

Assessment of optical coherence tomography angiography and multifocal electroretinography in eyes with and without nonproliferative diabetic retinopathy

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Purpose: To examine (i) the retinal structure and function using optical coherence tomography angiography (OCTA) and multifocal electroretinography (mfERG), respectively, in eyes with and without nonproliferative diabetic retinopathy (NPDR), (ii) and their interrelationship between retinal structure (OCTA) and function (mfERG) in the two groups independently. **Methods:** This was a prospective observational study. One hundred twenty-one eligible participants with type 2 diabetes with No DR ($n = 89$), or with mild or moderate NPDR ($n = 32$) underwent ophthalmic examination, ultrawide field-view fundus photography, OCTA, and mfERG. Group differences were assessed using a Mann–Whitney U test. Correlations were assessed using Spearman's rho. **Results:** There were no significant differences in OCTA measures between the two groups. The mfERG P1 implicit times (rings 1–6) were significantly delayed and P1 response densities in rings 5 and 6 were significantly lower in participants with NPDR compared to those with No DR. In those with No DR, P1 implicit times in almost all rings were delayed in relation to lower vessel density and perfusion (maximum variance noted was 13%). In individuals with NPDR, the P1 response density in rings 2 and 3 showed a positive nonsignificant correlation with macular perfusion. **Conclusion:** In those with diabetes with No DR, retinal neuronal function is influenced by lower macular vessel density and perfusion. The retinal neuronal function is abnormal in individuals with NPDR compared to those with No DR and is not correlated with OCT angiometric measures, suggesting the likelihood of a different retinal structural correlate.

Key words: Diabetic retinopathy, mfERG, OCTA

Diabetes is associated with alterations in multiple neuronal layers of the retina and is associated with alterations in retinal structure and visual function before the appearance of diabetic retinopathy (DR).^[1] Thinning of the outer and inner retinal layers^[2] and several attributes of visual function such as color vision, contrast sensitivity,^[3] dark adaptation,^[4] visual fields and electroretinography^[5] are compromised in the absence of clinical signs of DR.^[6,7] Advances in ophthalmic imaging techniques such as optical coherence tomography angiography (OCTA) have demonstrated subclinical vascular changes, such as enlargement of the foveal avascular zone (FAZ) in the absence of clinical signs of DR.^[8] Macular ischaemia has been proposed to be the underlying mechanism for the aforementioned finding.^[8] Nevertheless, there is lack of information regarding the relationship between retinal

neuronal and subclinical vascular changes in the absence of DR. The current study examined the retinal vascular angiometric measures using OCTA, retinal function using multifocal electroretinography (mfERG) and their interrelationship in individuals with diabetes without DR and mild or moderate nonproliferative DR (NPDR).

Methods

The study was conducted at a tertiary eye hospital in Chennai, south India, after obtaining ethics approval from the Institutional Review Board of our hospital. The study was conducted in accordance with the Declaration of Helsinki. Consecutive patients who visited our hospital for a regular ophthalmic check-up and hospital staff were screened for eligibility from March 2018 till June 2019. Participants provided written informed consent after explanation of the nature and possible consequences of the study. Consenting adults with type 2 diabetes and a minimum of 1-year duration of diabetes without DR and those with mild or moderate NPDR were

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eligible. Individuals with diabetes underwent comprehensive eye examination including visual acuity testing, refraction, slit-lamp examination, intraocular pressure measurement, pupillary dilatation, and retinal evaluation using ultrawide field view fundus photography. Individuals were excluded if they had media haze that compromised visual acuity or quality of imaging, coexisting ocular infection or inflammation, spherical refractive error greater than ± 6 D, astigmatism greater than ± 3 D, IOP >22 mmHg, a vertical and horizontal cup-disc ratio >0.6 , had undergone or were planned for vitreoretinal surgery in at least one eye, retinal vascular diseases other than DR, participating in any other interventional research trial [Fig. 1].

DR was graded by a single experienced operator (SS) based on the International Clinical Diabetic Retinopathy Disease Severity Scale^[9] into no DR-no retinopathy; mild NPDR-presence of microaneurysms only; and moderate NPDR-more than just microaneurysms but less than severe NPDR. Participants with untreated or treated severe NPDR, proliferative DR, and diabetic macular edema were excluded. One hundred and twenty-one eligible participants underwent OCTA, mfERG, and ultrawide field fundus photography in both eyes.

OCTA was performed using the Angioplex™ Cirrus HD-OCT 5000, Carl Zeiss meditec, USA, built on the previous Cirrus™ HD-OCT Model 5000 instrument that generates 104 high-resolution 3-D maps of the retinal and choroidal microvasculature from 68,000 A-scans per second 105 from the central 6 mm. Participants underwent angiography 6×6 mm for quantitative vascular and perfusion measures from the superficial capillary plexus layer and high-definition radial scans. Images with signal strength < 6.0 were excluded. FAZ measures such as area, perimeter, circularity, vessel density, and perfusion measures of the superficial vascular plexus from the angiography $6 \text{ mm} \times 6 \text{ mm}$ scans were recorded. Those with poorly defined FAZ borders ($n = 9$) were excluded.

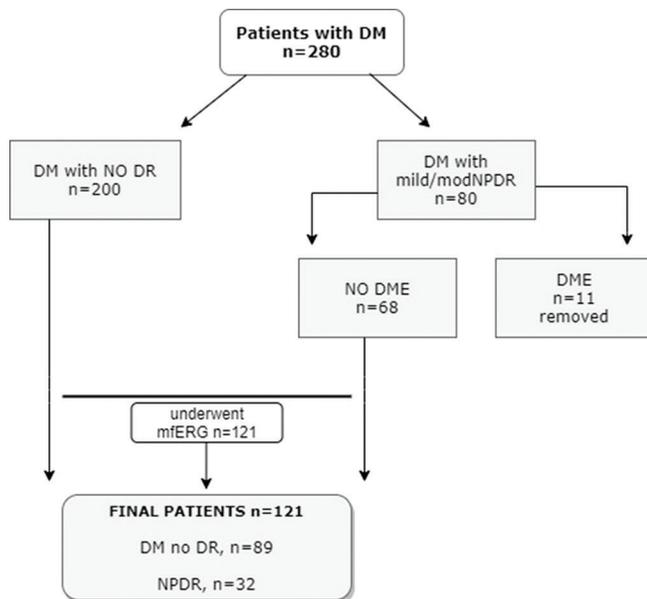


Figure 1: Flowchart of participant recruitment. (One participant (NPDR, $n=80$) in the NPDR group did not fixate well despite good visual acuity, so excluded, thus NPDR was $n=79$)

Participants underwent multifocal electroretinogram (Veris™ Science 6.4.8 app, California, USA) unilaterally, using a Burian Allen Electrode based on the guidelines of the International Society for Clinical Electrophysiology of Vision guidelines for basic mfERG. An array of 103 hexagons was displayed at a frame rate of 75 Hz. Testing was done using a Burian Allen Electrode, unilaterally with refractive correction in place, and the other eye was patched. A gold cup electrode attached to the earlobe served as a ground electrode. The stimulus for mfERG consisted of an array of 103 hexagons presented on a monitor at a frame rate of 75 Hz, subtending an angle of 35 degrees horizontally and 31 degrees vertically at a viewing distance of 53 cm, flickering according to a pseudorandom m-sequence at a mean luminance of 64 cd/m^2 . The luminance of the bright and the dark hexagons were 128 and 1 cd/m^2 , respectively. For fixation, a red cross of 2-mm diameter was used and an in-built camera enabled the operator to monitor fixation throughout the recording. An internal Grass amplifier (Grass Technologies, An Astro-Med, Inc, West Warwick, R. I.) amplified the recordings (6100 000) which were then band-pass filtered (10–100 Hz). The actual mfERG recording time was 7 min and 17 s per eye. The mfERG responses (response density and implicit times) were analyzed using the Veris software and first-order kernels were recorded and displayed in the form of a trace array of 103 local retinal responses, a three-dimensional topographical chart, and as group trace averages of six concentric rings. Amplitudes recorded per unit area were assessed as response density (in nanovolts per square degree, nV/deg^2) which provides a measure of the amplitude obtained in each ring, considering its size. The implicit time denotes the time taken to reach the maximum amplitude in the respective macular region assessed.^[10] The response densities and implicit times were displayed in six rings. In order to maintain steady fixation on the fixation target in mfERG and to read the contrast sensitivity chart, a minimum visual acuity of 20/60 was considered a prerequisite.

Optomap (Optos UWF™, Optos Inc, USA) was utilized for ultrawide field view fundus photography. Red-green and autofluorescence fundus images were obtained in the central, superior, inferior, nasal, and temporal fields.

The right eye of all participants was analyzed. The OCT and mfERG variables did not conform to normality. Therefore, differences between the two groups were assessed using a Mann-Whitney U test. Correlations were assessed using the Spearman's rank correlation (ρ).

Results

Of the 121 eyes, 89 had No DR and 32 had NPDR (mild NPDR, $n = 11$, moderate NPDR, $n = 21$) [Fig. 1]. Clinical variables in participants with diabetes with No DR and NPDR are shown in Table 1.

There were no significant differences in age between the No DR and the NPDR groups ($P = 0.631$). Those with NPDR had prolonged duration of diabetes (12.0 yrs vs. 9.6 yrs, $P = 0.034$) and higher HbA_{1c} levels than those with diabetes with No DR (9.7% vs. 9.1%, $P = 0.005$). The refractive error assessed as spherical equivalent ($P = 0.835$) and LogMAR visual acuity ($P = 0.700$) were comparable between the two groups.

Table 1: Clinical variables in No DR and NPDR groups

	DM No DR <i>n</i> =89		NPDR <i>n</i> =32		M-W U
	Mean	SD	Mean	SD	<i>P</i>
Age	58.9	8.9	58.1	6.9	0.631
DM duration	9.6	6.1	12.0	7.0	0.034
HbA _{1c}	9.1	2.5	9.7	2.1	0.005
Sp Eq	0.22	1.8	0.31	1.45	0.835
LogMAR units	0.04	1.32	0.14	0.65	0.700

DM, diabetes mellitus; DR, diabetic retinopathy; NPDR, nonproliferative DR; Sp Eq, spherical equivalent; M-W U, Mann-Whitney U test, LogMAR, Logarithm of the Minimum Angle of Resolution

Table 2: OCT angiometrics in No DR and NPDR groups

	DM No DR <i>n</i> =89		NPDR <i>n</i> =32		M-W U
	Mean	SD	Mean	SD	<i>P</i>
FAZ area	0.30	0.14	0.31	0.15	0.555
FAZ perimeter	2.42	0.68	2.56	0.84	0.514
FAZ circularity	0.62	0.11	0.62	0.13	0.949
Overall vessel density	14.57	2.93	14.13	3.08	0.410
Overall perfusion	35.37	7.65	34.82	8.24	0.740

DM, diabetes mellitus; DR, diabetic retinopathy; NPDR, nonproliferative DR, FAZ, foveal avascular zone; M-W U, Mann-Whitney U test

Table 3: mfERG variables in No DR and NPDR groups

	DM No DR, <i>n</i> =89		NPDR, <i>n</i> =32		M-W U
	Mean	SD	Mean	SD	<i>P</i>
1. P.Implicit time	31.900	2.942	33.745	4.669	0.009
1. P1.Amp	29.651	11.69	25.443	11.409	0.055
2. P1.Implicit time	31.129	2.627	32.99	4.399	<0.001
2. P1.Amp	21.551	8.2	19.774	7.694	0.327
3. P1.Implicit time	30.156	1.943	32.021	3.603	<0.001
3. P1.Amp	17.331	6.825	15.571	5.899	0.205
4. P1.Implicit time	29.966	2.023	31.678	3.689	<0.001
4. P1.Amp	14.732	5.879	12.684	4.801	0.06
5. P1.Implicit time	30.071	1.835	31.717	3.744	<0.001
5. P1.Amp	12.803	5.345	10.704	4.033	0.044
6. P1.Implicit time	30.559	2.276	32.148	3.865	0.001
6. P1.Amp	11.795	5.75	9.691	3.72	0.044

DM, diabetes mellitus; DR, diabetic retinopathy; NPDR, nonproliferative diabetic retinopathy; M-W U, Mann-Whitney U test; Amp, amplitudes assessed as response densities

The OCT angiometric parameters in the two groups are summarized in Table 2. The FAZ area ($P = 0.555$), perimeter ($P = 0.514$), circularity ($P = 0.949$), overall macular vessel density ($P = 0.410$), and perfusion ($P = 0.740$) did not show significant differences between the two groups.

The mfERG measures in the two groups are summarized in Table 3. The mfERG P1 implicit times are delayed in all rings in the NPDR compared to the No DR group. In addition, P1 response density in rings 5 ($P = 0.044$) and 6 ($P = 0.044$) were

Table 4: Correlation coefficients between OCT angiometrics and mfERG central ring parameters (ring 1)

OCTA variables	Spearman's rho	Ring 1.P.Lat.	Ring 1.P1.Amp.
DM No DR			
Area	Correlation Coefficient	-0.196	0.070
perimeter	Correlation Coefficient	-0.106	-0.005
Circularity	Correlation Coefficient	0.109	0.139
VD	Correlation Coefficient	-0.184	0.252*
Perfusion	Correlation Coefficient	-0.196*	0.276**
NPDR			
Area	Correlation Coefficient	0.225	-0.045
perimeter	Correlation Coefficient	0.273	0.050
Circularity	Correlation Coefficient	-0.009	-0.209
VD	Correlation Coefficient	-0.076	0.137
Perfusion	Correlation Coefficient	-0.135	0.150

DM, diabetes mellitus; DR, diabetic retinopathy; NPDR, nonproliferative diabetic retinopathy; VD, vessel density; Amp, amplitudes assessed as response densities; *Correlation is significant at the 0.05 level (two-tailed). **Correlation is significant at the 0.01 level (two-tailed)

significantly lower in the NPDR group compared to the No DR group.

The correlations between OCT angiometric measures and mfERG measures in rings 1–6 are summarized in Tables 4 and 5.

In patients with No DR, there were no significant correlations between implicit time and response density with the OCTA-derived area, perimeter, or circularity. However, the vessel density correlated inversely with the implicit time in ring 2 ($\rho = -0.260$, $P = 0.008$), ring 3 ($\rho = -0.198$, $P = 0.047$), ring 4 ($\rho = -0.229$, $P = 0.021$), and ring 5 ($\rho = -0.209$, $P = 0.035$) and directly with the response density in ring 1 ($\rho = 0.252$, $P < 0.011$), ring 2 ($\rho = 0.330$, $P < 0.001$), ring 3 ($\rho = 0.331$, $P < 0.001$), ring 4 ($\rho = 0.317$, $P < 0.001$), ring 5 ($\rho = 0.316$, $P = 0.001$) and ring 6 ($\rho = 0.303$, $P = 0.002$). In patients without DR, perfusion correlated inversely with the implicit time in ring 1 ($\rho = -0.196$, $P = 0.048$), ring 2 ($\rho = -0.273$, $P = 0.005$), ring 3 ($\rho = -0.226$, $P = 0.022$), ring 4 ($\rho = -0.252$, $P = 0.010$), and ring 5 ($\rho = -0.234$, $P = 0.018$) and directly with the response density in ring 1 ($\rho = 0.276$, $P = 0.005$), ring 2 ($\rho = 0.352$, $P < 0.001$), ring 3 ($\rho = 0.356$, $P < 0.001$), ring 4 ($\rho = 0.345$, $P < 0.001$), ring 5 ($\rho = 0.346$, $P < 0.001$), and ring 6 ($\rho = 0.331$, $P < 0.001$). There were no significant correlations between IT and response density with OCTA-derived area, perimeter, or circularity in those with diabetes with No DR.

In NPDR eyes, the P1 amplitude in rings 2 and 3 showed a positive nonsignificant correlation with the macular perfusion ($\rho = 0.275$, $P = 0.104$ and $\rho = 0.213$, $P = 0.212$, respectively).

Discussion

The main findings of the study are that (i) the OCT angiometric measures did not differ in the NPDR group compared to No DR group but the P1 implicit times in all rings were delayed and response density in rings 5 and 6 were significantly lower in those with NPDR compared to No DR. The above findings are already well established in DR.

Table 5: Correlation coefficients between OCT angiometrics and mfERG rings 2-6 measures in diabetes with No DR and NPDR groups

	2.P1.IT.	2.P1.Am.	3.P1.IT.	3.P1.Am.	4.P1.IT.	4.P1.Am.	5.P1.IT.	5.P1.Am.	6.P1.IT.	6.P1.Am.
DM No DR										
Area	-0.11	-0.01	-0.166	0.001	-0.214	0.021	-0.158	0.01	-0.109	0.032
perimeter	-0.163	-0.063	-0.132	-0.021	-0.146	0.004	-0.179	-0.004	-0.159	0.018
Circularity	0.128	0.073	-0.01	0.017	-0.056	0.018	-0.028	0.029	0.018	0.026
VD	-0.260**	0.330**	-0.198*	0.331**	-0.229*	0.317**	-0.209*	0.316**	-0.171	0.303**
Perfusion	-0.273**	0.352**	-0.226*	0.356**	-0.252*	0.345**	-0.234*	0.346**	-0.185	0.331**
NPDR										
Area	0.206	-0.101	0.186	-0.015	-0.018	-0.091	-0.052	-0.002	-0.084	0.072
Perimeter	0.252	-0.087	0.186	0.017	0.052	-0.064	0.04	-0.005	0.014	0.002
Circularity	-0.038	-0.095	0.015	-0.154	0.016	-0.038	0.032	0.023	0.069	0.112
VD	0.09	0.257	0.081	0.181	0.042	0.165	0.037	0.177	0.014	0.143
Perfusion	0.028	0.275	0.037	0.213	0.003	0.197	-0.013	0.207	-0.059	0.17

DM, diabetes mellitus, DR, diabetic retinopathy; NPDR, nonproliferative diabetic retinopathy; Correlation coefficients assessed by Spearman's rho; VD, vessel density; IT, implicit times; Am, amplitudes assessed as response densities. *Correlation is significant at the 0.05 level (two-tailed). **Correlation is significant at the 0.01 level (two-tailed)

Nevertheless, data in individuals with diabetes with No DR is scarce. Therefore, our primary focus was on patients with diabetes with no clinical signs of DR. Therefore, we examined the retinal structure–function correlation in each group independently. It was observed that in eyes with No DR, the P1 implicit time in almost all mfERG rings are delayed in relation to lower macular vessel density and perfusion. (ii) P1 response densities are positively correlated with the vessel density and perfusion index.

The mfERG P1 implicit times are delayed in all rings in the NPDR compared to the No DR group. With regards to the response density, the P1 response densities are reduced only in rings 5 and 6. One explanation could be that the implicit times may be more sensitive to vascular insult or ischemia when compared to response density.^[11,12] Another explanation could be that mfERG response densities are subject to wide variations, and therefore, may not be as reliable indicators as are the implicit times.^[11,12]

In eyes with No DR, we observed that a lower macular vessel density and perfusion are correlated with delayed P1 implicit times and lower response densities in almost all rings. Minimum variance in P1 implicit times was noted as 4% and maximum variance was noted to be 7% in relation to both, vessel density and perfusion. The minimum variance in P1 response density was noted to be 7–8% and the maximum variance ranged between 11% and 13% for vessel density and perfusion, suggesting that 13% of the variance in the P1 response density is attributed to lower macular perfusion in eyes with No DR. A likely explanation for the relatively lower correlations could be that we examined only the superficial capillary plexus. The deep capillary plexus has been demonstrated to correlate better with the retinal functional measure due to disruption of photoreceptors.^[13] A recent study by Kim *et al.*^[14] examined the correlation between OCT macular vessel density and full-field ERG. A positive correlation was observed between the ERG amplitude and vessel density, and a negative correlation between the implicit times and superficial vessel density in the scotopic and combined response b-wave.

These correlations between electrophysiological parameters and vascular parameters were not significant in the nondiabetic, healthy control group. The study by Kim *et al.*^[14] utilized a full-field ERG that evaluates the mass electrical activity from the entire retina. Our study utilized a multifocal ERG that allows recording and analysis of multiple focal retinal responses. The findings from our study indicate neuronal dysfunction in eyes with no clinical signs of DR and are related to subclinical vascular compromise. The mfERG responses were noted to be reduced in the No DR group. This could be caused by potential neuronal functional changes occurring earlier than the actual structural changes in DR.^[15,16]

The mfERG implicit times are delayed in the NPDR group compared to the No DR group, and this observation was made in the presence of no significant differences in angiometric measures. When examining correlations within each group, the response density is lower and implicit times are delayed in relation to a lower macular vessel density and perfusion only in the No DR group and were not significant in the NPDR group. The delay in mfERG implicit times in the NPDR group compared to the No DR group with absence of significant correlation with angiometric measures may suggest that there could be another retinal structural correlate that is outside of what is assessed in this study.

Studies involving primates have reported that the origin of first negative (N1) implicit times of mfERG is largely due to OFF-bipolar cells while depolarization of the ON-bipolar cells contributes to P1 implicit times.^[11,17,18] A significant correlation between a higher FAZ diameter and prolonged implicit times is often an early sign of capillary closure.^[19] The reduced macular vessel density and/or perfusion in our study may suggest occlusion or destruction of parafoveal retinal capillaries and it also influences the retinal function assessed by mfERG in the absence of DR. The assessment shows early functional impairment even before structural alterations are clinically visible. Similarly, a recent study by Ratra *et al.*^[20] examined OCT angiometric parameters and mfERG measures in those with prediabetes in comparison to those with no

diabetes. It was observed that OCT angiometric parameters did not show significant differences between the two groups. However, the mfERG amplitudes in rings 1–5 were reduced and the implicit times in rings 4–6 were significantly delayed in individuals with prediabetes compared to those with no diabetes. The study demonstrated changes in the mfERG parameters before retinal structural changes are noted on OCTA^[20] indicating that these changes might start as early as the prediabetic stage. We observed that in the NPDR group, the P1 response densities in rings 2 and 3 showed a tendency to be lower in relation to lower macular perfusion, but are not significant. The European Consortium for the Early Treatment of Diabetic Retinopathy study proposed the likely existence of two phenotypes in NPDR: one phenotype with visible microvascular changes but with no neuronal changes, and the other with neuronal changes in the absence of microvascular changes.^[21] Another likely explanation could be that the deep rather than the superficial capillary plexus is demonstrated to have the strongest correlation with DR severity as it may be more susceptible to oxygen deprivation.^[22]

The absence of significant correlations in the NPDR group in our study may likely be related to the aforementioned factors.

The mfERG assessment may selectively elicit responses from the ON- and OFF-bipolar cells, and the implicit times have been reported to be spatially linked to retinopathy lesions^[13,23,24] and may suggest glial activation.^[18] The observation that the P1 implicit times in all rings are significantly delayed in relation to reduced macular vessel density and perfusion in our study suggest that early neuronal functional compromise is evident in specific retinal layers in the presence of no clinical signs of DR.

Previous studies have demonstrated that the FAZ area and perimeter show an increasing trend in individuals with diabetes with DR compared to those with no diabetes and an increasing trend in various grades of NPDR.^[25,26] Our study shows a similar but a nonsignificant difference in angiometric measures between the two groups. In addition, in the No DR group, there were no significant correlations between implicit time and response density with the OCTA-derived area, perimeter, or circularity measures from the superficial capillary plexus rather than the superficial capillary plexus. One explanation could be that the deep capillary plexus is reported as being more susceptible to ischemic damage^[22] evident even before the appearance of clinical signs of DR.

In addition, a decrease in the vessel density and perfusion in the macula is reported in those with NPDR compared to those with diabetes with No DR.^[25,26] We observed that that the OCT angiometric measures did not differ in the NPDR group but demonstrated delayed implicit times in all rings when compared to the No DR group. This could likely be a sign of macular ischemia in the absence of visible signs of ischemia on OCTA.^[25,26]

Strength of this study is that ultrawide field photography was utilized to determine the presence and the severity of DR. In addition, a standardized grading scale was utilized and the photographs were graded by a single experienced operator. Participants with severe NPDR, PDR, and DME were excluded. Nevertheless, the study has certain limitations. Since we examined consecutive consenting patients who visited the hospital, we could not recruit an equal number

of participants for all categories of DR severity. A majority of those with NPDR had moderate NPDR. Therefore, the results reported here may be largely applicable to those with moderate NPDR, and may benefit from validation in a larger cohort of individuals in various stages of DR. We examined the quantitative vascular and perfusion measures from the superficial capillary plexus layer. Lack of quantitative measures of the deep capillary plexus in the OCTA utilized in our study may have limited our potential to deduce further conclusions. Another potential limitation of this study is that we did not examine axial length for participants in both the groups, but excluded spherical refractive errors greater than 6 D sphere and astigmatism greater than 3 D cylinder. Therefore, our OCT angiometric findings were not corrected for axial length^[27] in both the groups.

Conclusion

In individuals with diabetes with No DR, the retinal neuronal function seems to be influenced by a lower macular vessel density and perfusion. The abnormal retinal neuronal function in individuals with NPDR in the absence of significant correlation with the OCT angiometric measures may suggest the likelihood of a different retinal structural correlate.

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Conflicts of interest

There are no conflicts of interest.

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