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ORIGINAL ARTICLE

Optical coherence tomography angiography analysis of retinal vascular plexuses and choriocapillaris in patients with type 1 diabetes without diabetic retinopathy

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Abstract
Aims To analyze retinal vascular plexuses and choriocapillaris by optical coherence tomography angiography (OCT-A) and retinal nerve fiber layer and ganglion cell layer (GCL) by structural optical coherence tomography (OCT) in patients with type 1 diabetes mellitus (T1DM) without diabetic retinopathy (DR).
Methods A total of 25 eyes of 25 consecutive T1DM patients without signs of DR were prospectively recruited and compared to 25 healthy subjects (control eyes). All patients underwent OCT-A (CIRRUS HD-OCT model 5000, Carl Zeiss Meditec, Dublin, CA) and structural OCT. Qualitative and quantitative analyses with vessel density were performed on OCT-A images in the superficial capillary plexus (SCP), deep capillary plexus (DCP) and choriocapillaris for all patients.
Results By means of OCT-A, a rarefaction of the perifoveal capillary network in SCP was detected in 7 out of 25 eyes. No significant difference was found in FAZ area of both SCP and DCP comparing diabetic and control groups. By analyzing the DCP, diabetic eyes revealed a significant decreased vessel density compared to control eyes [0.464 ± 0.016 and 0.477 ± 0.014, respectively ($p = 0.005$)]. Instead, no significant difference was found in the vessel density of all-retina plexus, SCP and choriocapillaris. By RFNL and GCL thickness analysis, no significant differences were disclosed between diabetics and healthy subjects.
Conclusions We demonstrated the ability of OCT-A to disclose early vascular alterations in patients with T1DM diagnosed as without any signs of DR on the basis of fundus biomicroscopy. Our results also suggest that microvascular changes could precede detectable damage of diabetic neuroretinopathy.

Keywords Optical coherence tomography angiography · Diabetic retinopathy · Ganglion cell layer · Retinal imaging · Retinal nerve fiber layer

Introduction

Diabetic retinopathy (DR) is no longer considered the most common cause of new blindness among western adults in the middle, most productive years of life [1]. Timely diagnosis and prompt treatment are necessary to reduce the risk of visual loss [2]. The chance of developing DR is greater with increasing duration of diabetes [3, 4]. During the first two decades of disease, retinopathy develops in nearly all younger-onset patients [type 1 diabetes mellitus (T1DM)] and >60% of older-onset patients [type 2 diabetes mellitus (T2DM)] [5]. The exact mechanism by which diabetes causes retinopathy is still not clear, but several studies based on histopathology and imaging revealed that DR is a consequence of microvascular changes including

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capillary remodeling, regression and decreased density [6–9]. The exact molecular mechanism of microvascular changes is still not well understood. Genetic studies demonstrate that a single nucleotide polymorphism in mir-146 is positively associated with microvascular complications in patients with T1DM [10]. Furthermore, Mazzeo et al. [11] demonstrated that extracellular vesicles derived from mesenchymal stem cells maintained in diabetic-like conditions could play a role in vessel destabilization, thus contributing to angiogenesis through paracrine signaling. It seems that a well-recognized risk factor for foot diabetes, the body mass index, is inversely associated with the risk to develop retinopathy. Two large Asian studies demonstrated that compared with normal weight, overweight was associated with reduced risk of any DR; this finding is attributable to better β cell function in overweight patients [12, 13]. Even if diabetic retinopathy is still considered a primary microvascular disease, a recent finding has underlined the role of the neural retinal layer in microvascular change. It seems that there is a complex interplay between retinal pericytes and photoreceptors, and pharmacologic modulation of these cells with somatostatin is promising for the treatment of ocular diseases [14]. However, developments in optical coherence tomography (OCT) technologies have enabled to identify alterations in the inner retina of patients without or with minimal DR, suggesting an early retinal neurodegeneration before clinical signs of vasculopathy arise [15–19].

In the most recent years, the introduction of the OCT angiography (OCT-A), a dye-free imaging technique useful to visualize retinal and choroidal vasculature, has allowed to detect angiographic features of DR and changes in the macular capillary network, even before disease onset. In patients with DR, areas of nonperfusion and their localization in either superficial and deep plexuses, irregular capillaries and microaneurysms have been clearly analyzed [9, 20]. In addition to these qualitative features, OCT-A offers quantitative analysis of retinal blood vessel density and flow [21]. Dimitrova et al. [22] had reported OCT-A findings in retina and choriocapillaris in T2DM patients without DR, demonstrating decreased superficial and deep capillary density in parafoveal area. More recently, Simonett et al. [23] had showed a significant decrease in parafoveal vessel density in the deep capillary plexus (DCP) of patients with T1DM with mild DR, while only a trend in the same direction in young-onset diabetic patients without DR. Thus, the aim of our study is to analyze both qualitatively and quantitatively retinal vascular plexuses and choriocapillaris by OCT-A and, furthermore, to analyze retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL) by structural OCT in post-pediatric patients with T1DM without DR, to investigate the possible occurrence of vascular and/or neurodegenerative alterations in these young patients and gather insights into the DR pathogenesis.

Methods

Patients presenting with diagnosis of type 1 diabetes were prospectively enrolled between September 2016 and February 2017 at the Medical Retina and Imaging Unit of the Department of Ophthalmology, University Vita-Salute, Ospedale San Raffaele in Milan from a pool of patients previously followed at Pediatric Department. The study was conducted in agreement with the Declaration of Helsinki for research involving human subjects and was approved by the local institutional review board. Included patients signed a written consent to participate to observational studies. Inclusion criteria for the study group were: age greater than 18 years, diagnosis of T1DM from at least 5 years before the enrollment, absence of any signs of DR at fundus biomicroscopy, sufficiently clear ocular media, adequate pupillary dilation and fixation to permit high-quality OCT imaging. Ocular exclusion criteria included any other retinal diseases (including retinal vascular diseases, vitreoretinal diseases, history of central serous retinopathy or macular dystrophies), and any previous eye surgical intervention or laser photocoagulation in the study eye.

Each enrolled patient underwent a comprehensive ophthalmologic examination, including measurement of best-corrected visual acuity (BCVA) using early treatment diabetic retinopathy study (ETDRS) chart, dilated slit lamp anterior segment and fundus biomicroscopy, structural spectral domain-OCT (SD-OCT; Spectralis + HRA; Heidelberg Engineering, Heidelberg, Germany) and OCT-A (AngioPlex® CIRRUS HD-OCT model 5000, Carl Zeiss Meditec, Inc., Dublin, USA).

An additional age and sex cohort of healthy patients was used as control group; also for these patients, the study was conducted in agreement with the Declaration of Helsinki for research involving human subjects and was approved by the local institutional review board. Included patients signed a written consent to participate to observational studies.

Structural SD-OCT measurements

Structural SD-OCT minimum acquisition protocol included: 19 horizontal raster linear B-scans, each composed by 9 averaged OCT B-scans (1024 A-scans per line) at 240-μm intervals, covering an area of 20° by 15°; six radial linear B-scans, each composed by 25 averaged OCT B-scans (768 A-scans per line) at 30° centered on the fovea; 49 horizontal raster dense linear B-scans, each

composed by 16 averaged OCT B-scans (384 A-scans per line) at 30-μm intervals, covering an area of 15° by 5°. Central macular thickness (CMT) was recorded with the Spectralis software (Heidelberg Eye Explorer, version 1.9.11.0 Heidelberg Engineering, Germany) in the central 1-mm-diameter circle of the ETDRS thickness map. The Spectralis software was also used to obtain each retinal layer thickness, in particular RNFL and GCL, as performed in a previous published study [19]. In detail, 1-mm-diameter central circle (*C*) and 3-mm-diameter subfield of nasal (*N*), temporal (*T*), superior (*S*) and inferior (*I*) quadrants as defined by ETDRS were recorded and used for the analysis. To achieve a better visualization of the choroid, enhanced depth imaging (EDI) OCT was used in all acquisitions.

Choroidal thickness (ChT) was assessed by manually measuring subfoveal distance between Bruch's membrane interface and sclerochoroidal interface to identify the inner and outer boundaries of the choroid, respectively. A manual function was used because Spectralis OCT does not provide an automatic segmentation of the choroid.

OCT-A images acquisition and analysis

In all patients, a scanning area of 3 × 3 mm was adopted, centered on the foveal area.

Angioplex uses optical microangiography (OMAG), a recently developed imaging technique that produces 3D images of dynamic blood perfusion within micro-circulatory tissue beds at an imaging depth up to 2.0 mm [25–27]. Angioplex CIRRUS HD-OCT model 5000 contains an A-scan rate of 68,000 scans per second, using a superluminescent diode centered on 840 nm. The resultant 3 × 3 angio cube contains 245 B-scan slices repeated up to 4× at each B-scan position. Each B-scan is made of 245 A-scans, and each A-scan is 1024 pixels deep. All acquisitions were performed using FastTrac^{TM} retinal-tracking technology to reduce motion artifacts. All 3 × 3 OCT-A images were exported into the National Institutes of Health ImageJ 1.50 (National Institutes of Health, Bethesda, Maryland, USA) software. Foveal avascular zone (FAZ) area was manually measured using a previous published method [28, 29]. Briefly, FAZ area was manually outlined using the polygon selection tool in superficial capillary plexus (SCP) and DCP, and its dimension was expressed as square millimeters (mm^2).

Qualitative analysis

Two different readers (A.C and R.S.) evaluated the presence of one or more of the following specific angiographics features on OCT-A images in SPC and DPC: (A) microaneurysms, (B) rarefaction of perifoveal capillary, (C) capillary tortuosity and (D) disruption of the perifoveolar capillary arcade. Disagreement regarding interpretation of the different features of OCT-A images was resolved by open adjudication.

Quantitative analysis

Vessel density was calculated through image thresholding and binarization, according to previous studies [30, 31]. Specifically, the mean's thresholding was used to binarize each 3 × 3 OCT-A image; binarized image was converted from 8 bit into red green blue (RGB) color type, and FAZ area was contoured and colored to pure blue. White pixels were considered as vessel and black pixels as background, and blue pixel were automatically excluded from the analysis. Vessel density was calculated for full thickness retinal plexus (RP), SCP, DCP and choriocapillary plexus (CCP) as the ratio between the white pixel and the total pixels after FAZ exclusion.

Statistical analysis

Statistical analysis was performed using SPSS software 21 (SPSS, Inc., Chicago, IL, USA). All quantitative data were expressed as mean ± standard deviation. Categorical variables were analyzed using Chi-squared test. The Gaussian distribution of continuous variables was verified with the Kolmogorov–Smirnov test. Comparisons of mean BCVA, CMT, ChT, RNFL thickness, GCL thickness, FAZ area and vessel density between diabetic patients and control subjects were made using the Student's *t* test. Correlation between continuous variables was analyzed using Pearson's correlation analyses. p values <0.05 were considered to be statistically significant.

Results

A total of 25 eyes of 25 consecutive diabetic patients (11 females, 14 males) without signs of DR were included in the study. The mean age was 22 ± 2 years (median 22; range 18–26 years), and all patients were Caucasian. Mean duration of the disease was 11 ± 4 years (range 7–18 years), and mean HbA1c level was 7.3 ± 0.7% (range 6.2–8.6%). None of the patients was affected by systemic hypertension and renal dysfunction. Best-corrected visual acuity was 20/20 Snellen equivalent in all eyes, mean CMT was 270 ± 22 μm (range 224–315 μm), and mean subfoveal ChT was 320 ± 53 μm (range 204–416 μm).

Twenty-five healthy subjects were included in the control group. Patients included were homogenous for age and sex: The mean age was 23 ± 2 years (median 23; range 18–26 years; $p = 0.115$), with 9 females and 16 males

Table 1 Retinal nerve fiber layer and ganglion cell layer thickness analysis of diabetic eyes compared with control group

Subfield analyzed	Diabetic eyes ($n = 25$)	Control eyes ($n = 25$)	
	Mean ± SD	Mean ± SD	p value (&)
RNFL thickness (μm)			
1-mm central circle	12.52 ± 2.58	12.56 ± 1.78	0.949
3-mm *S* subfield	24.32 ± 2.76	24.76 ± 3.28	0.611
3-mm *I* subfield	25.00 ± 2.71	25.28 ± 3.37	0.748
3-mm *N* subfield	21.28 ± 2.30	21.12 ± 2.71	0.823
3-mm *T* subfield	16.20 ± 0.82	16.64 ± 1.78	0.266
GCC thickness(μm)			
1-mm central circle	15.64 ± 4.49	16.56 ± 3.62	0.429
3-mm *S* subfield	54.64 ± 4.33	54.00 ± 3.14	0.552
3-mm *I* subfield	54.16 ± 3.98	53.56 ± 2.92	0.546
3-mm *N* subfield	53.68 ± 4.79	53.76 ± 3.24	0.945
3-mm *T* subfield	49.32 ± 4.63	49.16 ± 3.93	0.896

RNFL retinal nerve fiber layer, *GCL* ganglion cell layer, *n* number, *SD* standard deviation

& Student independent samples *t* test

($p = 0.564$). Control group BCVA was 20/20 Snellen equivalent in all eyes, CMT was 278 ± 11 μm (range 258–300 μm; $p = 0.108$), and subfoveal ChT was 293 ± 63 μm (range 190–466 μm; $p = 0.101$).

By RFNL and GCL thickness analysis, no significant differences were disclosed comparing each subfield (*C*, *S*, *I*, *N* and *T*) of diabetic patients with the corresponding subfield of control subjects (Table 1).

Qualitative analysis

Considering OCT-A images, a rarefaction of perifoveal capillary network in SCP was detected in 7 out of 25 eyes (Fig. 1). No other abnormalities (i.e., microaneurysms, capillary tortuosity or disruption of the perifoveolar capillary arcade) were observed in diabetics. No alterations were disclosed in the control group (Fig. 2). There was agreement of 100% between readers.

Quantitative analysis

FAZ area was well detectable in all diabetic and control eyes. No significant difference was found in FAZ area at both SCP and DCP by comparing diabetics and control group. In SCP, FAZ area was 0.223 ± 0.100 mm^2 and 0.251 ± 0.104 mm^2 [diabetic and healthy subjects, respectively ($p = 0.341$)]. Mean FAZ area at DCP was 0.747 ± 0.199 mm^2 in diabetic eyes and 0.762 ± 0.231 mm^2 in control eyes ($p = 0.808$).

With regard to the retinal vasculature, no difference was detected in vessel density of RP between patients affect by diabetes and control subjects [0.446 ± 0.019 and 0.439 ± 0.017, respectively ($p = 0.213$)]. Also, by analyzing only SCP, no difference was disclosed in vessel density between the two groups [0.432 ± 0.023 and 0.430 ± 0.020, diabetics and control subjects, respectively ($p = 0.805$)]. At the DCP, diabetic eyes revealed a significantly decreased vessel density compared to control eyes of healthy subjects [0.464 ± 0.016 and 0.477 ± 0.014, respectively ($p = 0.005$)]. Instead, with regard to CCP, no difference was disclosed in vessel density between two groups analyzed [0.490 ± 0.013 and 0.487 ± 0.015, respectively ($p = 0.359$)].

No significant correlation was found between CMT and vessel density in RP, SCP and DCP ($p = 0.647$, $p = 0.796$ and $p = 0.601$, respectively). Sex, patient age, HbA1c level and duration of the disease did not influence significantly the FAZ area and vessel density in RP, SCP, DCP and CCP. Furthermore, no difference was detected in vessel density of DCP between patients with diabetes for more than 10 years and patients with diabetes for less than 10 years [VD 0.464 ± 0.015 and 0.463 ± 0.017, respectively ($p = 0.930$)].

Discussion

In this study, we analyzed both qualitatively and quantitatively different OCT-A plexuses in post-pediatric patients with T1DM without DR compared to healthy subjects, to investigate the possible occurrence of vascular alterations in these young patients and gather insights into DR pathogenesis. In 7 out of 25 diabetic patients, a rarefaction of perifoveal capillary network in SCP was disclosed in the qualitative analysis. However, these alterations were mild and this might explain why the vessel density of SCP was not reduced in diabetic patients compared to control group.

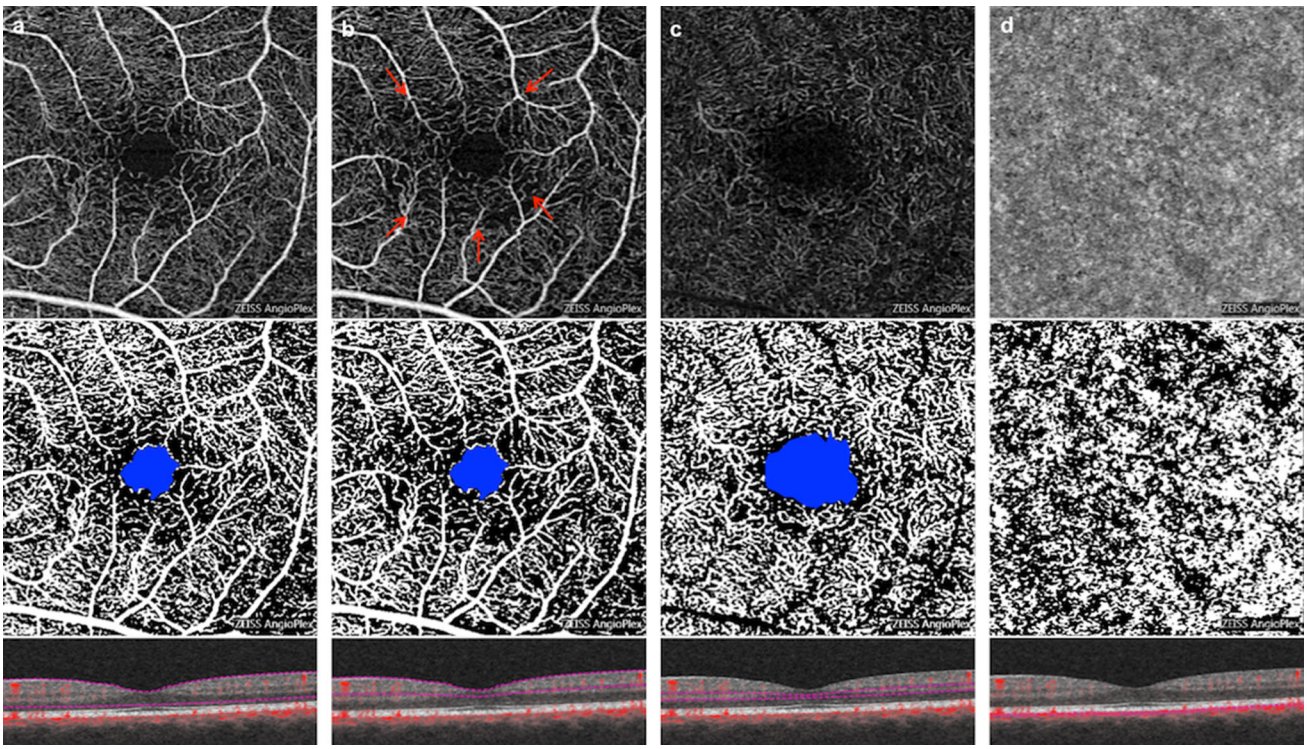

Fig. 1 Optical coherence tomography angiography (OCT-A) of a type 1 diabetic patient**. a** Full retinal thickness on 3 × 3 en-face OCT-A, corresponding binarized image and B-scan with flow. In binarized image, foveal avascular zone (FAZ) area was colored with *pure blue*. **b** Superficial capillary plexus on 3 × 3 en-face OCT-A shows a clearly visible rarefaction of perifoveal capillary (*red arrows*); corresponding binarized image and B-scan with flow. In binarized image, FAZ area was colored with *pure blue*. **c** Deep capillary plexus on 3 × 3 en-face OCT-A, corresponding binarized image and B-scan with flow. In binarized image, FAZ area was colored with *pure blue*. **d** Choriocapillary plexus on 3 × 3 en-face OCT-A, corresponding binarized image and B-scan with flow

Compared to healthy subjects, diabetic patients had a significant ($p = 0.005$) decreased vessel density in the DCP, while no difference was found in the full thickness RP, SCP and CCP as well as in ChT and CMT, and no significant difference was found in FAZ area of both SCP and DCP by comparing diabetics and control group. These findings suggest that a decreased vessel density is an early process in the T1DM disease and initially occurs in the DCP, although probably the reduction in DCP thickness is so mild compared to all retinal thickness resulting in no significant modification of the CMT. In addition, the choroid circulation is not impaired in the earliest stages of the disease.

Moreover, we hypothesize that an alteration of the vessel density in perifoveal capillaries is a sign that precedes the enlargement and remodeling of FAZ because there were no significant differences in the analysis of the FAZ area between the two study groups.

Recently, different studies have used OCT-A to study early vascular changes in diabetic patients with DR [32–35]. Scarinci et al. [32] reported outer retinal structural changes associated with macular capillary nonperfusion at the level of DCP in diabetic patients and showed that macular photoreceptor disruption on SD-OCT in patients with DR corresponds to areas of capillary nonperfusion at the level of the DCP. Couturier et al. [33] reported the presence of vascular abnormalities in both SCP and DCP in all eyes with DR. However, these studies do not differentiate between type 1 and type 2 DM and differ in the included stages of DR. For this reason, it remains unknown whether initial involvement of the DCP is unique to DM1 patients without any signs of DR.

In past studies, fluorescein angiography has demonstrated macular capillary nonperfusion in patients with no or mild DR [36, 37]. OCT-A can show in much greater detail that can be seen on FA microaneurysms, enlarged foveal avascular zone, area of retinal nonperfusion, reduced capillary density, capillary tortuosity and dilatation, identifying their localization in the superficial and deep capillary plexuses [21].

Currently, OCT-A and analytic software have facilitated automated and manual processing of macular perfusion data [30, 31, 38]. Recently, Simonett et al. [23] investigated quantitative differences in OCT-A data between DM1 patients with no or mild signs of retinopathy and nondiabetic subjects and reported a trend in reduction in DCP parafoveal vessel density in diabetic patients with no DR. De Carlo et al. [34] demonstrated that OCT-A was

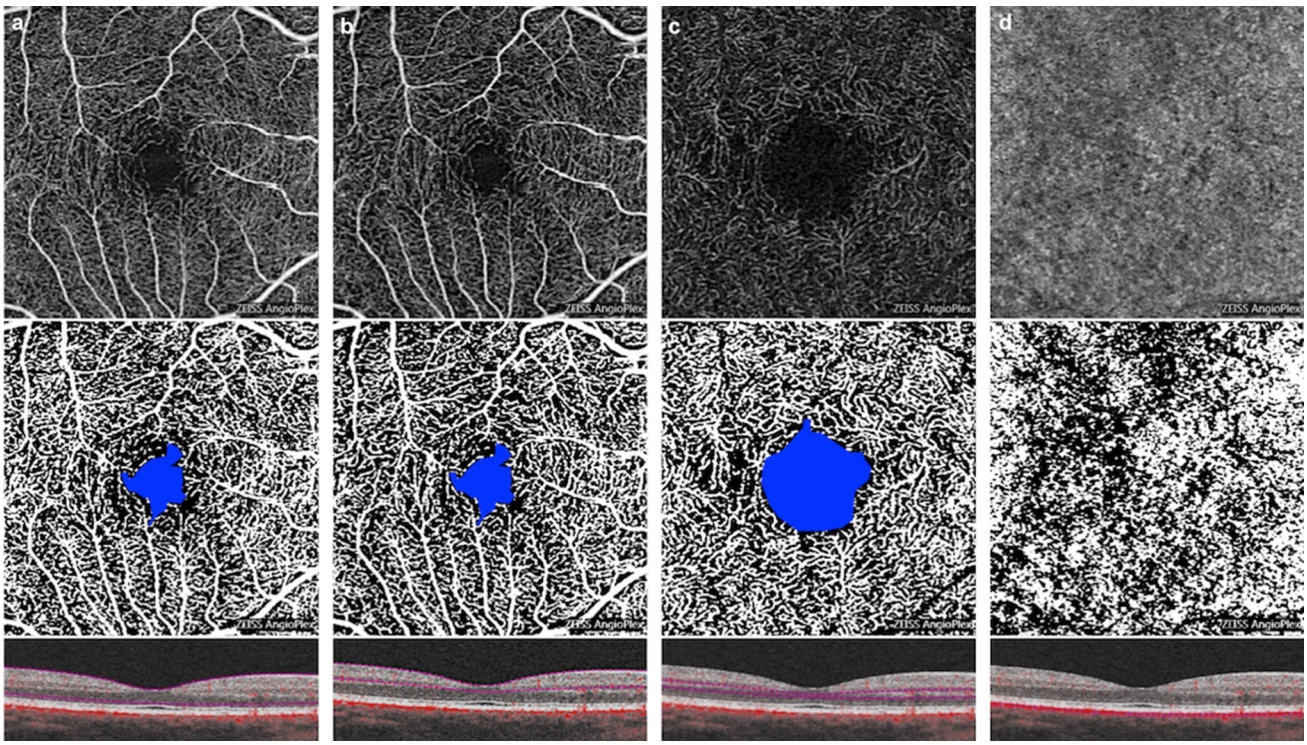

Fig. 2 Optical coherence tomography angiography (OCT-A) of a healthy subject**. a** Full retinal thickness on 3 × 3 en-face OCT-A, corresponding binarized image and B-scan with flow. In binarized image, foveal avascular zone (FAZ) area was colored with *pure blue*. **b** Superficial capillary plexus on 3 × 3 en-face OCT-A, corresponding binarized image and B-scan with flow. In binarized image, FAZ area was colored with *pure blue*. **c** Deep capillary plexus on 3 × 3 en-face OCT-A, corresponding binarized image and B-scan with flow. In binarized image, FAZ area was colored with *pure blue*. **d** Choriocapillary plexus on 3 × 3 en-face OCT-A, corresponding binarized image and B-scan with flow

able to image foveal microvascular changes that were not detected by clinical examination in diabetic eyes. Freiberg et al. [35] evaluated FAZ area and symmetry in patients with DR compared to healthy controls and demonstrated that OCT-A can detect disintegration of the vascular arcades surrounding the FAZ, thus allowing to differentiate DM from healthy eyes; of note, vascular abnormalities were more pronounced in the DCP.

To the best of our knowledge, this is the first study that demonstrated a significant decreased vessel density in the DCP, in patients with T1DM without RD compared to healthy subjects.

Furthermore, in our results no significant differences analyzing RNFL and GCL thickness were found by comparing diabetics and control group. Up to now, the exact relationship between vascular DR and diabetic retinal neuropathy is not yet known. Previous studies indicated that neuroretinal degeneration could be one of the earliest detectable retinal abnormalities in DM patients and could precede vascular alterations [39, 40]. El Fayoumi et al. [15] demonstrated a significant reduction in macular GCL thickness and in peripapillary RNFL thickness in children with T1DM without vascular detectable damage at fundus examination. Also Picconi et al. [24] reported an early structural damage of neuroretina (RFNL and inner nuclear layer), using structural OCT, in T1DM subjects with no signs or with mild nonproliferative DR. Probably, in our series the lack of differences in RNFL and GCL thickness between diabetics and control group was due to the young age of the subjects and the low mean duration of the disease. Nevertheless, the introduction of OCT-A in clinical practice has allowed identifying vascular abnormalities in RP before visualization of fundus biomicroscopy and dye angiography alterations. Based on this rationale, our results, in particular the decreased DCP vessel density associated with no changes in RNFL and GCL thickness, supported that probably a vascular damage to the DCP is the earliest detectable retinal abnormality in patients with T1DM, preceding detectable neuroretinal damage.

Interestingly, no correlation between duration of diabetes and vessel density of DCP was disclosed in our series. We believe that this lack of association was due to the small standard deviation of the duration of the disease, to the homogeneity of the sample and to its relative small size.

We acknowledge several limitations of this study. The series we analyzed is relatively small. Moreover, projection artifacts may also limit accurate evaluation as the light

beam that encounters the superficial retinal plexus may pass through the moving blood cells (45% of photons are estimated to pass across the vessel), and the projection of the scanned vessel may appear on the reconstruction of the DCP [41]. Finally, the lack of follow-up data did not allow to analyze the role of HbA1c for a longer period.

In conclusion, current OCT-A applications could revolutionize our daily clinical practice [42] and OCT-A is able to detect the early alterations and the changes in macular capillary network. We demonstrated the ability of OCT-A to disclose early vascular alterations in patients with T1DM diagnosed as without any signs of DR on the basis of fundus biomicroscopy. Our main findings suggest also that microvascular changes in DCP, detected by means of OCT-A, could precede detectable damage of diabetic neuroretinopathy.

Future studies investigating the progression of T1DM are warranted to better understand the pathophysiology.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Ethical standard All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Human and animal rights All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent Informed consent was obtained from all patients for being included in the study.

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