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Early microvascular and neural changes in Patients with Type 1 and Type 2 Diabetes Mellitus with no Clinical Signs of Diabetic Retinopathy

Stela Vujosevic MD, PhD, [1] Andrea Muraca MD,[1] Micol Alkabes MD, [1] Edoardo Villani MD, [2, 3] Fabiano Cavarzeran ScD, [4] Luca Rossetti MD,[5] Stefano De Cilla' MD, [1, 6]

1. University Hospital Maggiore della Carita', Ophthalmology Clinic, Novara, Italy
2. Department of Clinical Science and Community Health, University of Milan, Milan, Italy
3. Eye Clinic San Giuseppe Hospital, Milan, Italy.
4. Department of Neuroscience, University of Padova, Padova, Italy
5. ~~Eye Clinic,~~ Eye Clinic, University of Milan, San Paolo Hospital, Milan, Italy
6. Ophthalmology Unit, Department of Health Science, University East Piedmont "A. Avogadro", Novara, Italy

Corresponding Author: Stela Vujosevic MD, PhD, FEBO
University Hospital Maggiore della Carita', Ophthalmology Clinic,
Novara, Italy
Corso Mazzini 36, 28100 Novara
Phone: +390321805401
Email: stela.vujosevic@gmail.com

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Abstract:

Purpose:To assess early modifications in inner retinal layer (IRL) thickness and OCT angiography(OCT-A) parameters in patients with diabetes mellitus(DM) Type 1 and 2 without clinical signs of diabetic retinopathy (DR).

Methods**:**90 eyes of 90 subjects (24 Type 1 DM, 36 Type 2 DM and 30 healthy controls) were prospectively evaluated with SD-OCT, swept-source OCT–A and color fundus photo (on the same day). Retinal nerve fiber layer(NFL), ganglion cell layer(GCL+) and NFL+GCL+ (GCL++)thickness were automatically determined by the instrument in the 1, 3 and 6 central millimeters. On OCT-A, the following parameters were evaluated: area of foveal avascular zone(FAZ), number of microaneurysms, presence of: regular/irregular FAZ, capillary loss and capillary network irregularities in the superficial(SCP) and deep capillary plexuses(DCP).

Results:GCL++ was thinner in DM Type 1 versus controls within 3mm. GCL+ (p=0.0099) and GCL++ (p=0.0367) were significantly thicker in DM type 1 vs DM Type 2 in 1 central mm.The area of FAZ was significantly larger: in DM Type 1 vs controls in both SCP and DCP and in DM Type 1 vs Type 2 only in DCP($p<0.05$ for all); the number of microaneurysms was higher in DM Type 1 vs controls in both SCP and DCP ($p<0.01$ for all); and in DM type 2 vs controls only in DCP(p=0.007). Perifoveal capillary loss in SCP and IRL thickness had the highest correlation in both DM types.

Conclusions:There are specific neural and microvascular modifications even before clinical signs of DR in DM type 1 and 2. In DM Type 1,both SCP and DCP were affected, whereas in DM Type 2 mostly DCP was affected. IRL thickness and perifoveal capillary loss in the SCP had the highest correlation. This data may help in characterization of patients at preclinical stage of DR.

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Introduction:

Diabetic retinopathy (DR) is the most important ocular complication in diabetes mellitus (DM). [1] Chronic hyperglycemia leads to increased oxidative stress, inflammation and hypoxia, all inducing alterations of the neurovascular unit of the retina. [2] The neurovascular unit of the retina consists of retinal neurons and glial cells, endothelial cells, and pericytes. [2] All of these cell types undergo specific modifications induced by DM, even before the onset of clinical signs of DR, as reported by the experimental studies in animals and humans with DM. [3-8]

With recent advent of non invasive retinal imaging techniques such as spectral domain optical coherence tomography (SD-OCT) and SD-OCT angiography, changes in thickness of single retinal layers as well as modifications of macular capillary network have been evaluated in patients with DM with or without DR. A decrease in retinal nerve fiber layer and ganglion cell layer has been reported in patients with DM, even without clinical signs of DR. [9-13] Alterations of the macular capillary network (especially foveal avascular zone enlargement and presence of areas of capillary loss in the perifoveal area in deep capillary plexus and or superficial capillary plexus) have been reported in patients with DM without clinical signs of DR, when compared to subjects with no DM. [14-16]

There are very limited data on the direct evaluation and comparisons between microvascular changes detected on OCT angiography and retinal layer thickness changes in patients with DM and no clinical signs of DR. [15, 16] Dimitrova et al, evaluated changes in deep capillary plexus (DCP) and choroid in patients with DM Type 2 and no clinical signs of DR. [15] Simonett et al. evaluated a population of patients with DM Type 1 and no clinical signs of DM. [16] In this study we aimed to compare changes in inner retinal layer thickness and early microvascular changes detected on OCT angiography in patients with DM Type 1 and DM Type 2 and no clinical signs of DR and compare to healthy controls.

Material and Methods:

Patients:

This is a prospective, cross-sectional, comparative and consecutive case-control series of 90 subjects (90 eyes), consisting of normal subjects (no DM) and patients with DM with no clinical signs of DR. All subjects underwent blood pressure measurement, anamnestic collection of data including type of DM, value of haemoglobin A1c (HbA1c) complete ophthalmologic examination with best corrected visual acuity determination (BCVA), intraocular pressure (IOP) measurement, slit lamp fundus examination with 90D lens, color fundus photo of the macula, spectral domain optical coherence tomography (SD-OCT), and OCT angiography performed on the same day. Inclusion criteria were: patients with DM (type 1 and type 2, confirmed by the Diabetologist) with no clinical signs of DR determined on slit lamp fundus examination and confirmed on color fundus photo of the macula; healthy subjects (control group) and patients with DM older than 18 years; no history of ocular hypertension (IOP>21mmHg), glaucoma, uveitis, or neurodegenerative disease (for example Alzheimer disease, Parkinson, etc…); patients/subjects that accepted to participate. Major exclusion criteria were: previous intraocular treatment (laser, intravitreal injections, vitreo-retinal surgery); cataract surgery within 6 months; any antiinflammatory/steroids topical therapy; refractive error ≥ ±6D; any stage of DR or diabetic macular edema; and significant media opacity that precluded good quality fundus imaging and examination. The study was conducted in accordance with the tenets of the Declaration of Helsinki. All patients accepted to participate and signed the consent form.

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Visual acuity:

Best-corrected distance visual acuity (BCVA) for each eye was measured by a certified tester using standard ETDRS protocol at 4 m distance with a modified ETDRS distance chart illuminator (Precise vision, Bloomington, IL). Visual acuity was scored as the total number of letters read correctly (ETDRS score) and (also expressed in logMar).

Imaging:

Spectral domain optical coherence tomography and OCT-angiography:

Spectral domain optical coherence tomography (SD-OCT) and OCT angiography were performed using the swept source OCT, DRI OCT Triton plus (Topcon Medical Systems Europe, Milano, Italy). This instrument uses a 1050nm wavelength, with a scanning speed of 100.000 A-Scans/second. Following scan-patterns were performed: a linear B-scan (12mm length) centered on the fovea at 0°; 3D Macula map covering central area of 7mm x 7mm; and OCT-angiography maps covering central 3mmx3mm area and 6mmx6mm area.

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SD-OCT Segmentation and measurement:

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DRI OCT Triton plus OCT instrument with software version 10.07.003.03 allows for automatic segmentation of following retinal layers from 3D map: retinal nerve fiber layer (NFL) from inner limiting membrane (ILM) to NFL; ganglion cell layer (GCL)+ complex – from NFL/GCL interface to inner plexiform layer (IPL)/inner nuclear layer (INL); GCL++ complex- from ILM/NFL interface to IPL/INL; outer retina- from IPL/INL interface to interdigitation zone (Verhoeff's membrane); [17] (full) retina thickness- from ILM to interdigitation zone. Automatic evaluation of the specific retinal layer thickness and full retinal thickness was performed in 9 ETDRS areas and evaluated as (central subfield retinal thickness (CSF) – circular area with 1 mm diameter centered on the fovea; inner rings (mean value of 4 inner quadrants with diameter of 3mm; and outer rings (mean value of 4 outer quadrants with 6 mm diameter). After automated segmentation, each scan was checked for the presence of segmentation errors, and in that case a manual correction was performed.

OCT angiography scans evaluation:

OCT angiography (OCT-A) scans obtained within 3mmx3mm were used for both quantitative and qualitative evaluation. All evaluations were separately performed at the level of SCP and DCP. The built-in software segmentation algorithm was used to define SCP and DCP on *En face* OCT angiograms. The SCP slab was obtained from the ILM to 15.6 micron above the IPL/INL interface. The DCP slab was obtained from the IPL/INL interface (above 15.6 micron) to the IPL/INL interface (below 70.2 micron). Only good quality OCT-A images were considered for analyses, excluding those that had presence of artifacts such as double vessel pattern, dark areas from blinks, or motion artifacts, and those with signal strength index below 50.

Following parameters were evaluated: 1) area of the foveal avascular zone (FAZ) delimitated using the tool caliper area available within software IMAGENET 6 (Version 1.17.9720, Topcon Medical Systems, Inc, Oakland, NJ). After manual delimitation of the FAZ , the software automatically calculates the area in square milimeters (mm^2); 2) number of microaneurysms counted within 3mm area; 3) presence of regular (oval or round shaped with regular and clear contour) [18] or irregular (asymmetrical) FAZ 4) presence of area of capillary loss/reduced capillary density; 5) alterations of the capillary network (presence of tortuosity and/or beadings).

All measurements and evaluations were performed by two graders, independently, masked to the clinical data of patients, on 3 mm scan area, zooming the image in order to have greater magnification and evaluate more easily details, and (if necessary) using also the reverse mode (in which vessels are black and the background is white, for better definition of details). In case of disagreement the experienced medical retina specialist made a final adjudication.

Statistical methods:

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The summary of parameters has been made by means of usual methods used for descriptive statistics: mean value, standard deviation, and range for continuous numeric variables; frequency distribution, absolute and relative (percentage), for qualitative variables.

Sample characteristics (age, systolic pressure, diastolic pressure, HbA1c, BCVA, and IOP) have been compared between patients with DM and controls by means of t-Student test. Comparisons among groups (DM Type 1 and DM Type 2, and controls) have been made by means of one-way Analysis of Variance (ANOVA) followed by post-hoc Bonferroni test for multiple comparisons.

OCT parameters:

Mean values of retinal thickness (full retinal thickness, NFL thickness, GCL+ thickness, GCL++ thickness, and Outer Retinal thickness) have been compared between patients with DM and controls, and among DM type 1, DM type 2, and controls by means of three factor with interaction ANOVA for repeated measures analysis, adjusted for age. Comparison between DM Type 1 and DM Type 2 has been adjusted also for the duration of DM. Retinal thickness determined in the 9 ETDRS quadrants was summarized into three values referred as CSF (central sub-field quadrant), inner ring (average value of inner superior, inner nasal, inner inferior, and inner temporal quadrant values), and outer ring (average value of outer superior, outer nasal, outer inferior, and outer temporal quadrant values). Factors of the model were Group (patients with DM and Controls in one analysis, DM Type 1, DM Type 2, and Controls in the other), Sector (CSF, inner ring, and outer ring), and the interaction Group by Sector. However particular interest has been placed to significance of Group factor (which tested differences among overall mean retinal thicknesses) and to interaction factor (which tested differences among retinal thickness profiles). Comparisons have been adjusted for patient's age and in case of

significant result, post-hoc tests for multiple comparisons with Bonferroni correction were applied.

OCT Angiography parameters:

Mean values of FAZ area extension and number of microaneurysms in the SCP and DCP have been compared among groups (patients with DM vs. controls, and DM Type 1 vs. DM Type 2 vs. controls) by means of one-way ANOVA analysis adjusted for patient's age and blood pressure and followed by Bonferroni post-hoc test for multiple comparisons.

Association between Group and presence of regular/irregular FAZ, presence of area of capillary loss/reduced capillary density, and presence of tortuosity/beadings have been assessed by means of logistic regression model adjusted for patient's age and blood pressure. Association was expressed in terms of Odds-Ratios and relative 95% Wald confidence interval.

Correlations among OCT and OCT Angiography parameters:

Multiple linear regression models with stepwise selection of independent variables have been used to assess the relationship between retinal thickness (dependent variable) – one model for each parameters: full retina thickness, NFL layer, GCL+, GCL++, and Outer retinal thickness – and OCT Angiography parameters. Patient's age and blood pressure (systolic and diastolic) were taken into account. Three models have been estimated, one for each of the following sectors: CSF, inner ring, and CSF plus inner ring. Qualitative variables have been coded as follows before entering the model: FAZ regularity, 0=regular, 1=irregular; Capillary loss, 0=absent, 1=present, Tortuosity/Beadings, 0=absent, 1=present.

All the analyses have been performed by means of SAS® v.9.3 (SAS, Cary, NC) statistical software. All statistical tests have been considered significant when $P<0.05$.

Results:

Of 90 examined subjects (90 eyes), 60 were patients with DM with no clinical signs of DR and 30 were healthy subjects (with no DM). Of 60 patients with DM, 24 patients had DM Type 1 and 36 patients had DM Type 2 (of which 9 patients were on insulin whereas 27 were oral hypoglycemic drugs). Systemic and ocular findings (mean age, HbA1c, duration of DM, blood pressure, BCVA and IOP values) are reported in Table 1.

Table 2 shows mean values of retinal thickness (automatically measured by the instrument) of full retina, NFL, GCL+ complex, GCL++ complex and outer retina divided by rings (CSF, inner ring and outer ring) and groups (normal subjects, all patients with DM and separately DM type 1 and DM type 2). After adjusting for age there was a different thickness profile in GCL++ complex among three examined groups (control, patients with DM type 1 and DM type 2), p=0.049, (ANOVA) in the three different rings. Such a model estimated that GCL++ complex in the inner ring was thinner in patients with DM Type 1 (114.9µm±8.3) versus controls with borderline statistical significance (118.0µm±8.2, Bonferroni post-hoc test for multiple comparison, p=0.0981).

After adjusting for age and DM duration, GCL+ complex (49.4µm±7.3 vs. 43.6µm ±9.6, *p*=0.0099) and GCL++ complex (57.2 µm±10.8 vs. 50.5±10.2 µm, p=0.0367) were significantly thicker in patients with DM type 1 vs DM Type 2 in the CSF (Table 2).

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Table 3 shows data of parameters evaluated on OCT angiography, adjusted for patient's age and blood pressure. All evaluated parameters both quantitative and qualitative were significantly different in patients with DM versus controls in both SCP and DCP. (Table 3). In particular, the area of FAZ was significantly larger: in patients with DM Type 1 versus controls in both SCP (p=0.05) and DCP (p<0.001) and in patients with DM Type 1 versus DM Type 2 only in DCP (p<0.0001); the number of microaneurysms was higher in patients with DM Type 1 versus controls in both SCP (p<0.001) and DCP (p<0.0001); and in patients with DM type 2 versus controls only in DCP (p=0.007). (Table 3) Logistic regression analysis (adjusted for patient's age and blood pressure), showed greater

association between all evaluated qualitative parameters in patients with DM type 1 and almost all (exception for irregularities in the SCP) for patients with DM type 2 versus control group. (Table 3)

Table 4 shows statistically significant correlations between OCT and OCT angiography parameters. Statistically significant correlations to retinal thickness include almost all parameters evaluated on OCT angiography in the SCP in patients with DM type 1, whereas only the presence of areas of capillary loss and FAZ area in the SCP in patients with DM type 2. In particular, the area of FAZ in SCP inversely correlated to NFL thickness in the CSF in patients with DM type 2 whereas, the area of FAZ in DCP inversely correlated to NFL thickness in the CSF in patients with DM type 1.

The highest correlations were found between perifoveal capillary loss in the SCP and GCL+ and GCL++ thickness in the inner ring in patients with DM Type 1 and perifoveal capillary loss in the SCP and NFL thickness in the CSF and the inner ring in patients with DM Type 2.

Discussion:

In this study we report on early microvascular and retinal thickness changes in patients with DM and no clinical signs of DR, evaluating further and separately patients with DM Type 1 and DM Type 2 vs healthy subjects. All quantitative and qualitative parameters evaluated on OCT-A in the SCP and DCP were significantly different in patients with DM versus healthy controls. In particular, in patients with DM and no clinical signs of DR, FAZ area was larger and irregular, the number of microaneurysms was higher, areas of perifoveal capillary loss and perifoveal capillary network irregularities (such as tortuosity and beadings) were more numerous in both SCP and DCP when compared to healthy subjects. Simonett et al. evaluated patients with DM type 1 and no DR (9 eyes) or mild non proliferative DR (19 eyes) and reported decreased parafoveal vessel density (that could be compared to parafoveal capillary nonperfusion) only in the DCP, and no changes in the

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FAZ area were found in neither SCP nor DCP. [16] Dimitrova at al. documented decreased parafoveal vessel density in the SCP and DCP and FAZ area increase in the SCP in patients with DM (mostly type 2) and no DR when compared to control subjects. [15]

In the present study, specific changes are documented separately in patients with DM Type 1 and DM Type 2. All quantitative and qualitative parameters evaluated on OCT angiography resulted altered in both SCP and DCP in patients with DM type 1 vs controls. In DM type 2, the only quantitative parameter found different, compared to controls, was the higher number of microaneurysms in the DCP. Thus, the other evaluated quantitative parameters (the area of the FAZ in SCP and DCP and the number of microaneurysms in SCP) were not found different in patients with DM type 2 vs controls. Moreover, the area of the FAZ was significantly larger in patients with DM type 1 vs patients with DM type 2 in the DCP. Besides modifications in quantitative parameters, also all evaluated qualitative parameters were found different in patients with DM type 1 vs controls, and almost all (except the presence of capillary irregularities in the SCP) in patients with DM type 2 compared to controls. Although data from the present study cannot be directly correlated to the data available in the literature on OCT angiography parameters in patients with DM and no clinical signs of DR, due to different population of patients (considering together patients with DM type 1 and 2, or only separately patients with DM type 1 but also with some signs of DR), some similarities or differences could be drawn. De Carlo et al. reported on increased prevalence in changes to the FAZ (increased FAZ area and presence of FAZ remodeling) and capillary nonperfusion in patients with DM (considering together type 1 and type 2) and no clinical signs of DR. [14] The same authors reported that vascular tortuosity was present in similar percentage in both controls and patients with DM, concluding that this microvascular abnormality may be a variant of normal, and thus cannot be used as the OCT-A screening parameter for retinal vascular change in DM. [14] However, the authors did not report on blood pressure data and differences between the

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groups. On the contrary, in the present study, after adjusting for blood pressure differences both venous beading and vascular tortuosity were found more frequent in patients with DM type 1 and DM type 2 versus non diabetic subjects. Thus the significance of microvascular abnormalities in patients with DM and no clinical signs of DR may warrant further evaluation.

Results from the present study confirm data reported by Dimitrova et al, who found changes in parafoveal vessel density in both SCP and DCP in patients with DM (mostly type 2) and no clinical signs of DR. [15] Simonett et al, evaluated a cohort of 28 patients with DM type 1 and no or minimal signs of DR using an automated algorithm software. [16] These Authors reported a decrease in parafoveal vessel density only in the DCP. [16] Choi et al, using an ultrahigh speed swept source OCT angiography prototype, documented retinal microvascular abnormalities (such as capillary dropout, dilated capillary loops, tortuous capillary branches, patches of reduced capillary perfusion, irregular FAZ contours, and/or FAZ enlargement) in both SCP, intermediate and DCP, in 18 of the 51 eyes with DM and no clincial signs of DR (with no specification of DM type). [19] Moreover, these Authors reported focal or diffuse choriocapillaries flow impairment in almost half of the evaluated patients without DR.

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Data from the present study may indicate that both SCP and DCP are precociously altered in patients with DM type 1 and no clinical signs of DR, whereas in patients with DM type 2, DCP is more precociously involved. To the best of our knowledge, no data are currently available on direct comparison between patients with DM type 1 and type 2 at pre-clinical stage of DR.

Patients with DM type 1 had thinner inner parafoveal retinal thickness (GCL++ complex in the inner ring) when compared to normal subjects (when adjusted for age). Scarinci et al, recently reported a significant thinning of the GCL layer in patients with DM type 1 and no

DR. [13] Van Dijk et al documented no significant difference in any layer thickness in the macula in patients with DM but no DR compared to normal controls and a decrease in the NFL, GCL and IPL thickness in the pericentral area of the macula, in patients with minimal DR compared to controls [10] Vujosevic and Midena documented decreased NFL thickness in the macular area in patients with no DR or with non proilferative DR, mostly with DM type 2. [11] In the present study, after adjusting for age and DM duration, GCL+ and GCL++ complex thicknesses were significantly higher in patients with DM type 1 vs DM Type 2 in the CSF. The exact mechanism leading to this finding is not known. One hypothesis may implicate a protective role of more intensive insulin treatment on retinal ganglion cells already reported in experimental studies, thus thicker inner retina in DM type 1.[20, 21] Another hypothesis may see reported differences in complication characteristics in patients with DM type 2 versus DM type 1, that included more prevalent peripheric neuropathy and cardiovascular disease in DM type 2, independent of disease duration, whereas no differences were found for the presence of DR. [22] This may explain thinner inner retina in DM type 2 when corrected for age difference and DM duration in the present study. [22]

In conclusion this study documents very early and specific microvascular and neuronal changes both in patients with DM type 1 and DM type 2, when compared to non diabetic controls. OCT-A allows for detection of microvascular changes even before clinical signs of DR are present. Thus, OCT-A may help in earlier diagnosis and new classification of DR. Further, larger studies are needed to better evaluate and correlate microvascular and neural changes in the retina in DM.

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Figures' Legend:

Figure 1.

Right eye of a patient with diabetes mellitus type 1 and no clinical signs of diabetic retinopathy. First raw: Left - color fundus photo; middle-OCT-angiography (OCT-A) of the superficial capillary plexus (SCP); right- OCT-angiography of the deep capillary plexus (DCP); modifications of the foveal avascular zone-FAZ including enlaragment and presence of irregular form presence of area of capillary loss/reduced capillary density, and alterations of the capillary network are present both the SCP and DC**;** Second raw: OCT linear scan in the macula showing automatic segmentation (green lines) of the nerve fiber layer; ganglion cell layer (GCL)+ complex – from NFL/GCL interface to inner plexiform layer (IPL)/inner nuclear layer (INL); GCL++ complex- from ILM/NFL interface to IPL/INL; outer retina- from IPL/INL interface to interdigitation zone**.**

Figure 2.

Right eye of a patient with diabetes mellitus type 1 and no clinical signs of diabetic retinopathy. First raw: Left - color fundus photo; middle-OCT-angiography (OCT-A) of the superficial capillary plexus (SCP); right- OCT-angiography of the deep capillary plexus (DCP); enlargament of the foveal avascular zone as well as the presence of microaneurysm is better observed in the DCP. presence of area of capillary loss/reduced capillary density, and alterations of the capillary network are present both the SCP and DC**;** Second raw: OCT linear scan in the macula showing automatic segmentation (green lines) of the nerve fiber layer; ganglion cell layer (GCL)+ complex – from

NFL/GCL interface to inner plexiform layer (IPL)/inner nuclear layer (INL); GCL++ complex- from ILM/NFL interface to IPL/INL; outer retina- from IPL/INL interface to interdigitation zone.

Table 1 – Sample characteristics. Mean (SD, range)

Variable	*Controls (N=30)*	*Diabetics (N=60)*	*Diabetes Type I (N=24)*	*Diabetes Type II (N=36)*
Age	44.4 (14.8, 21-72)	57.4[a] (15.4, 19-81)	36.9 (12.1, 19-53)	64.2[e,f] (8.9, 41-81)
HbA1c	n.a.	7.3 (1.0, 5.8-10.3)	7.2 (0.9, 5.9-9.1)	7.3 (1.1, 5.8-10.3)
Duration	n.a.	9.9 (8.7, 0.5-40)	16.3 (11.3, 2-40)	7.7[d] (6.4, 0.5-21)
SBP	120.2 (8.9, 90-130)	126.1[b] (10.0, 100-150)	119.2 (11.6, 100-140)	128.5[g,h] (8.3, 120-150)
DBP	76.7 (7.8, 60-90)	77.3 (5.8, 60-85)	73.3 (8.1, 60-85)	78.6[i] (4.2, 70-85)
ETDRS	85.0 (0.0, 85-85)	83.6 (4.3, 55-88)	84.9 (0.3, 84-85)	82.8[j] (5.4, 55-88)
IOP	14.6 (2.1, 12-19)	13.9[c] (1.2, 12-18)	13.2[k] (1.1, 12-15)	14.3[l] (1.0, 13-18)

Legend: Duration=duration of diabetes (years); SBP=systolic blood pressure (mmHg); DBP=diastolic blood pressure (mmHg); ETDRS=number of letters; IOP=intraocular pressure (mmHg); n.a.=not applicable. Diabetics vs. Controls t-Student test: a) P=0.0010; b) P=0.0161; c) P=0.0315. Bonferroni post-hoc test: d) Type I vs. Type II , P=0.0020; e) Type II vs. Controls, P<0.0001; f) Type II vs. Type I, P<0.0001; g) Type II vs. Controls, P=0.0029; h) Type II vs. Type I, P=0.0092; i) Type II vs. Type I, P=0.0425; j) Type II vs. Controls, P=0.0326; k) Type I vs.Controls, P=0.0020; l) Type II vs. Type I, P=0.0143.

Table 2 – Retinal thickness. Mean (SD)

Parameter	*Controls (N=30)*	*Diabetics (N=60)*	*Diabetes Type I (N=24)*	*Diabetes Type II (N=36)*
Total thickness				
CSF	243.2 (21.4)	238.4 (20.9)	241.2 (20.1)	236.5 (21.5)
Inner ring	313.0 (12.2)	307.7 (16.1)	309.1 (16.5)	306.8 (16.0)
Outer ring	273.0 (12.9)	267.0 (13.0)	268.9 (12.8)	265.8 (13.2)
NFL layer				
CSF	7.3 (3.6)	7.3 (3.3)	8.0 (3.6)	6.8 (3.1)
Inner ring	27.0 (2.0)	26.9 (2.4)	26.4 (1.3)	27.1 (2.9)
Outer ring	40.5 (2.9)	39.6 (3.3)	39.4 (2.7)	39.8 (3.7)
GCL+ layer				
CSF	47.6 (8.7)	46.0 (6.7)	48.7[c] (6.1)	44.1 (6.5)
Inner ring	92.7 (5.4)	90.0 (6.7)	90.2[a] (7.2)	89.8 (6.5)
Outer ring	66.5 (3.8)	65.2 (5.3)	66.1 (4.4)	64.6 (5.8)
GCL++ layes				
CSF	54.8 (12.0)	53.2 (9.2)	56.8[D] (9.0)	50.9 (8.6)
Inner ring	119.7 (6.8)	116.8 (8.2)	116.7[b] (7.9)	116.9 (8.4)
Outer ring	107.0 (5.2)	104.8 (7.3)	105.6 (6.5)	104.3 (7.8)
Outer retina				
CSF	188.3 (11.5)	185.2 (14.2)	184.5 (13.2)	185.6 (15.1)
Inner ring	193.3 (7.2)	190.9 (10.3)	192.5 (10.9)	189.9 (9.8)
Outer ring	165.9 (8.6)	162.2 (8.6)	163.3 (9.9)	161.5 (7.6)

Legend:NFL: retinal nerve fiber layer; GCL+ - ganglion cell layer; GCL++- NFL and GCL+ ; CSF: central subfield retinal thickness; inner ring- retinal thickness within central 3mm; outer ring: retinal thickness within central 6mm.

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537 **Table 3** – OCT-Angiography parameters.

Parameter	*Controls (N=30)*	*Diabetics (N=60)*	*Diabetes Type I (N=24)*	*Diabetes Type II (N=36)*
FAZ area[1]				
Superficial plexus	286.4 (137.0)	359.3[a] (129.1)	377.2[e] (150.6)	347.5 (113.2)
Deep plexus	363.7 (142.1)	497.0[b] (150.3)	557.1[f,g] (139.6)	456.9 (145.9)
Number of microaneurisms[1]				
Superficial plexus	0.10 (0.40)	1.40[c] (1.61)	1.38[h] (2.10)	1.42 (1.20)
Deep plexus	0.07 (0.37)	2.07[d] (1.89)	1.83[i] (2.16)	2.22[j] (1.71)
Irregular quality[2]				
Superficial plexus	17 (56.7)	50[A] (83.3)	21[G] (87.5)	29 (80.6)
Deep plexus	8 (26.7)	50[B] (83.3)	23[H] (95.8)	27[M] (75.0)
Capillary loss[2]				
Superficial plexus	12 (40.0)	55[C] (91.7)	22[I] (91.7)	33[N] (91.7)
Deep plexus	7 (23.3)	58[D] (96.7)	23[J] (95.8)	35[O] (97.2)
Presence of Tortuosity/Beadings[2]				
Superficial plexus	1 (3.3)	47[E] (78.3)	14[K] (58.3)	33[P] (91.7)
Deep plexus	3 (10.0)	54[F] (90.0)	19[L] (79.2)	35[Q] (97.2)

538 Legend: 1) Mean (standard deviation); 2) Frequency (%). ANOVA adjusted for patient's age and
539 blood pressure, test vs. Control group: a) *P*=0.0582; b) *P*=0.0003; c) *P*=0.0005; d) *P*<0.0001. *Post-*
540 *hoc* Bonferroni test vs. Control group after ANOVA adjusted for patient's age and blood pressure:
541 e) *P*=0.0189; f) *P*<0.0001; g) vs. Type II group, *P*=0.0012; h) *P*=0.0013; i) *P*<0.0.001; j) *P*=0.0118.
542 Logistic regression analysis adjusted for patient's age and blood pressure, Odds-Ratios (95%CI)
543 vs. Control group: A) 4.0 (1.3 to 11.7); B) 22.0 (6.0 to 81.5); C) 13.1 (3.8 to 44.3); D) 87.5 (14.8 to
544 517.2); E) 97.1 (11.0 to 858.3); F) 168.1 (19.0 to +∞); G) 5.6 (1.3 to 23.0); H) 68.2 (7.7 to 606.9); I)
545 27.9 (4.4 to 177.1); J) 179.3 (12.6 to +∞); K) 68.3 (6.4 to 788.2); L) 352.4 (16.3 to +∞); M) 8.9 (1.9
546 to 42.4); N) 5.4 (1.0 to 29.8); O) 36.9 (3.5 to 389.5); P) 136.8 (11.7 to +∞); Q) 87.7 (7.3 to +∞).
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553 **Table 4** – Correlation between OCT parameters and OCT-Angiography parameters.

Quadrant	*Total thickness*	*NFL layer*	*GCL+ layer*	*GCL++ layer*	*Outer retina*
Controls					
CSF	FAZs (-0.145)	FAZd (-0.020) CAPd (+2.516)	FAZs (-0.036) FAZd (-0.023)	FAZs (-0.046) FAZd (-0.036)	FAZs (-0.073) QUAd (-6.692)
Inner	FAZs (-0.060) QUAs (-8.472)	FAZd (-0.008)	FAZs (-0.026) QUAs (-2.695) TORd (+7.891)	FAZs (-0.033) QUAs (-3.748)	FAZs (-0.030) QUAs (-4.653)
CSF+Inner	FAZs (-0.082) QUAs (-6.930)	FAZd (-0.011)	FAZs (-0.031) TORd (+7.016)	FAZd (-0.043) QUAs (-2.938)	FAZs (-0.038) QUAd (-3.968)
DM					
CSF	FAZs (-0.069) MICd (-3.487)	FAZs (-0.009) TORs (-2.552)	FAZs (-0.021) MICs (+1.427) MICd (-1.110) TORs (-6.681) TORd (+5.111)	FAZs (-0.031) TORs (-7.267)	FAZd (-0.036) MICd (-2.576) QUAd (+7.720)
Inner	MICd (-2.627)	FAZd (-0.003) CAPs (-2.307)	FAZs (-0.019)	FAZs (-0.023)	MICd (-1.764)
CSF+Inner	FAZs (-0.033) MICd (-2.456)	FAZd (-0.004) QUAd (+1.390) CAPs (-2.601)	FAZs (-0.020)	FAZs (-0.022) TORs (-3.818)	MICd (-2.075)
Type I					
CSF	FAZd (-0.083)	FAZd (-0.013)	FAZd (-0.027)	FAZd (-0.038)	MICs (-3.357)
Inner	MICs (-2.899)	QUAs(-1.835) TORs (-1.114)	CAPs (-10.273)	CAPs (-11.011)	-
CSF+Inner	MICs (-3.305)	MICs (-0.357)	CAPs (-8.936)	MICs (-1.522)	MICs (-1.783)
Type II					
CSF	FAZs (-0.119) MICd (-3.853)	FAZs (-0.009) CAPs (-4.401)	FAZs (-0.042)	FAZs (-0.056)	FAZs (-0.067) MICd (-3.749)
Inner	FAZd (-0.041) MICd (-2.995)	CAPs (-4.053)	FAZd (-0.022)	FAZd (-0.027)	MICd (-2.766)
CSF+Inner	FAZs (-0.067) MICd (-3.456)	CAPs (-4.388)	FAZd (-0.023)	FAZs (-0.038)	FAZs (-0.029) MICd (-2.912)

Legend: In brackets statistically significant (P<0.05) regression coefficients from the multiple regression model of each OCT parameter vs. Angio-OCT parameters (stepwise selection criterion) adjusted for patient's age and blood pressure (systolic and diastolic). FAZ = extension of area of the foveal avascular zone FAZ (mm^2); MIC = microaneurysms (number); QUA = quality of the FAZ area (0=regular,1=irregular; CAP = capillary loss (0=no, 1=yes); TOR = presence of tortuosity/beadings (0=no, 1=yes); suffix 's' = superficial plexus; suffix 'd' = deep plexus. CSF = central subfield retinal thickness; Inner = the mean of superior, nasal, inferior and temporal inner quadrant thickness (central 3mm); CSF+Inner = the average of CSF and Inner quadrants' thickness.

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