

In vivo assessment of associations between photoreceptors structure and macular perfusion in type 1 diabetes

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ABSTRACT

Purpose To explore the potential relationships between macular vascular network and different adaptive optics (AO) metrics in patients with type 1 diabetes mellitus (DM1) with no signs (NoDR) or mild non-proliferative diabetic retinopathy (NPDR).

Design Observational cross-sectional study.

Methods Forty eyes of consecutive patients with DM1 (12 NoDR and 28 NPDR) and 10 healthy age-matched control subjects were included. All patients and controls were imaged using AO retinal camera and PLEX Elite 9000 optical coherence tomography (OCT) angiography (OCTA). The AO outcome measures to evaluate the cone photoreceptor mosaic characteristics were as follows: (1) Cone density (CD); (2) Linear Dispersion Index (LDi) and (3) Heterogeneity Packing Index (HPI). The OCTA outcome measures included: (1) superficial capillary plexus (SCP) perfusion density (PD); (2) deep capillary plexus (DCP) PD and (3) the choriocapillaris (CC) flow deficit percentage (FD%).

Results NPDR group exhibited a close relationship between cone metrics and CC FD. Notably, CC FD% increase along with LDi ($p=0.035$), while the increasing CC FD% were associated with reducing CD ($p=0.042$) and the HPI ($p=0.017$). Furthermore, the OCTA parameters, including PD SCP and DCP, showed a significant negative correlation with CD.

Conclusions Our results demonstrated the relationship between macular perfusion at both retinal and choroidal levels and the cone mosaic in patients with DM1 interpolating swept-source-OCTA and AO metrics. In NPDR eyes, the photoreceptor damage was accompanied by CC insufficiency since the early stages of the disease.

INTRODUCTION

Diabetic retinopathy (DR) represents the leading cause of blindness and reduced macular function among working-age individuals in the Western world.¹ Neurodegeneration has been considered the earliest pathogenic event in DR,² but microvascular changes precipitate macular dysfunction leading to outer retinal layers and photoreceptor damage.^{3–6} During retinal hypoxia, the retinal microvascular network represents the leading vascular supply, as the choroidal circulation lacks autoregulation.^{7,8}

Previous studies hypothesised an association between the reduced flow in the deep capillary plexus (DCP) and the photoreceptor damage.^{8,9} Recent

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Previous important imaging studies revealed that macular ischaemia in diabetic retinopathy (DR) may be associated with photoreceptors damage. In particular, an interpolation of cone metrics was performed exclusively with the choriocapillaris layer of type 2 diabetic eyes. Therefore, the current literature lacks evidence regarding the direct interpolation of cone photoreceptors morphometric changes in response to microvascular changes at retinal and choroidal levels in preclinical and/or early DR stages in type 1 diabetes mellitus (DM1) and the corresponding topographical evaluation.

WHAT THIS STUDY ADDS

⇒ By interpolating optical coherence tomography angiography (OCTA) and adaptive optics (AO) findings, this study aimed to quantify and correlate macular perfusion and photoreceptor damage in patients with DM1 with no or mild non-proliferative DR (NPDR). The results of this study demonstrated the relationship between macular perfusion (both retinal and choroidal) and AO metrics in patients with DM1 diabetes using a combination of OCTA and AO.
⇒ In addition, the study revealed topographical differences among sectors suggesting a regional predilection for the temporal sector in NPDR, which appeared more vulnerable to diabetic damage.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These findings may provide precious insights into the pathophysiology of structural and functional damage in patients with DM1.

studies have explored more in detail the close connection between choriocapillaris (CC) flow¹⁰ and the ellipsoid zone (EZ) reflectivity, which can be considered a surrogate for photoreceptor damage.^{11–14}

Adaptive optics (AO) provides a high-resolution in vivo assessment of the cone photoreceptors and mosaic metrics.^{4,15} Recent studies have shown disturbance of the cone mosaic in patients with diabetes even without any signs of DR.^{4,5} More



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recently, the interpolation of cone metrics was performed exclusively with the CC layer of type 2 diabetic eyes.¹⁶

Therefore, the current literature lacks evidence regarding the direct interpolation of cone photoreceptors morphometric remodelling in response to macular microvascular changes in preclinical and/or early DR stages in type 1 diabetes mellitus (DM1). Further, a topographical evaluation may reveal sectors early affected in a population of DM1. This particular cohort also has the advantage of having fewer confounding factors derived from systemic comorbidities.

This study aimed to explore the potential relationship between AO metrics and macular microvascular changes in DM1 eyes, focusing on the association of retinochoroidal vascular network and photoreceptor structural changes. Furthermore, to assess microvascular and AO variations in patients with DM1 with no diabetic signs or mild non-proliferative diabetic retinopathy (NPDR) and healthy controls.

METHODS

Study participants

In this observational cross-sectional study, patients with DM1 with no signs of diabetic retinopathy (NoDR) or mild NPDR were consecutively enrolled at IRCCS-Bietti Foundation between July and December 2020.

Of the 60 eyes (60 subjects) initially evaluated, 50 eyes fulfilled the inclusion criteria, of whom 40 patients with DM1 and the remaining 10 eyes served as healthy controls. Inclusion criteria for DM patients were age > 18 years, best-corrected visual acuity (BCVA) of 20/20, a diagnosis of DM1 from at least 4 years NoDR or mild NPDR according to the modified ETDRS retinopathy severity scale.¹⁷ Mild NPDR was defined as the presence of at least one microaneurysm and/or mild haemorrhages. Two experienced examiners (MP and EC) identified the eligible eyes based on clinical and instrumental examinations (colour fundus photographs using the Topcon TRC-50DX and optical coherence tomography (OCT)).

Inclusion criteria for the control group consisted of age-matched healthy subjects without DM1 or any other retinal disorders. Exclusion criteria were: (1) evidence or history of any other ocular disease; (2) previous ocular surgery; (3) axial length > 26 mm and/or

refractive error > 6 dioptres (D); (4) evident or subclinical macular oedema detected on OCT B-scans; (5) significant media opacities and (6) poor quality images (signal strength index (SSI) < 8) with either significant motion artefacts and/or incorrect segmentation. If both eyes were eligible, the right eye was chosen by default.

All patients received a complete ophthalmologic examination, including BCVA, intraocular pressure evaluation, and dilated ophthalmoscopy. All enrolled subjects were imaged with swept-source OCT angiography (SS-OCTA) PLEX Elite 9000 device (Carl Zeiss Meditec, Dublin, CaliforniaA, USA) and the AO retinal camera (rtx1; Imagine Eyes, Orsay, France) (figure 1).

Image processing

Adaptive optics

AO retinal camera uses infrared illumination (wavelength, 850 nm) with a spatial resolution of 2 μ m. Images were acquired at different locations within the central retina, covering an area of 5°×4° centred on the preferred locus of fixation (coordinates x=0° and y=0°). On average, five frames were obtained for each session to maximise the acquisition quality. A proprietary programme provided by the manufacturer has been used to adjust the raw image sequence for distortions and to correlate frame-average to produce a final image with an enhanced signal-to-noise ratio before further analysis.¹⁸ The final images were registered, aligned and montaged using the montage tool in i2k Retina Pro (DualAlign, Clifton Park, New York, USA) (figure 2).

Optical coherence tomography angiography

A 3×3 mm OCTA volume scan was acquired for each eye using FastTrac motion correction software to remove shadowing and/or segmentation artefacts. En face OCTA images automatically segmented by PLEX Elite 9000 built-in software at the superficial capillary plexus (SCP), DCP and CC levels. The OCTA slabs exported as PNG format with a dimension of 1024×1024 pixels were thus imported into ImageJ software V.1.50 (National Institutes of Health, Bethesda, MD; <http://rsb.info.nih.gov/ij/index.html>) and a 2.25 mm diameter square centred on the fovea was cropped (figure 3A). Then, using the 'Landmark correspondences

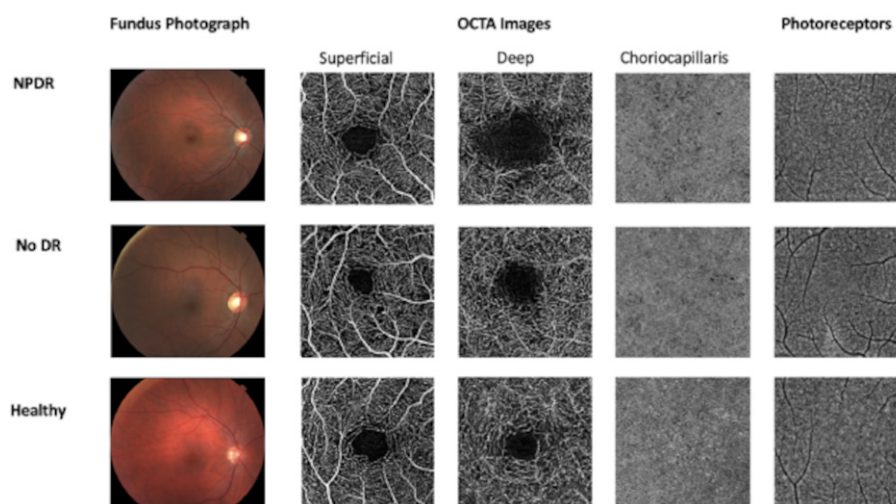


Figure 1 Representative colour fundus photographs, optical coherence tomography angiography (OCTA) and adaptive optics of healthy patients and diabetic patients. The severity of diabetic retinopathy was assessed by our group based both on the clinical ocular fundus evaluation and on the evaluation of routine instrumental examinations. Colour fundus Photographs were obtained using the Topcon TRC-50DX. En face OCTA images automatically segmented by PLEX elite 9000 built-in software at the SCP, DCP and CC levels. Adaptive optics were imaged using an AO retinal camera (rtx1; Imagine eyes, Orsay, France). AO, adaptive optics; CC, choriocapillaris; DCP, deep capillary plexus; NPDR, non-proliferative diabetic retinopathy; SCP, superficial capillary plexus.

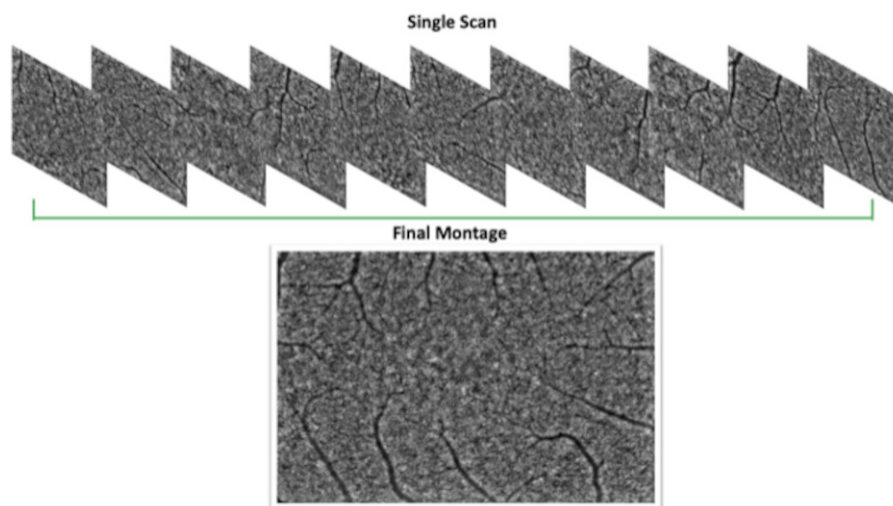


Figure 2 Representation of the algorithm used to obtain final AO image of a diabetic patient without signs of diabetic retinopathy. Images were acquired at different locations in the central retina covering an area of $5^\circ \times 4^\circ$ (single scan). Consequently, a proprietary programme provided by the manufacturer has been used to adjust the raw image sequence for distortions and to correlate and frame average to produce a final image with an enhanced signal-to-noise ratio. The final images were montaged using the Montage tool in i2k retina pro (final Montage). AO, adaptive optics.

method', the refined OCTA images were registered with the AO montage obtaining a square of the same size, which was precisely overlaid using the retinal vasculature as reference (figure 3B).¹⁹ The outer diameter of 2.25 mm was used to ensure that the macular vascular slabs were correctly overlapped with the AO montage image (figure 3B).

AO and OCTA quantitative analysis

The ETDRS grid was used to customise the quantitative analysis for each slab. Two different rings were plotted: (1) foveal region, which was defined as a 1 mm circle centred on the fovea, (2) parafoveal region, consisting of a 2 mm ring-shaped area surrounding the foveal region.²⁰ On OCTA analysis, the potential physiological increase in the intercapillary distance along the temporal sector was overcome by only considering the central 3 mm in the present analysis.²¹

The parafoveal region was analysed in different sub-fields: superior, inferior, nasal, and temporal areas consisting of 0.65 mm^2 each (figure 4A), excluding the foveal region from the analysis. AO transverse resolution can resolve up to $2 \text{ }\mu\text{m}$ cones, limiting the ability to analyse the fovea where the photoreceptors are densely packed (figure 4A). Therefore, the parafoveal region was adopted to estimate the cone metrics precisely, considering the microvascular parameters in the same regions.

Imaging analysis

AO metrics

The AO measures analysed were: (1) Cone density (CD), which represents the number of cones in the sampling area (cones/ mm^2); (2) Cone spacing (CS), denoting the distance between neighbouring cones measured through the Linear Dispersion

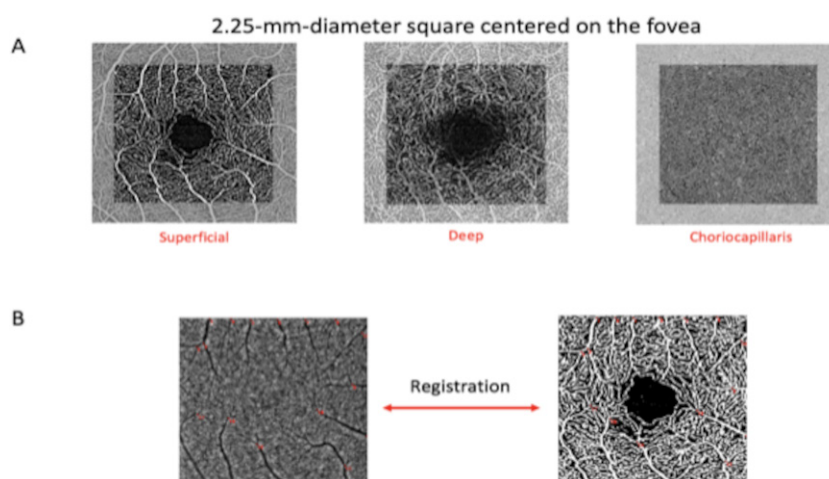


Figure 3 Representation of the OCTA and AO image processing of a patient with diabetes without signs of diabetic retinopathy. En face OCTA images automatically segmented by PLEX Elite 9000 device at the superficial capillary plexus (SCP), DCP and CC levels were imported into ImageJ software and a 2.25 mm diameter square centred on the fovea was cropped (A). then, the refined slabs were registered with the AO Montage image obtaining a square of the same size, which was precisely overlaid using the retinal vasculature as reference (red dotted numbers). The outer diameter of 2.25 mm was used to ensure that the macular vascular slabs were correctly overlapped with the AO Montage image (B). AO, adaptive optics; CC, choriocapillaris; DCP, deep capillary plexus; OCTA, optical coherence tomography angiography.

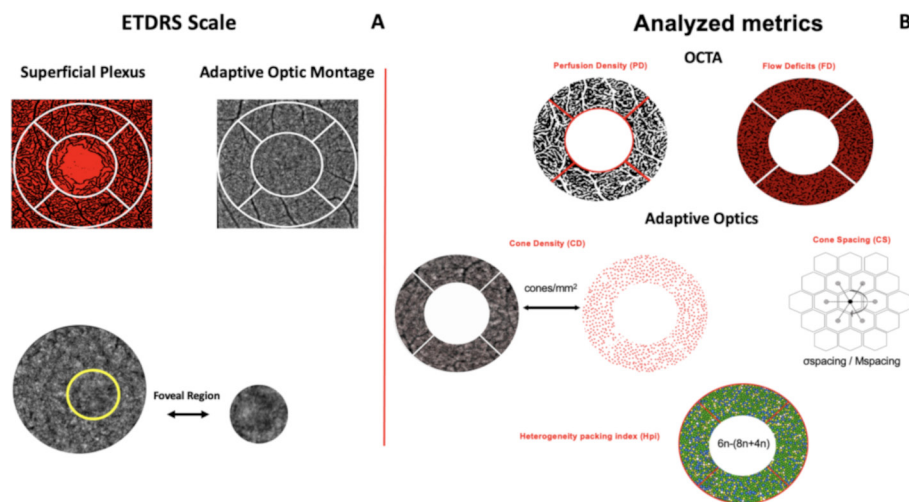


Figure 4 Representation of the investigated regions and of the analysed OCTA and AO metrics of a patient with diabetes without signs of diabetic retinopathy. regions used to investigate OCTA and AO variables. For each slab, the ETDRS grid was used to customise the quantitative analysis. Two different rings were plotted: (1) foveal region, which was defined as a circle centred on the fovea with a diameter of 1 mm, (2) parafoveal region, which consists of a ring-shaped area of the 2 mm surrounding the foveal region. Furthermore, the analysis of the parafoveal region was performed in different subfields: the superior, inferior, nasal and temporal areas consisting of 0.65 mm² area each. Of note, the quantitative analysis was made in the parafoveal region sub-fields, excluding the foveal region. The reason resides in the AO ability to resolve up to 2 µm cones in the retina, which results in a significant decrease in cone resolution within the foveal region. this effect may be due to the increased density and packing of the photoreceptors in the central fovea. Therefore, the parafoveal region estimation can provide more accurate cone parameters. the two obtained thresholded images then were merged to obtain the final image. OCTA and AO analysed metrics. The OCTA measures analysed were as follows: (1) the SCP perfusion and (PD); (2) the DCP PD and (3) the CC flow deficit percentage (FD %). PD defines the ratio of the area occupied by the vessels divided by the total area; the FD percentage (%) represents the absence of flow of the CC vascular layer. The main AO measures analysed were as follows: (1) Cone density (CD), which represents the number of cones in the sampling area (cones/mm²); (2) Cone spacing (CS), which determines the distance between neighbouring cones measured through the Linear Dispersion Index, (LDi) (M±SD) and (3) the cone packing (CP) arrangement showing the disposition of the cells within the mosaic calculating the Heterogeneity Packing Index, (Hpi) expressed in percentage, (%), which describes the fractional increase in four-sided and eight-sided non-hexagonal Voronoi tiles for six-sided cells $6n-(8n+4n)$. A decreased HPI results in a greater deviation of the cone mosaic from the normal arrangement. All metrics were tested in parafoveal region. AO, adaptive optics; CC, choriocapillaris; DCP, deep capillary plexus; OCTA, optical coherence tomography angiography.

Index, (LDi) (M±SD) and (3) The cone packing (CP) arrangement showing the cells arrangement within the mosaic calculating the Heterogeneity Packing Index (Hpi). (figure 4B).

A previously reported algorithm with the image processing toolbox in MATLAB (The Mathworks, Natick, Massachusetts, USA) was applied to evaluate these cone metrics.^{4 5} To allow an optimal validation of the cone identification, an independent masked observer manually identified and corrected the cones erroneously selected by the algorithm.^{4 5}

The CD was detected automatically by calculating cones number per square millimetre (cones/mm²). The Ldi was defined by the ratio of $\sigma_{\text{spacing}}/\text{Mspacing}$, where σ_{spacing} is the SD of the mean Mspacing, representing the distance between neighbouring cones in the given mosaic.²¹ The Hpi (%) describes the fractional increase in four-sided and eight-sided non-hexagonal Voronoi tiles for six-sided cells 3–5 ($6n-(8n+4n)$), used to find spatial-distribution pattern alterations of the cones (ie, the CP); the CP arrangement was assessed graphically using Voronoi diagrams. Finally, according to the good AO metrics reproducibility²² the x,y coordinates of the cones were stored and used to calculate CD, Ldi and Hpi.

Optical coherence tomography angiography

The OCTA measures analysed were: (1) the SCP perfusion density (PD); (2) the DCP PD and (3) the CC flow deficit percentage (FD%).

PD defines the ratio of the area occupied by the vessels divided by the total area; the FD represents the absence of flow or FD appreciable in the CC (figure 4B).²² The SCP, DCP and CC slabs were automatically segmented by the PLEX Elite 9000 device and then imported into ImageJ software. PD was calculated after binarizing the exported images with a previously validated thresholding algorithm.^{23–25}

CC FD% was calculated after using the 'Phansalkar' threshold to obtain binarised images, as previously described.^{11 26} DCP and CC areas beneath major superficial retinal vessels were excluded from the analysis to avoid potential shadows or projection artefacts.^{25 26}

Statistical analysis

Statistical evaluation was performed using SPSS (IBM SPSS Statistic V.25). All results were expressed as the mean±SDs. Pearson's correlation was computed to analyse the degree of correlation between data in each group at different region of interest. The data distribution was tested through the one-sample Kolmogorov-Smirnov test. Analysis of variance (ANOVA) one-way and Kruskal-Wallis (KW) tests were used to assess differences among the study groups, as appropriate. Post hoc comparisons were performed with Bonferroni correction for multiple tests. A multiple-comparison correction was applied to counteract repeated measures on the four retinal regions (superior, inferior, temporal and nasal) using Bonferroni correction. In brief, the p

Table 1 Clinical characteristics of subjects included in the analysis

Variables	NPDR (n=28)	No DR (n=12)	Controls (n=10)	P value
Age (years)	41.7±5.77	36.7±5.62	38.1±2.97	0.180
Gender (male, %)	14 (50)	7 (58)	3 (30)	0.290
Duration of diabetes (years)	21.4±8.34	12.3±4.66	N/A	<0.001
HbA1c (%)	7.41±0.8	6.9±0.7	N/A	0.079

Quantitative data are presented as mean±SD.
HbA1c, glycated haemoglobin (A1c); N/A, not available; NPDR, non-proliferative diabetic retinopathy.

values resulted from ANOVA and KW tests were corrected by a 4 factor (p value multiplied × 4). A p of 0.05 was considered statistically significant.

RESULTS

Characteristics of subjects included in the analysis

Of the 60 eyes (60 individuals) initially enrolled, 10 patients were excluded either because of a low OCTA (SSI<8) or incorrect segmentation at the CC level, or whether the average of the AO frames exhibited significant motion artefacts.

Of the 50 subjects (50 eyes) included, 28 had mild NPDR (14 males), 12 NoDR (7 males) and 10 were healthy controls (3 males). The characteristics of subjects included in the analysis are shown in [table 1](#).

Cone metrics analysis

The parafoveal average LDi was significantly higher in NPDR eyes (0.10±0.02) than controls (0.09±0.0, p=0.002). No differences were found with the NoDR group ([table 2](#)). In addition, the topographical subanalysis highlighted statistically significant differences in all the subfields analysed. Particularly, the LDi temporal region in the NPDR subgroup was significantly higher in comparison with both the NoDR (p=0.005) and the control group (p=0.002) ([table 3](#)).

The parafoveal average HPi (%) was significantly lower in the NPDR subgroup (33.69±6.67) compared with control eyes (43.93±3.97; p<0.001) and NoDR eyes (38.88±4.16; p=0.036) ([table 2](#)).

CD was not significantly different between groups (online supplemental file 1). The mean (±SD) parafoveal CD in the NPDR, NoDR, and control groups was 16265±1073, 16592±2822 and 16953±1245 cells/mm², respectively. Although not statistically significant, a density reduction trend was observed in the NPDR and NoDR groups compared with the controls ([table 2](#)).

OCTA analysis

In the parafoveal SCP, the NPDR group showed a lower parafoveal PD in comparison with the control group (35.73±1.88% and 37.86±0.7%, respectively (p=0.0008)). Within the parafoveal SCP region, the NPDR group showed a greater PD reduction in the temporal region than in the healthy eyes (p=0.017). Similarly, the parafoveal DCP PD was lower in the NPDR group compared with the control group (33.72±3 and 36.32±1.75%, respectively (p=0.043)).

Contrariwise, no significant difference was found in PD between the NoDR group with control and NPDR groups. (p>0.05) ([table 2](#)). For further details, see also the supplementary file 1.

Otherwise, the parafoveal CC FD was significantly increased in the NPDR group compared with control group (16.89±6.80% and 10.78±3.56%, respectively (p=0.039)) and NoDR group (10.76±4.52%, p=0.013).

The topographical CC subanalysis highlighted statistically significant FD% increase in the inferior and temporal parafoveal regions between NPDR and the control group (16.90±6.92% and 10.60±3.29%, (p=0.016); 16.78±6.66% and 10.07±3.57%, (p=0.008), respectively). Likewise, the NPDR group compared with the NoDR showed CC FD% increase in all four regions considered (p<0.05). All significant values are summarised in [table 3](#).

Relationship between OCTA parameters and AO metrics

The NPDR group was characterised by a close relationship between cone metrics and CC FD. Notably, CC FDs increase along with LDi, while the increasing CC FDs were associated with reducing CD and the HPi ([table 4](#)).

The OCTA parameters, including PD SCP and DCP, showed a significant negative correlation with CD, indicating that the retinal PD tended to increase along with a CD reduction. The NPDR temporal sector was characterised by a higher negative

Table 2 Cone metrics and OCTA analysis for parafoveal region

	NPDR	NoDR	Controls	P value
Parafoveal CD	16265±1073	16592±2822	16953±1245	0.307
Parafoveal LDi	0.10±0.02 ^{*A}	0.09±0.0	0.09±0.0	0.001
Parafoveal HPi (%)	33.69±6.67 ^{*A}	38.88±4.16 ^{*B}	43.97±3.97	<0.001
Parafoveal SCP PD (%)	35.73±1.88 ^{*A}	36.85±1.94	37.86±0.70	0.007
Parafoveal DCP PD (%)	38.95±3.97 ^{*A}	38.56±4.30	38.83±1.92	0.043
Parafoveal CC FD (%)	16.89±6.80 ^{*A}	10.76±4.52 ^{*B}	10.78±3.56	0.004

Data are presented as mean±SD with the p value calculated using ANOVA one-way or Kruskal-Wallis tests. Post hoc test results are specified as follow: in particular, A=NPDR versus controls; B=NPDR versus NoDR.

*P<0.05 and superscript letters denote the compared groups.

ANOVA, analysis of variance; CC, choriocapillaris; CD, cone density; DCP, deep capillary plexus; FD, flow deficits; HPi, Heterogeneity Packing Index; LDi, Linear Dispersion Index; NoDR, no signs of diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; OCTA, optical coherence tomography angiography; PD, perfusion density; SCP, superficial capillary plexus.

Table 3 Cone metrics and OCTA parameters topographic analysis for the four regions of interest

	NPDR	NoDR	Controls	P value
Cone metrics				
Superior LDI	0.101±0.017 ^{*A}	0.093±0.007	0.086±0.005	0.004
Temporal LDI	0.097±0.017 ^{*A}	0.086±0.004 ^{*B}	0.085±0.003	<0.001
Nasal LDI	0.098±0.016 ^{*A}	0.088±0.006	0.084±0.003	0.016
Superior HPI (%)	33.32±7.79 ^{*A}	38.05±6.44	46.10±7.19	0.004
Inferior HPI (%)	35.17±7.15 ^{*A}	41.26±4.89 ^{*B}	43.63±5.93	0.004
Temporal HPI (%)	33.53±6.47 ^{*A}	39.09±4.25	43.15±4.59	<0.001
Nasal HPI (%)	32.73±6.97 ^{*A}	37.11±4.58	42.86±4.71	0.004
OCTA parameters				
Superior SCP PD (%)	34.82±1.92 ^{*A}	35.79±2.40	37.24±1.19	0.028
Temporal SCP PD (%)	36.53±2.20 ^{*A}	38.15±1.76	38.67±0.85	0.024
Nasal SCP PD (%)	35.90±3.26 ^{*A}	37.08±1.94	38.35±1.21	0.04
Superior CC FD (%)	16.62±6.83 ^{*B}	10.87±4.86	11.12±3.44	0.032
Inferior CC FD (%)	16.90±6.92 ^{*A}	10.81±4.77 ^{*B}	10.60±3.29	0.016
Temporal CC FD (%)	16.78±6.66 ^{*A}	10.60±4.89 ^{*B}	10.07±3.57	0.008
Nasal CC FD (%)	17.27±7.12 ^{*B}	10.78±3.97	11.33±4.36	0.028

Data are presented as mean±SD with the corrected p value calculated using ANOVA one-way or Kruskal-Wallis tests, the applied correction deals with the repetition of the analysis in the four areas. Post hoc test results are specified as follow: in particular, A=NPDR versus Controls; B=NPDR versus NoDR.

*P<0.05, corrected with Bonferroni, and superscript letters denote the compared groups.

ANOVA, analysis of variance; CC, choriocapillaris; CD, cone density; DCP, deep capillary plexus; FD, flow deficits; HPI, Heterogeneity Packing Index; LDI, Linear Dispersion Index; NoDR, no signs of diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; OCTA, optical coherence tomography angiography; PD, perfusion density; SCP, superficial capillary plexus.

correlation of the OCTA variables in the parafoveal subfields. Contrariwise, the NoDR and control eyes did not show any correlations between cone metrics and OCTA parameters (table 4).

DISCUSSION

This study revealed a microvascular impairment accompanied by cone metrics alterations occurring early in DM1 eyes with NPDR, whereas no evident changes were appreciable in the NoDR group.

Our findings supported a strong connection between cellular impairment of photoreceptors and macular vascularity, demonstrated by a significant relationship between OCTA and AO parameters. Both the CD and HPI tended to reduce with increasing CC

flow impairment in the parafovea, while the LDI, expressing the distance between cells, increased. These findings corroborate the previous results on the association between microvascular changes and the EZ reflectivity.^{11 12} By contrast, a more recent study showed no direct association between CC FDs and the HPI, after interpolating choroidal microcirculation and cone photoreceptors mosaic in type 2 DM eyes suffering from NPDR and proliferative DR.¹⁶ Possible explanations for such discrepancy are represented by the cohort examined, consisting of patients with type 2 DM suffering from many systemic comorbidities affecting primarily the microvasculature and possibly the cone arrangement. Further, only the HPI has been analysed among cone metrics with a different regional evaluation, interesting the entire fovea and parafovea.

A topographic subanalysis within the parafoveal regions revealed novel and intriguing insights, pointing towards a regional predilection for the temporal sector in NPDR. Notably, the retinal PD was significantly lower in the NPDR temporal field. This finding is in agreement with a previous study showing that the temporal region was the most sensitive area to predict the onset of mild NPDR.²⁷

Several studies have pursued the well-known microvascular theory as the main responsible for the progressive visual loss in DR eyes using OCTA.^{8 28 29} In a previous study using OCTA, Nesper *et al*²⁹ have observed significant correlation between retinal and CC vascular nonperfusion with disease severity in eyes with DR. Likewise, the reduced amount of SCP and DCP flow demonstrated a correlation with DR severity.²⁸ Scarinci *et al*⁸ reported a direct relationship between the flow rarefaction at the DCP level and the photoreceptors disruption using a qualitative assessment of the ellipsoid band integrity. Choroidal microvascular abnormalities mainly limited to the CC were also described in DR.^{30 31}

In this study, we have considered a combination of microvascular retinal and choroidal flow parameters in the early stages of the disease to detect potential microvascular changes affecting the photoreceptors.

Table 4 Correlation between OCTA parameters and AO metrics in NPDR group

	Cone density	Cone packing (Hpi)	Cone spacing (LDi)
Parafoveal SCP PD	−0.38 (p=0.04)	−0.01	0.21
Parafoveal DCP PD	−0.15	0.26	−0.12
Parafoveal CC FD	−0.39 (p=0.042)	−0.48 (p=0.017)	0.40 (p=0.035)
Superior CC FD	−0.31	−0.43	0.40 (p=0.03)
Inferior SCP PD	−0.40 (p=0.033)	−0.10	0.18
Inferior CC FD	−0.48 (p=0.010)	−0.54 (p=0.003)	0.55
Temporal SCP PD	−0.47 (p=0.012)	−0.08	0.20
Temporal DCP PD	−0.44 (p=0.049)	0.09	0.03
Temporal CC FD	−0.39 (p=0.04)	−0.35	0.39
Nasal CC FD	−0.29	−0.42 (p=0.03)	0.33

Pearson correlation values and p values are specified in the table as followed: (p value), p values are specified only if <0.05.

AO, adaptive optics; CC, choriocapillaris; DCP, deep capillary plexus; FD, flow deficit; HPI, Heterogeneity Packing Index; LDI, Linear Dispersion Index; NPDR, non-proliferative diabetic retinopathy; OCTA, optical coherence tomography angiography; PD, perfusion density; SCP, superficial capillaries plexus.

Our study further displayed that the CD in the NPDR tended to reduce with increasing parafoveal SCP PD. This unexpected finding needs to be carefully interpreted. In fact, it implies a perfusion increase in the retinal superficial microvascular plexus related to a decreased CD. One possible explanation relies on the increased oxygen and nutrients demand towards the outer retinal layers in the early stages of DR.^{7 32 33} Accordingly, the parafoveal SCP could increase flow as a compensatory mechanism when photoreceptors started suffering, leading to an apparent overflow.

The topographical subanalysis further corroborates these findings by revealing a CD reduction in the temporal parafovea correlated to increasing flow in the SCP and DCP.

We hypothesise that this transient compensatory mechanism is more concentrated in the temporal sector as it is most sensible to predict the onset of mild NPDR.²⁷ Furthermore, the population analysed suggests that this overflow precedes the typical diabetic vascular damage.

Regarding the cone metrics alterations using AO, our results have identified a significant disruption of the parafoveal cone mosaic in NPDR patients. The cone photoreceptors distancing was significantly increased (LDi) with an impaired cone arrangement as demonstrated by a reduction of HPI. The CD seemed to be reduced in diabetic eyes (NDPR and NoDR), although it did not reach a statistical significance. These findings support a relevant photoreceptor reorganisation in terms of spacing and rearrangement in the early stages of the disease, as previously demonstrated.^{4 5} The novelty of the present work regards the interpolation of the macular microcirculation at retinal and CC levels with photoreceptor morphometry, allowing us to identify a significant impact of the impairment CC microcirculation on the photoreceptors organisation in the NPDR eyes. The increasing CC ischaemia was associated with a reduced CD and packing (ie, CD and Hpi), and a larger spatial distance (Ldi). Such findings were not recognisable in the NoDR and healthy controls, suggesting a CC hypoxic status related to vascular modifications in NPDR affecting the cone mosaic. These findings may result from the cohort examined, consisting of patients with DM1 without systemic or cardiovascular comorbidities, suggesting that other systemic diseases may contribute to microvascular damage in patients with type 2 DM.

On a previous OCTA study¹¹ the EZ reflectivity, obtained by postprocessing the EZ en-face slabs through an image software analysis, was combined with CC perfusion in type 2 diabetic eyes demonstrating an association between CC ischaemia and reduced EZ reflectivity. However, the EZ band intensity can be influenced by the waveguiding of the photoreceptors and by the scattering of light, representing an 'indirect' surrogate for the photoreceptors health.³⁴ The use of AO in our study has overcome these limitations, as this sophisticated instrumentation allows to resolve the cellular-resolution imaging of rod and cone structures.^{7 34}

Our study has several limitations to consider when interpreting our findings. First, the sample size was relatively small with a cross-sectional design. In addition, the images can contain artefacts, including motion, projection or shadowing, leading to incorrect OCTA and AO parameters estimation.

Strengths are represented by a combination of high-resolution imaging modalities and high-spatial-resolution to assess the relationship between macular perfusion (both retinal and choroidal) and a set of photoreceptor damage biomarkers.

In conclusion, by interpolating SS-OCTA and AO, we demonstrated that NPDR eyes showed microvascular hypoperfusion and impaired CD, CS and CP arrangement. Importantly,

the CC ischaemia and the increased retinal flow seem to be strongly correlated with photoreceptor damage. The topographical analysis revealed that the temporal parafoveal sector is most sensitive to microvascular and photoreceptors impairment in NPDR. The methodology presented in this study can also serve in future studies and artificial intelligence algorithms to permit an easier matching of the AO images with OCTA slabs, thus facilitating high-resolution imaging of cells/retinal structures. These findings may provide precious insights into the pathophysiology of structural and functional damage in patients with DM1.

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