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A gender-based analysis of retinal microvascular alterations in patients with diabetes mellitus using OCT angiography

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ABSTRACT

Purpose: To assess the difference in microvascular changes between males and females with diabetes mellitus (DM) without diabetic retinopathy (NoDR) and with mild-to-moderate non-proliferative diabetic retinopathy (NPDR) using Optical Coherence Tomography Angiography (OCT-A).
Design: Retrospective cross-sectional study.
Methods: 267 DM patients, 133 females (49.81 %), 111 with NoDR (41.57 %) and 156 NPDR (58.43 %) were included. Foveal-centered 3 × 3 mm OCT-A images corresponding to the superficial (SCP), intermediate (ICP) and deep capillary plexus (DCP), and full retinal (RET) slab were used for analysis. For each slab, FAZ area, perimeter, and circularity index (CI) were determined, following manual delineation of the FAZ; perfusion (PD) and vessel density (VD), fractal dimension (FD), vessel length density (VLD), geometric perfusion deficits (GPD) were also computed. Flow voids (FV) were determined in the choriocapillaris plexus; and perfused capillary density (PCD) in the RET slab.
Results: Females showed larger FAZ CI in SCP and greater FAZ area and perimeter than males in NPDR group. Males had higher central macular thickness than females in NPDR group. All density metrics at the level of ICP and DCP were affected in the NPDR group with no gender differences. Of note, the same significant findings were found in type 1 DM patients, and not in type 2 DM patients.
Conclusions: Our OCT-A findings suggest significant microvascular changes in females with NPDR compared to males, but no such differences in patients without DR. Therefore, gender-related vascular alterations might be present in early stages of DR with potential role.

1. Introduction

Precision medicine approaches and multimodal retinal imaging have advanced our understanding of the pathophysiological processes involved in diabetic retinopathy (DR) that could serve in the development of novel targeted therapies over the last decade.[1]

In contrast to fluorescein angiography (FA), the traditional dye-based technique of imaging retinal vasculature in clinical practice, OCT-angiography (OCT-A) is a non-invasive imaging modality that provides depth-resolved images of retinal microvasculature by detecting motion contrast.[2] OCT-A enables separate, high-resolution visualization of the three retinal capillary plexuses within the macular area, known as the superficial (SCP), intermediate (ICP), and deep capillary plexuses (DCP), and the choriocapillaris (CC). It can also image the foveal

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avascular zone (FAZ), in more detail and with greater resolution than FA.[2] Thus, early microvascular pathological features associated with DR, such as capillary dropout, perifoveal capillary loss, irregularity and enlargement of the FAZ can be imaged in vivo and more accurately classified with OCT-A.[2,3] This, coupled with the availability of software-based image processing provides a range of possibilities for the quantitative and qualitative analysis of DR, and important insights into its pathophysiology.[1]

Although retinal vascular manifestations of DR have been described using OCT-A, there have not been investigations on gender- and sex-based differences in retinal imaging findings in DR so far. The term "sex" is used to describe the biological status as female, or male depending on their sex chromosomes (XX versus XY), reproductive organs and other physical characteristics. Even though gender is assigned at birth according to biological sex, the term "gender" refers to socio-cultural roles, behaviors and identities.[4] An increasing amount of evidence suggests that both sex and gender can impact the development, progression and treatment outcomes of various diseases.[4] In this paper, the two terms can be interchangeably used.

Epidemiological studies have found sex differences in microvascular disease burden in patients with DM, with males having a higher incidence of DR and higher risk for disease progression than females.[5–10] However, other studies suggested that females might have a higher prevalence of DR than males.[11,12]

Thus, the question of whether the risk for microvascular alteration in DR is higher in females as compared with males and whether gender differences may play a role in disease progression are under debate. In this scenario, it may be useful to determine whether these differences could impact the detection, the severity and early progression of microvascular abnormalities in the macula of patients with diabetes mellitus (DM) using OCT-A. Such advances are key to optimizing healthcare utilization and guiding personalized approaches toward the development of ocular biomarkers for early detection and monitoring of DR.

In the present study, we investigated macular vascular changes in patients with DM type 1 (T1DM) and 2 DM (T2DM) at early stages of DR using OCT-A and compared quantitative metrics differences between males and females with DM without DR (NoDR) and with mild-to-moderate non-proliferative DR (NPDR).

2. Methods

2.1. Study participants

This observational cross-sectional retrospective study was performed at the Retina & Imaging Unit, IRCCS MultiMedica in Milan, and at the Medical Retina Research Unit, IRCCS Fondazione Bietti in Rome between March 2022 and December 2022. The study adhered to the tenets of the Declaration of Helsinki and each subject gave informed consent for the use of the images from DR institutional registry after approval by the Institutional Ethics Committee.

Patients with T1DM and T2DM were included in the study.

Diagnosis of DR was determined through comprehensive chart review and diagnostic multimodal retinal imaging. DR stage was graded[13] by two retina specialists (S.V. and M.P.) in either absent DR (NoDR) and mild-to-moderate NPDR. All included patients needed to have a well-documented BCVA at 4 m using standard Early Treatment Diabetic Retinopathy Study (ETDRS) charts, dilated fundus examination, ultra-widefield color fundus photography (UWF-CFP) (Optos California, Optos plc, UK), OCT and OCT-A with HRA + OCT device (Spectralis, Heidelberg Engineering, Germany). A detailed recorded medical history for each subject was available.

In all patients, a single eye was selected at random for inclusion in the study if both eyes had the same grade of DR, or the eye with more severe grade of DR was chosen.

The inclusion criteria were: 1) DM type 1 or 2 and diagnosis of NoDR or mild-to-moderate NPDR, 2) best corrected visual acuity (BCVA) of $\geq$ 0.3 logarithm of the minimum angle of resolution (LogMAR), 3) good quality CFP, OCT and OCT-A images. The exclusion criteria included: 1) presence of any other retinal or macular disease, 2) severe NPDR or proliferative DR, 3) presence of diabetic macular edema, 4) refractive error $\geq \pm 2$ diopters, 5) previous vitreoretinal surgery, 6) significant media opacities that precluded good quality fundus imaging.

The entire cohort of patients with DM was then divided into two groups according to gender: i) male and female and ii) in NoDR and NPDR according to DR severity.

2.2. OCT and OCT-A scans

A single 10°x10° scan (512 B-scans at 6 μm spacing; automatic real-time 5) centered at the fovea, was acquired from each study participant. Following image acquisition, SCP, ICP and DCP slabs were generated by the Spectralis OCT-A module. For image processing, TIFF images with a resolution of 320 × 320 pixels were imported into ImageJ (ImageJ 1.53v; National Institutes of Health, Bethesda, Maryland, USA). The first step involved manual contouring of the FAZ on the full vascular slab and the three individual vascular plexuses. Then FAZ area, perimeter, and circularity index (CI) were calculated. Retinal thickness was measured as central macular thickness (CMT).

2.3. Image analysis

The automatic OCT-A image analysis was performed through the Matlab image processing toolbox and custom scripts (version 2020a, MathWorks, Natick, MA).

Following parameters have been evaluated on en-face OCT-A images:

- Perfusion Density (PD), Vessel Density (VD) and Fractal Dimension (FD) for the SCP, ICP, DCP images;
- Flow Voids (FV) in the choriocapillaris plexus;
- Vessel length density (VLD) and Geometric Perfusion Deficits (GPD) in (RET, full retina slab, SCP, ICP, DCP);
- Perfused Capillary Density (PCD) in the full retinal slab (RET).

Details of the OCT-A image analysis are included in the Supplemental Methods.[14–18]

2.4. Statistical analysis

For statistical testing, SPSS Statistic (Version 25.0; Armonk, NY: IBM Corp.) was used. Only one randomly selected eye from each subject was included for data analysis and image processing to avoid interocular correlation. All results were expressed as the mean ± SD or frequencies, as appropriate. The data distribution was tested through the one-sample Kolmogorov-Smirnov test. The quantitative demographic differences between genders were investigated by using the independent sample *t*-test or the Mann–Whitney test; and for studying the relationship between gender and the qualitative clinical patients' parameters the chi-square or the Fisher's test were used. Pearson correlation was used to explore the relationship between CMT and OCTA vascular parameters.

Analysis of one-way variance (ANOVA) and Kruskal-Wallis (KW) tests were used to assess differences among the four study groups and further in the stratified groups, by type of DM and age intervals (32–66; 67–76; 77–95). All post hoc comparisons were performed with Bonferroni correction for multiple tests. A $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Clinical features of patients with DR

A total of 267 eyes of 267 patients with DM were included in the study. The study population consisted of 87 (32.58 %) patients with T1DM and 180 (67.42 %) with T2DM, with a mean duration of disease of 16.83 ± 0.85 years and a mean HbA1c level of 7.31 % ±1.43 %. In the whole cohort, 133 were females (49.81 %) and 134 were males (50.18 %); the mean age was 60.90 ± 21.34 years. Regarding DR severity across the whole cohort, we found that NoDR was more prevalent among female patients ($n = 65$, 58.55 %) ($p = 0.016$) while NPDR among males ($n = 88$, 56.41 %) (p = 0.016) (Table 1); these differences were lost in the subgroup analysis of T1DM and T2DM patients.

Patient demographics, visual acuity, and systemic comorbidities are summarized in Table 1.

3.2. Comparison between patients stratified for DR severity and type of DM in both females and males

3.2.1. Overall population (T1DM and T2DM)

Females with NPDR showed significantly higher values of the following parameters than females with NoDR: FAZ perimeter in ICP ($p = 0.032$), GPD% in ICP ($p = 0.014$) and DCP ($p < 0.001$). For the male group, the analysis showed similar results to the females (NPDR>NoDR) only for GPD% in DCP ($p = 0.006$). On the other hand, the females with NoDR had the following higher values than NPDR: FAZ CI in the RET ($p = 0.048$), FD in the SCP ($p = 0.020$), VLD ($p = 0.014$), PD% ($p = 0.007$), VD% ($p = 0.018$) and FD ($p < 0.001$) in ICP and VLD, PD%, VD% and FD in DCP (all p < 0.001). These differences were also found in males in which the FAZ CI in the RET ($p = 0.005$), FD in SCP ($p = 0.004$), FAZ CI, PD and FD in ICP ($p = 0.032$, $p = 0.013$ and p < 0.001) and VLD, PD%, VD% and FD in DCP (all p < 0.001) were higher in NoDR than NPDR. All data are extensively reported in Table 2.

In the supplementary material, the analysis of T1DM group (Table 1s) and of T2DM group (Table 2s) is reported and then stratified for T2DM ages' groups: 32–66 (Table 3s); 67–76 (Table 4s) and 77–95 years (Table 5s). Considering the amount of data collected and analyzed, only the significant results were discussed in the text.

Table 1
demographic and clinical patients' characteristics.

	Overall population ($n = 267$)	M ($n = 134$)	F ($n = 133$)	*p*-value
Age, years m ± SD	60.90 ± 21.34	62.03 ± 17.83	58.25 ± 22.06	0.125
Disease duration, years m ± SD	16.83 ± 0.85	17.39 ± 11.44	16.35 ± 10.22	0.477
HbA1c, % m ± SD	7.31 ± 1.43	7.00 ± 1.56	7.28 ± 1.27	0.280
BCVA, logMAR m ± SD	0.07 ± 0.08	0.02 ± 0.11	0.81 ± 7.78	0.239
Type of DM, n (%)				
T1DM	87 (32.58)	38 (43.67)	49 (56.32)	0.139
T2DM	180 (67.42)	96 (53.33)	84 (46.66)	
DR severity, n (%)				
NPDR	156 (58.43)	88 (56.41)	68 (43.59)	0.016*
NoDR	111 (41.57)	46 (41.44)	65 (58.55)	
Stroke History, n (%)	9 (3.37)	6 (66.66)	3 (33.33)	0.250
Yes	258 (96.63)	125 (48.44)	128 (49.61)	
No				
Cardiopathy, n (%)				
Yes	85 (31.83)	48 (56.5)	37 (43.52)	0.147
No	177 (66.29)	83 (46.89)	94 (53.10)	
Nephropathy, n (%)	27 (10.11)	15 (55.55)	12 (44.44)	0.542
Yes	235 (88.01)	116 (49.36)	119 (50.63)	
No				

M = male; F = female; m = mean; SD = standard deviation; BCVA = best corrected visual acuity; DM = diabetes mellitus; DR = diabetic retinopathy; NPDR = non proliferative diabetic retinopathy; NoDR = no signs of DR.

3.2.2. T1DM subgroup

The subgroup analysis of patients with T1DM showed significantly higher value of GPD - RET in females with NPDR vs NoDR and higher values of GPD of all layers in NPDR males vs NoDR. The T1DM subgroup also showed these significant differences, stratified for disease severity: VLD, PD%, VD% and FD at the ICP and DCP in patients with NoDR were higher than NPDR for both males and females; VLD in RET, VLD, PD% and VD% at the SCP in NoDR were found higher than NPDR only in the male group (Table 1s, supplemental material).

3.2.3. T2DM subgroup

In addition, the subgroup T2DM and gender analysis showed that males with mild-to-moderate NPDR had significantly higher CMT compared to females (M: 281.79 ± 28.29 F: 263.60 ± 24.41 μm $p = 0.001$). Greater FAZ area (0.51 ± 0.18 vs 0.39 ± 0.16, $p = 0.002$) in DCP was found in females than in males in mild-to-moderate NPDR.

After stratification by age, in the T2DM subgroup with a range age 67–76 years the following measurements were significantly higher in females than in males with NPDR: greater FAZ area (0.47 ± 0.17 vs 0.32 ± 0.12, $p = 0.011$) and perimeter (3.26 ± 0.61 vs 2.60 ± 0.56, $p = 0.007$) in ICP, greater FAZ area (0.58 ± 0.22 vs 0.40 ± 0.17, $p = 0.012$) in DCP. In the same subgroup patients with NoDR, higher GPD% values in ICP were found in males vs females (25.71 ± 14.82 vs 10.94 ± 10.19, $p = 0.032$) (Table 2s, supplemental material).

3.3. Comparison between females and males with NoDR and NPDR

The analysis of CMT showed lower values in female than in male patients (as reported in the Table 2), and this difference reached the statistical significance in NPDR group (F 265.03 ± 23.27 vs M 281.90 ± 28.19 μm, $p = 0.000$).

The following OCT-A measurements were significantly higher in females than in males in NPDR: higher FAZ CI in SCP ($p = 0.017$), greater FAZ area ($p = 0.006$) and perimeter in DCP ($p = 0.030$). No other OCT-A parameter showed any significant difference between the groups (Table 2). A significant negative relationship was found between CMT and FAZ area mm^2 and perimeter in females in SCP ($r = -0.663$ $p < 0.001$, $r = -0.520$ $p < 0.001$), ICP (r = −0.663 $p < 0.001$, $r = -0.525$ $p < 0.001$), DCP ($r = -0.584$ p < 0.001, $r = -0.529$ p < 0.001). By differentiating with DR severity, the same relationship was confirmed but the Pearson correlation reaches higher value in NoDR female group (in SCP $r = -0.708$ p < 0.001, $r = -0.553$ p < 0.001), ICP ($r = -0.737$ p < 0.001, $r = -0.592$ p < 0.001), DCP ($r = -0.637$ p < 0.001, $r = -0.625$ p < 0.001) in comparison with NPDR female group (in SCP $r = -0.641$ p < 0.001, $r = -0.502$ p < 0.001), ICP ($r = -0.640$ p < 0.001, $r = -0.515$ p < 0.001), DCP ($r = -0.560$ p < 0.001, $r = -0.475$ p < 0.001).

3.4. Correlation between gender and systemic conditions

In the present study, we have explored three systemic variables, namely stroke history, cardiopathy and nephropathy, and their relationship with gender, finding no differences between genders. No differences have been found when considering patients with T2DM, also stratified for age (32–66; 67–76 and 77–95 years). Nevertheless, the subgroup analysis of T1DM patients showed that 88.9 % of patients with T1DM with cardiopathy were males ($p = 0.009$); no significant differences were found for stroke history and nephropathy in the same subgroup.

Table 2
OCT and OCT-A parameters comparison between male and female, NoDR and NPDR groups in the overall population (T1DM and T2DM).

OCT and OCT-A parameters	Male		Female		ANOVA/KW	Post-hoc	
	NoDR (n = 46)	NPDR (n = 88)	NoDR (n = 65)	NPDR (n = 68)	p-value	NoDR F vs NoDR M	NPDR F vs NPDR M
CMT (microns)	273.52 ± 22.48	281.90 ± 28.19	263.95 ± 19.20	265.03 ± 23.27	0.000*		0.000*
FV CC %	46.70 ± 2.97	47.59 ± 3.65	47.52 ± 3.41	48.34 ± 3.65	0.018*		
FAZ Area, mm2 (RET)	0.33 ± 0.11	0.36 ± 0.15	0.35 ± 0.14	0.39 ± 0.15	0.042*		
FAZ Perimeter mm (RET)	2.64 ± 0.46	2.89 ± 0.77	2.63 ± 0.59	2.91 ± 0.62	0.004*		
FAZ CI (RET)	0.58 ± 0.08 (0.005#)	0.53 ± 0.10	0.61 ± 0.08 (0.048§)	0.57 ± 0.07	0.000*		
VLD (RET)	0.11 ± 0.03	0.11 ± 0.02	0.12 ± 0.03	0.12 ± 0.02	0.276		
GPD % (RET)	27.08 ± 20.69	26.36 ± 19.78	26.69 ± 23.58	26.44 ± 19.59	0.357		
PCD %(RET)	32.69 ± 6.54	33.02 ± 5.31	33.82 ± 6.10	34.42 ± 5.01	0.209		
FAZ Area, mm2 (SCP)	0.43 ± 0.15	0.45 ± 0.23	0.47 ± 0.14	0.50 ± 0.17	0.063		
FAZ Perimeter mm(SCP)	3.01 ± 0.58	3.18 ± 1.14	3.12 ± 0.53	3.19 ± 0.59	0.387		
FAZ CI (SCP)	0.59 ± 0.09	0.56 ± 0.10	0.60 ± 0.07	0.61 ± 0.08	0.012*		0.017*
VLD (SCP)	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.02	0.462		
GPD % (SCP)	27.34 ± 20.18	26.06 ± 19.51	23.75 ± 18.89	24.11 ± 15.14	0.204		
PD % (SCP)	37.39 ± 3.71	35.69 ± 4.09	36.90 ± 3.54	36.14 ± 3.74	0.037*		
VD %(SCP)	11.22 ± 3.44	11.00 ± 2.69	11.63 ± 3.02	11.15 ± 2.33	0.400		
FD (SCP)	1.52 ± 0.04 (0.004#)	1.49 ± 0.03	1.51 ± 0.03 (0.020§)	1.49 ± 0.03	0.000*		
FAZ Area, mm2 (ICP)	0.31 ± 0.12	0.33 ± 0.15	0.33 ± 0.13	0.39 ± 0.15	0.016*		
FAZ Perimeter mm (ICP)	2.55 ± 0.47	2.69 ± 0.74	2.58 ± 0.60 (0.032Ç)	2.87 ± 0.61	0.004*		
FAZ CI (ICP)	0.60 ± 0.08 (0.032#)	0.55 ± 0.10	0.61 ± 0.09	0.58 ± 0.10	0.001*		
VLD (ICP)	0.12 ± 0.04	0.12 ± 0.03	0.13 ± 0.03 (0.014§)	0.12 ± 0.03	0.000*		
GPD % (ICP)	15.34 ± 13.16	15.03 ± 12.80	12.25 ± 11.92 (0.014Ç)	13.46 ± 10.96	0.001*		
PD % (ICP)	36.29 ± 3.02 (0.013#)	34.53 ± 3.05	37.00 ± 2.54 (0.007§)	35.42 ± 2.62	0.000*		
VD %(ICP)	12.64 ± 3.80	12.13 ± 2.60	13.43 ± 3.38 (0.018§)	12.58 ± 2.52	0.000*		
FD (ICP)	1.53 ± 0.03 (0.000#)	1.51 ± 0.03	1.53 ± 0.02 (0.000§)	1.51 ± 0.02	0.000*		
FAZ Area, mm2 (DCP)	0.40 ± 0.12	0.40 ± 0.18	0.45 ± 0.14	0.48 ± 0.17	0.001*		0.006*
FAZ Perimeter mm (DCP)	2.84 ± 0.46	2.86 ± 0.74	2.99 ± 0.54	3.13 ± 0.60	0.009*		0.030*
FAZ CI (DCP)	0.61 ± 0.07	0.59 ± 0.09	0.62 ± 0.06	0.61 ± 0.07	0.219		
VLD (DCP)	0.15 ± 0.02 (0.000#)	0.13 ± 0.01	0.15 ± 0.02 (0.000§)	0.13 ± 0.02	0.000*		
GPD % (DCP)	9.10 ± 3.08 (0.006¶)	11.82 ± 4.83	9.21 ± 3.33 (0.000Ç)	12.50 ± 4.67	0.000*		
PD % (DCP)	38.48 ± 2.67 (0.000#)	36.06 ± 2.53	38.42 ± 3.05 (0.000§)	36.24 ± 3.03	0.000*		
VD %(DCP)	15.12 ± 1.94 (0.000#)	13.59 ± 1.48	15.21 ± 1.70 (0.000§)	13.68 ± 1.56	0.000*		
FD (DCP)	1.52 ± 0.02 (0.000#)	1.51 ± 0.02	1.52 ± 0.02 (0.000§)	1.51 ± 0.02	0.000*		

CMT: central macular thickness, FV CC: Flow Voids in the choriocapillaris, FAZ: foveal avascular zone, CI: circularity index, PD: Perfusion Density, VD: Vessel Density, FD: Fractal Dimension, VLD: Vessel length density, GPD: Geometric Perfusion Deficits, PCD: Perfused Capillary Density, RET: full retinal projection; SCP: superficial capillary plexus, ICP: intermedia capillary plexus, DCP deep capillary plexus.

* Asterisk shows a statistically significant difference ($p < 0.05$).

Statistically significant difference between male NoDR vs male NPDR (NoDR value > NPDR value).

§ Statistically significant difference between female NoDR vs female NPDR (NoDR value > NPDR value).

¶ Statistically significant difference between male NoDR vs male NPDR (NPDR value > NoDR value).

Ç Statistically significant difference between female NoDR vs female NPDR (NPDR value > NoDR value).

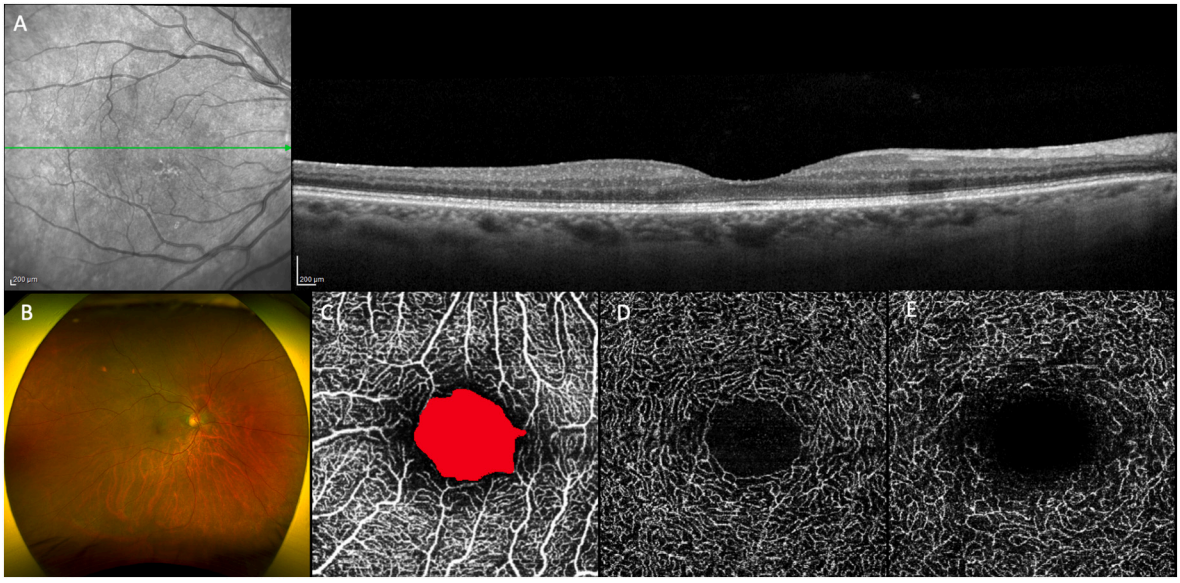

Fig. 1. Optical Coherence Tomography (OCT) B-scan, corresponding OCT-Angiography images of the macula and Ultra-Wide Field Color Fundus Photography (UWF-CFP) of female patient with NoDR. A) OCT B-scan; B) UWF-CFP; C) Superficial capillary plexus with highlighted Foveal Avascular Zone; D) Intermediate Capillary Plexus; E) Deep Capillary Plexus.

4. Discussion

Recent studies using OCT-A have reported on macular microvascular alterations and morphological changes of FAZ in DM patients with or without DR,[19–25] however, there is a lack of quantitative data describing whether females exhibit differences as compared with their male counterparts which limits our understanding on early gender-related changes in DM and DR.

This study confirmed the impairment of microvascular metrics within different retinal plexuses and the alterations of FAZ characteristics in DCP layer in presence of mild-to moderate NPDR compared to NoDR. A similar trend was observed in male and female patients (Fig. 1).

In our present study, the overall analysis revealed greater FAZ metrics within SCP and DCP among females compared to males in eyes affected by NPDR.

Multiple studies have investigated the correlation between vascular changes on OCT-A and DR severity regardless the gender, finding similar results. In particular, OCT-A studies have shown that the size of the FAZ tends to enlarge with increasing severity of DR,[19,20] aligning with findings from studies using FA conducted decades earlier.[26] It has also been documented that vessel and perfusion densities decrease in the different retinal layers[21–24] and the pattern of vessels in DCP is altered with increasing DR severity.[25]

The observed decrease in VD and increase in FAZ area as DR severity progresses can be attributed to capillary loss and reduced blood flow within existing vessels.[3] Overall, these changes on OCT-A could serve as useful biomarkers for assessing the severity of DR. Our data showed that all density metrics at the level of ICP and DCP were affected in the presence of signs of DR with no gender differences. Interestingly, the same significant results were found in T1DM sub-analysis and not so expressed in T2DM patients. This result could also suggest the evaluation of OCTA parameters in T1DM as useful biomarkers for diabetic retinal microvascular changes.

The present study documented that in both females and males, DCP GPD% was significantly higher in eyes with mild-to-moderate NPDR than in eyes with NoDR. GPD is an OCT-A metric recently proposed by Chen et al.[27] that is related to the underlying physiology of oxygen diffusion and ischemia and is computed using microvascular geometry. The authors showed that for all en-face projections, DR eyes had significantly higher GPD percentages than normal eyes and significantly lower vessel density percentages in all but DCP projection.[27] Also, a strong negative correlation between the GPD and vessel density percentages was reported. Therefore, they suggested that GPD based on oxygen diffusion could be used to quantitatively identify focal perfusion deficits occurring during DR progression, serving as a useful OCT-A biomarker for detecting and monitoring DR. In line with this study, we documented and reported a similar trend, at the DCP level, but in the subgroup analysis of T2DM patients aged between 67 and 76 years old (Table 4s, supplemental materials), we also found higher value of GPD% at the ICP in NoDR male compared with NPDR male patients and in male vs female comparison; we did not find the same results in the female T2DM population (67–76 years old). Furthermore, the meaning of this interesting finding strengthens the already known reduction of vascular density metrics (VLD, PD, VD and FD) found in NPDR compared with NoDR at the level of DCP and ICP, regardless of the gender.

While vascular changes with DR progression using OCT-A have become more common in the medical literature, gender-related influences on these quantitative data remain unclear given the lack of comparison. So far, to our knowledge, this study is the first to analyze gender-related changes in macular microvasculature and FAZ parameters across initial stages of DR.

Investigating gender-related differences in patients with mild-to-moderate NPDR using OCT-A, the key observations were that multiple FAZ metrics in different retinal plexuses were significantly increased in female compared to male patients. More specifically, our results showed that significant differences occurred more often within the SCP and DCP parameters of the FAZ in females. We found that females with mild-to-moderate NPDR exhibited a greater increase in SCP CI, and a greater FAZ area and perimeter in DCP. In addition, among T2DM patients aged between 67 and 76 years old, females with mild-to-moderate NPDR exhibited a greater FAZ area in ICP and DCP and perimeter in ICP than females with NoDR.

These findings could suggest that ICP may be affected more profoundly by sex-based differences in T2DM patients, suggesting that variations related to gender could lead to notable differences in how this microvascular network is affected by DM. Yet, the existing literature is still limited on this topic, highlighting the need for further research to better understand the extent and implications of these findings.

It is interesting to consider the open question of whether these changes could be induced by sex-related hormonal variations, where insulin, estrogen or other substances may have either an adverse or protective role.[28,29] However, inflammatory protein biomarker concentrations have been shown to be higher in males with DM[30] and for males with DM and without DR, neuroretinal function appears to be comparatively more abnormal than in females.[31] Furthermore, in the present study a significant association between the presence of cardiopathy in males with T1DM was found in comparison with females. Other possible explanations for these disparities could be in relation to behavioral, cultural and economic differences, different treatments for DM, or associated comorbidities between sexes. Future studies may clarify the role of these factors on the foveal microvascular gender-related disparities we found.

Notably, OCT-A findings from the present study have documented only a few statistically significant differences for comparisons of FAZ metrics or microvascular density and morphology between males and females with NoDR.

Several authors have reported that the increase in FAZ size is more significant in the later stages of retinopathy when compared to mild or no retinopathy. This may be attributed to the interindividual variability in the size of the FAZ among no DR patients and healthy individuals.[19–24] In the general population, FAZ has been shown to be larger in females than in males[32] however other studies have failed to find consistent differences related to gender in macular vasculature, in terms of vascular density or FAZ area.[19–24]

The major limits of the study include the retrospective nature of the study limited the data to those found in the charts and routinely assessed. Second, as we excluded patients with known confounding ocular factors such as refractive errors than >2 diopters, diabetic macular edema, uncontrolled hypertension, glaucoma, age-related macular degeneration, and other vitreoretinal diseases, our findings may not be generalizable to individuals with these comorbidities.

Third, although the larger FAZ areas could be related to an earlier and more advanced perifoveal capillary dropout in females once the DR is present, individual differences in baseline FAZ size need to be considered. These differences can affect the extent to which changes in FAZ are indicative of disease progression. Future studies should account for this variability and adjust the analysis for baseline FAZ size when comparing individuals or groups.

Overall, although the findings from the present study suggested that patient gender was not associated with retinal microvasculature modification differences in patients with DM with no DR using OCT-A imaging, we demonstrated a greater impact of macular structural changes in female compared with male patients in the early stages of DR. This suggests that there could be a significant difference in the way retinal microvasculature changes in DR affects males and females, contributing to the mounting body of evidence on sex- and gender-based epidemiological variations in diabetes.[5,6,8–12]

This could have crucial implications in clinical practice, highlighting the need for further research to explore the possibility of establishing separate threshold cutoffs based on gender to better categorize observed retinal vascular changes in the future.

The variety of metrics that could be applied to en-face OCT-A images,

together with the high-resolution and non-invasiveness support OCT-A as a well-suited modality for better studying gender-related vascular alterations in the pathophysiology of DR.

In conclusion, our results support the role of a noninvasive evaluation of the retinal microvasculature of DM patients by means of OCTA particularly in the early phases of the disease to detect the presence of early signs of DR and to modify the glycometabolic control accordingly. Specifically, we were able to highlight the differences in OCTA metrics between males and females showing a greater impairment of the perifoveal microvasculature in females in NPDR eyes.

The detection of this kind of gender difference is becoming more important as we are going toward a precision medicine approach and more precisely a gender-specific medicine to better clarify different disease progression, different responses to treatment and personalized follow-up.

Prospective investigations on microvascular DR could potentially derive an advantage from incorporating considerations of gender-associated differences, since they may act as confounding factors. To gain a better understanding of the pathophysiology that underlies sex and gender-based microvascular alterations in the retina of diabetic patients, further studies are needed to elucidate the contributions of specific environment and genetic factors and the link with retinal imaging biomarkers.

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CRediT authorship contribution statement

Stela Vujosevic: Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Celeste Limoli:** Writing – original draft, Project administration, Methodology, Investigation, Data curation. **Gabriele Piccoli:** Writing – original draft, Software, Project administration, Methodology, Investigation, Data curation. **Eliana Costanzo:** Writing – original draft, Project administration, Methodology, Investigation, Data curation. **Elisa Marenzi:** Writing – original draft, Software, Formal analysis. **Emanuele Torti:** Writing – original draft, Software, Methodology. **Daniela Giannini:** Writing – original draft, Formal analysis. **Maria Sole Polito:** Writing – original draft, Resources, Methodology, Investigation, Data curation. **Livio Luzi:** Writing – review & editing, Supervision. **Paolo Nucci:** Writing – review & editing, Supervision. **Mariacristina Parravano:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

Stela Vujosevic: Consultant to Abbvie, Apellis, Bayer, Novartis, Hoffman La Roche, Zeiss;

Paolo Nucci: Thea, Hoya, Essilor Luxottica e Fondazione One-sight, Sifi, Bausch& Lomb, Alfa Intes, Alcon, Doc, Santen, Eyerising.

Mariacristina Parravano reports personal fees from Abbvie, Novartis, Bayer, La-Roche, Zeiss outside the submitted work.

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List of abbreviations

ANOVA	Analysis of one-way variance
BCVA	best corrected visual acuity
CC	choriocapillaris
CI	circularity index
CFP	color fundus photography
CMT	central macular thickness
DCP	deep capillary plexus
DM	diabetes mellitus
DR	diabetic retinopathy
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	fluorescein angiography
FAZ	foveal avascular zone
FD	fractal dimension
FV	Flow voids
GPD	geometric perfusion deficits
ICP	intermediate capillary plexus
KW	Kruskal-Wallis
NoDR	without diabetic retinopathy
NPDR	non-proliferative diabetic retinopathy
OCT	Optical Coherence Tomography
OCT-A	Optical Coherence Tomography Angiography
PCD	perfused capillary density
PD	perfusion density
RET	full retinal slab
SCP	superficial capillary plexus
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
UWF-CFP	ultra-widefield color fundus photography
VD	vessel density
VLD	vessel length density

References

1. Szeto SK, Hui VWK, Siu V, et al. Recent advances in clinical applications of imaging in retinal diseases. *Asia Pac J Ophthalmol (Phila)*. 2023;12:252–263.
2. Spaide RF, Fujimoto JG, Waheed NK, Sadda SR, Staurenghi G. Optical coherence tomography angiography. *Prog Retin Eye Res*. 2018;64:1–55.
3. Choi W, Waheed NK, Moult EM, et al. Ultrahigh speed swept source optical coherence tomography angiography of retinal and choriocapillaris alterations in diabetic patients with and without retinopathy. *Retina*. 2017;37:11–21.
4. Institute of Medicine (US). Committee on understanding the biology of sex and gender differences. In: Wizemann TM, Pardue ML, eds. *Exploring the Biological Contributions to Human Health: Does Sex Matter?* Washington (DC): National Academies Press; 2001.
5. Klein R, Knudtson MD, Lee KE, et al. The Wisconsin epidemiologic study of diabetic retinopathy: XXII the twenty-five-year progression of retinopathy in persons with type 1 diabetes. *Ophthalmology*. 2008;115:1859–1868.
6. Pradeepa R, Anitha B, Mohan V, et al. Risk factors for diabetic retinopathy in a South Indian Type 2 diabetic population–the Chennai Urban Rural Epidemiology Study (CURES) Eye Study 4. *Diabet Med*. 2008;25:536–542.
7. Hammes HP, Kerner W, Hofer S, et al. Diabetic retinopathy in type 1 diabetes-a contemporary analysis of 8,784 patients. *Diabetologia*. 2011;54:1977–1984.
8. Looker HC, Nyangoma SO, Cromie D, et al. Diabetic retinopathy at diagnosis of type 2 diabetes in Scotland. *Diabetologia*. 2012;55:2335–2342.
9. Al-Rubeaan K, Abu El-Asrar AM, Youssef AM, et al. Diabetic retinopathy and its risk factors in a society with a type 2 diabetes epidemic: a Saudi National Diabetes Registry-based study. *Acta Ophthalmol*. 2015;93:e140–e147.
10. Cherchi S, Gigante A, Spanu MA, et al. Sex-gender differences in diabetic retinopathy. *Diabetology*. 2020;1:1–10.
11. Kajiwara A, Miyagawa H, Saruwatari J, et al. Gender differences in the incidence and progression of diabetic retinopathy among Japanese patients with type 2 diabetes mellitus: a clinic-based retrospective longitudinal study. *Diabetes Res Clin Pract*. 2014;103:e7–10.
12. Li M, Wang Y, Liu Z, et al. Females with type 2 diabetes mellitus are prone to diabetic retinopathy: a twelve-province cross-sectional study in China. *J Diabetes Res*. 2020;2020:5814296.
13. Wilkinson CP, Ferris 3rd FL, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology*. 2003;110:1677–1682.
14. Zahid S, Dolz-Marco R, Freund KB, et al. Fractal dimensional analysis of optical coherence tomography angiography in eyes with diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2016;57:4940–4947.

15. Zhang Q, Zheng F, Motulsky EH, et al. A novel strategy for quantifying choriocapillaris flow voids using swept-source OCT angiography. *Invest Ophthalmol Vis Sci*. 2018;59:203–211.
16. Vujosevic S, Toma C, Villani E, et al. Diabetic macular edema with neuroretinal detachment: OCT and OCT-angiography biomarkers of treatment response to anti-VEGF and steroids. *Acta Diabetol*. 2020;57:287–296.
17. Vujosevic S, Toma C, Villani E, et al. Subthreshold micropulse laser in diabetic macular edema: 1-year improvement in OCT/OCT-angiography biomarkers. *Transl Vis Sci Technol*. 2020;9:31.
18. Rosen RB, Andrade Romo JS, Krawitz BD, et al. Earliest evidence of preclinical diabetic retinopathy revealed using optical coherence tomography angiography perfused capillary density. *Am J Ophthalmol*. 2019;203:103–115.
19. Di G, Weihong Y, Xiao Z, et al. A morphological study of the foveal avascular zone in patients with diabetes mellitus using optical coherence tomography angiography. *Graefe's Archive Clin Exp Ophthalmol*. 2016;254:873–879.
20. Takase N, Nozaki M, Kato A, et al. Enlargement of foveal avascular zone in diabetic eyes evaluated by En face optical coherence tomography angiography. *Retina*. 2015;35:2377–2383.
21. Agemy SA, Scripsema NK, Shah CM, et al. Retinal vascular perfusion density mapping using optical coherence tomography angiography in normals and diabetic retinopathy patients. *Retina*. 2015;35:2353–2363.
22. de Carlo TE, Chin AT, Bonini Filho MA, et al. Detection of microvascular changes in eyes of patients with diabetes but not clinical diabetic retinopathy using optical coherence tomography angiography. *Retina*. 2015;35:2364–2370.
23. Hwang TS, Gao SS, Liu L, et al. Automated quantification of capillary nonperfusion using optical coherence tomography angiography in diabetic retinopathy. *JAMA Ophthalmol*. 2016:1–7.
24. Freiberg FJ, Pfau M, Wons J, et al. Optical coherence tomography angiography of the foveal avascular zone in diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol*. 2016;254:1051–1058.
25. Couturier A, Mane V, Bonnin S, et al. Capillary plexus anomalies in diabetic retinopathy on optical coherence tomography angiography. *Retina*. 2015;35:2384–2391.
26. Bresnick GH, Condit R, Syrjala S, et al. Abnormalities of the foveal avascular zone in diabetic retinopathy. *Arch Ophthalmol*. 1984;102:1286–1293.
27. Chen S, Moult EM, Zangwill LM, et al. Geometric perfusion deficits: a novel OCT angiography biomarker for diabetic retinopathy based on oxygen diffusion. *Am J Ophthalmol*. 2021;222:256–270.
28. Reiter CE, Gardner TW. Functions of insulin and insulin receptor signaling in retina: possible implications for diabetic retinopathy. *Prog Retin Eye Res*. 2003;22:545–562.
29. Ketchem JM, Bowman EJ, Isales CM. Male sex hormones, aging, and inflammation. *Biogerontology*. 2023;24:1–25.
30. Haq Z, Yang D, Psaras C, Stewart JM. Sex-based analysis of potential inflammation-related protein biomarkers in the aqueous humor of patients with diabetes mellitus. *Transl Vis Sci Technol*. 2021;10:12.
31. Ozawa GY, Bearse Jr MA, Bronson-Castain KW, et al. Neurodegenerative differences in the retinas of male and female patients with type 2 diabetes. *Invest Ophthalmol Vis Sci*. 17 2012;53:3040–3046.
32. Gómez-Ulla F, Cutrin P, Santos P, et al. Age and gender influence on foveal avascular zone in healthy eyes. *Exp Eye Res*. 2019;189, 107856.