	0.92	0.44	0.00	0.41	0.00	-0.08	0.55
	Average vessel density	-0.09	0.59	0.02	0.90	-0.25	0.07	-0.21	0.11
	Average perfusion density	-0.07	0.66	0.01	0.92	-0.22	0.10	-0.26	0.05
CS	CFT	0.07	0.69	0.17	0.21	0.03	0.84	-0.37	0.01
	SFCT	0.10	0.56	0.28	0.04	0.08	0.59	-0.35	0.01
	FAZ AREA	-0.03	0.88	-0.27	0.04	-0.07	0.60	-0.03	0.85
	Average vessel density	-0.11	0.50	0.19	0.15	0.07	0.62	0.29	0.04
	Average perfusion density	-0.14	0.39	0.20	0.14	0.08	0.56	0.30	0.03
CFT	SFCT	-0.20	0.22	0.03	0.85	-0.10	0.47	0.51	0.00
	FAZ AREA	-0.19	0.24	-0.55	0.00	-0.29	0.04	-0.37	0.00
	Average vessel density	-0.07	0.65	-0.07	0.64	0.17	0.23	-0.01	0.95
	Average perfusion density	-0.09	0.57	-0.05	0.74	0.17	0.23	0.01	0.97
SFCT	FAZ AREA	0.05	0.75	-0.01	0.97	-0.11	0.45	-0.07	0.59
	Average vessel density	0.15	0.35	-0.16	0.24	-0.15	0.27	-0.10	0.43
	Average perfusion density	0.11	0.51	-0.14	0.32	-0.09	0.52	-0.08	0.53

FAZ: foveal avascular zone, CFT: central foveal thickness, SFCT: subfoveal choroidal thickness, BCVA: best-corrected visual acuity, CS: contrast sensitivity, DR: diabetic retinopathy

the inner retinal layers has been noted in prediabetic subjects, even without overt vascular or inflammatory changes.[11,12] De Clerck *et al*.[13] noted that about half the thinning observed in diabetic patients with no DR was already present at the prediabetic stage. However, with the help of multifocal electroretinography (mfERG) Ratra *et al*.[6] showed early functional neurodegenerative changes expressed as reduced amplitudes in prediabetic subjects without any structural evidence of neurodegeneration such as thinning of the ganglion cell layer. They concluded that neuronal dysfunction is apparent in prediabetes even before the onset of structural damage. These changes precede vascular changes and might even play a role in its pathogenesis. It is hypothesized that the retinal neurodegeneration can trigger an autoregulatory mechanism in the retinal vessels leading to formation of microaneurysms and other changes.[14] In fact increased implicit time in mfERG is considered a predictor for the development of visible vascular abnormalities over 1-year and 3-year periods.[15-17]

There was no significant difference in the CFT between controls and NDR patients, whereas the CFT was increased in DR patients indicating the contributory role of retinal vascular changes.[18] The retinal thickness at the fovea was not seen to correlate with age. In DR eyes, understandably it was correlated with HbA1c, BCVA, and contrast sensitivity. We did not find any correlation between CFT and VD; however, Dimitrova *et al*.[18] and Yu *et al*.[19] have noted significant positive correlation between parafoveal retinal tissue thickness and VD in controls. This suggests that in healthy retina, the VD increases with increasing thickness of the retina. However, the same was not applicable in diabetic retina. This discrepancy is difficult to

Table 5: The significant correlations between the various parameters are highlighted using the shaded squares

Scan size	Parameter	Area	Age		HbA1c		BCVA		CS		CFT		SFCT			
3 × 3	Vessel density	Center					PDM	NDR					PDM		DR		DR
		Inner						NDR	DR		DR	CTR				
		Full		NDR				NDR			DR		PDM			
	Perfusion density	Center		NDR			PDM	NDR					PDM		DR		DR
		Inner		NDR				NDR	DR		DR	CTR				
		Full		NDR				NDR			DR		PDM		DR		
	FAZ	Area					PDM	NDR		PDM			PDM	NDR	DR		
		Perimeter	PDM				PDM	NDR		PDM			PDM		DR		
		Circularity				NDR			NDR				PDM			NDR	
6 × 6	Vessel density	Center					DR	PDM	NDR	DR	PDM		PDM		DR		DR
		Inner				NDR											
		Outer				NDR				DR		DR					
		Full			DR	NDR						DR					
	Perfusion density	Center					DR		NDR	DR			PDM		DR		DR
		Inner			DR	NDR											
		Outer			DR	NDR				DR		DR					
		Full			DR	NDR											
	FAZ	Area					PDM	NDR					PDM		DR		
		Perimeter		NDR			PDM	NDR					PDM		DR		
		Circularity		NDR		NDR		NDR						NDR			

FAZ: foveal avascular zone, CFT: central foveal thickness, SFCT: subfoveal choroidal thickness, BCVA: best-corrected visual acuity, CS: contrast sensitivity, DR: diabetic retinopathy, NDR: no diabetic retinopathy, PDM: prediabetic patients, CTR: controls

Table 6: The odds ratio calculation for risk of developing diabetic retinopathy in diabetic patients with no diabetic retinopathy

Parameter	Median value	Odds ratio	95% Confidence interval
Age	>55 years	2.45	1.15-5.19
HbA1c	>9.18	3.04	1.42-6.52
BCVA	>0.1	13.19	4.61-37.73
CS	<1.55	53.3	11.77-241.25
CFT	>183.5 μ	2.81	1.32-5.99
SFCT	<299 μ	1.47	0.71-3.07
FAZ area	>0.41 mm^2	3.86	1.78-8.39
FAZ perimeter	>3.91 mm	9.84	4.17-23.23
FAZ circularity	<0.55	8.16	3.53-18.87
VD 3 × 3 scan	<17.25 mm^{-1}	20.42	7.86-53.02
PD 3 × 3 scan	<0.33	8.16	3.53-18.88
VD 6 × 6 scan	<16.6 mm^{-1}	6.83	3.01-15.50
PD 6 × 6 scan	<0.416	6.21	2.76-13.98

FAZ: foveal avascular zone, CFT: central foveal thickness, SFCT: subfoveal choroidal thickness, BCVA: best-corrected visual acuity, CS: contrast sensitivity, VD: vessel density, PD: perfusion density

explain. Yu *et al.*[19] postulate that increased retinal thickness might lead to increased nutrient demand leading to increased perfusion or the reverse might also be true that increased VD might translate into increased retinal thickness. In fact, Shen *et al.*[20] noted a negative correlation between CFT and VD in DR eyes, which is very well explained by the retinal ischemia associated with DR changes.

We noted a negative correlation between CFT and FAZ area in PDM, NDR, and DR eyes. Similar findings have been reported in healthy eyes as well as DR eyes.[21,22] In healthy eyes, this inverse relationship might be due to the centripetal displacement of cones and glial cells during foveal development. Lupidi *et al.*[22] further hypothesize that the tendency of the vessels to be codistributed along with glial cells might be responsible for this finding. Thus, an eye with larger FAZ is likely to have lesser concentration of glial cells at fovea making them more susceptible for vascular injury. They suggest that a simultaneous assessment of FAZ and CFT would be a good screening tool to detect damage at the earliest.

The FAZ indices denoting the parafoveal perfusion were seen to be significantly related to diabetic macular ischemia grading with a moderate agreement between OCTA images and the conventional fluorescein angiography images.[23] Some researchers have found increase of FAZ area in eyes without DR, suggesting a compromised retinal circulation before manifest clinical changes of DR.[18,24,25] We noted significant changes in the FAZ circularity in NDR eyes compared to CTR or PDM eyes as the earliest diabetic changes result in irregularity of the FAZ. Not surprisingly, the FAZ area was significantly positively correlated with logMAR vision in PDM as well as NDR eyes, suggesting worse vision with increasing FAZ area indicating macular ischemia. Similar findings have been noted in other studies too.[26,27] However, in DR eyes, such correlation was absent probably due to wide variation in the FAZ.

The VD, PD were reduced from the stage of prediabetes itself, but it showed statistical significance in the DR eyes. The decrease in the VD, PD correlated well with the disease severity. Almost all the previous studies have uniformly reported reduced VD in DR, first appearing in the deep

capillary plexus.[18,24,26,28,29] Interestingly, although the vascular changes correlated with severity of DR, they did not correlate with the duration of diabetes.[28] A regression model identified the FAZ area in superficial plexus, VD in deep plexus, and FAZ acircularity as parameters that best distinguished between DR severity groups.[30] Lei *et al*.[31] noted that among all metrics analyzed, vessel length density of superficial capillary plexus had the highest sensitivity and specificity in detecting mild to moderate diabetic retinopathy. Our study found VD and FAZ perimeter to be strongly predictive of DR changes.

We noted these significant vascular changes in 3 mm cube scans but not in the 6 mm cube scans, suggesting more of a parafoveal involvement in early stages. Vujosevic *et al*.[32] demonstrated early changes in superficial VD in the peripapillary region rather than in the macular region, indicating coexistence of early neuronal damage.

The VD has been seen to be significantly negatively correlated with logMAR visual acuity indicating worse vision with reduced VD.[26] The current study however failed to note any significant correlation between VD and BCVA. Among the systemic parameters VD was significantly correlated with HbA1c, which is in agreement with previous studies.[33,34]

There are a few limitations in our study. We included only the superficial capillary plexus in analysis and did not study the deep plexus. It is possible that the deep plexus might show changes different from those in the superficial plexus. The reason for excluding the deep plexus were difficulties in visualization, and unavailability of automated indices calculation in Angioplex 5000 machine. Manual measurements at the deep capillary plexus are difficult due to poor demarcation of the vessels and it is prone to errors due to projection artifacts. There are certain limitations on account of the machine. The automated FAZ delineation was not possible in a few scans with DR. Hence, manual marking was done. Also, the automated segmentation would have suffered errors in a few patients with diabetic macular edema with gross retinal thickening. Only one single reading was taken for each of the patients, whereas two or more readings would help in reducing the within the subject variation giving better agreement. Nevertheless, only a single operator did all the measurements eliminating interobserver errors.

Conclusion

Quantitative measurements of the microvascular changes on OCTA can identify preclinical early DR changes before the manifestation of clinically apparent retinopathy. These changes correlate well with severity of the retinopathy, and thus serial scans can be helpful in follow-up of DR eyes. They are also directly correlated with functional parameters such as vision and contrast sensitivity and can act as a reliable objective method of assessment. The microvascular changes on OCTA reflect the patient's systemic status as well by showing significant correlation with HbA1c levels. The changes in prediabetic stage point to an early neuronal damage preceding the actual structural or vascular changes, which lead us to believe that early neuroprotective measures in prediabetic stage itself may go a long way in ultimately preventing vision loss due to diabetic retinopathy.

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Conflicts of interest

There are no conflicts of interest.

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