FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA

TRABALHO FINAL DO 6º ANO MÉDICO COM VISTA À ATRIBUIÇÃO DO GRAU DE MESTRE NO ÂMBITO DO CICLO DE ESTUDOS DE MESTRADO INTEGRADO EM MEDICINA

JOÃO HEITOR PEIXOTO ALEGRE MARQUES

CHOROIDAL THICKNESS IN DIABETIC RETINOPATHY ASSESSED WITH SWEPT-SOURCE OPTICAL COHERENCE TOMOGRAPHY

ARTIGO CIENTÍFICO ORIGINAL

ÁREA CIENTÍFICA DE OFTALMOLOGIA

TRABALHO REALIZADO SOB A ORIENTAÇÃO DE:
PROFESSOR DOUTOR RUFINO SILVA
DOUTORA INÊS LAÍNS

MARÇO DE 2016

This work has been submitted to the 2016 ARVO Imaging in the Eye Conference and to the 16th EURETINA Congress.

Index

Background

Diabetes mellitus (DM) is a serious and increasing global health problem. According to the most recent estimates, it is expected to affect 592 million people worldwide in 2035 (1). Among its complications, stands out diabetic retinopathy (DR), which affects about a third of diabetic patients (2) and represents the leading cause of visual loss in the working age populations (3).

The current knowledge on DR pathophysiology suggests that it is a complex process (4). Retinal vascular changes play a key role (5). However, the choroid - the major blood supplier of the outer retina (6) - also seems to be involved, as initially suggested by histological (7) and laser doppler flowmetry studies (8). More recently, data obtained with optical coherence tomography (OCT) - a noninvasive, *in vivo*, imaging technology with a resolution approaching histological details - has also contributed to the comprehension of the role of the choroidal vasculature in DR. This was primarily achieved due to the description of the enhanced-depth imaging protocol (EDI) by Spaide (9), which allows an improved transversal view of the choroid and the assessment of its thickness (CT).

The available studies using EDI on DR produced controversial results: some authors described a reduced CT in patients with diabetes (10), with DR (11–13) and with the advanced stages of the disease (diabetic macular edema - DME - or proliferative diabetic retinopathy - PDR) (14–18). Conversely, others reported a thicker choroid in diabetic patients (19,20) and in the advanced stages of DR (21). Explanations for these controversial findings include the inherent limitations of the EDI protocol, namely the lack of software for automatic segmentation of the choroid, thus requiring a manual delineation of the inner and outer borders of this vascular layer. This is particularly relevant as some studies (12,16) did not even include two independent observers. Moreover, this protocol requires the averaging of 50 to 100 B-scans to achieve high-contrast and low-speckle noise (22) and the wavelength of the

spectral domain OCT (SD-OCT) – 870 nm - does not allow to detect the choroidal-scleral boundary in some cases, due to scattering and low penetration through the retinal pigment epithelium (RPE) (23).

The recently developed swept-source OCT (SS-OCT) is a new device that may allow overcoming some of these limitations. SS-OCT has a longer wavelength (1050 nm), and thus a better penetration and lower scattering at the RPE. Theoretically, these properties allow a more accurate high-resolution transversal view of the choroidal limits, without loosing detail for the distinct retinal layers' assessment (23,24). Furthermore, one of the commercially available SS-OCT devices in Europe - Topcon® DRI SS-OCT - has the ability to create automatic, user-independent CT maps. Interestingly, the United States Food and Drug Administration (FDA) has not yet approved this device. Despite this, its utility has already been described in the assessment of CT in different diseases, including age-related macular degeneration, central serous chorioretinopathy, angioid streaks and others (25–30).

To our knowledge, no studies have explored the potentialities of SS-OCT to assess CT on DR, which, as detailed, remains a controversial topic - it remains unclear whether diabetic patients present or not a reduced CT and the effect of the severity of the disease.

Thereby, the aim of this study was to analyze the CT of DR patients with different severity stages of disease and to compare it with age-matched controls, using SS-OCT.

Materials and methods

Study design

This was a prospective, cross-sectional, observational, multicenter study, including patients from the Ophthalmology Department, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal, in collaboration with the Association for Innovation and Biomedical Research on Light and Image (AIBILI), and from the Massachusetts Eye and Ear Infirmary (MEEI), Boston, United States. This research adhered to the tenets of the Declaration of Helsinki. The Institutional Review Board (IRB) of MEEI and of AIBILI approved the study protocol. As required by law, in Portugal, the Portuguese National Committee of Data Protection (CNPD) also approved the study. All included participants provided written informed consent.

Study subjects

Consecutive subjects with the diagnosis of DM, with or without DR, were identified and invited to participate in this study when they were coming to their regular appointments at CHUC and MEEI. Exclusion criteria included: refractive error equal or superior to 6 spherical equivalent diopters; diagnosis of ocular hypertension or glaucoma with a optic nerve cup-disc ratio superior to 0.6; laser capsulotomy, focal laser, panretinal photocoagulation or intravitreal injections in the 90 days prior to inclusion; any previous retinal surgery; diagnosis of other retinal or choroidal pathology, namely age-related macular degeneration, vitreomacular traction, epiretinal membrane, macular hole, uveitis; systemic diseases that might affect CT, such as uncontrolled hypertension, systemic lupus erythematosus, anemia, leukemia and obstructive sleep apnea; and decreased media transparency that precluded appropriate OCT imaging.

Simultaneously, subjects without diabetes and without any diagnosed vitreoretinal disease were also invited to participate in the study, as a control group. The same exclusion criteria applied.

Study protocol

All included participants were submitted to a complete bilateral ophthalmologic exam, as part of their regular clinical visit with an experienced ophthalmologist, including best-corrected visual acuity (BCVA) with Snellen charts (for analysis converted to logMAR), current refraction, intra-ocular pressure with Goldmann tonometry, biomicroscopy and dilated fundus exam. The diagnosis and staging of DR was established according to the Early Treatment Diabetic Retinopathy Study (31), based on the described clinical findings in this visit, combined with OCT imaging and, in selected cases, fluorescein angiography. For analysis, diabetic eyes were grouped as: NDR - eyes with no DR; NPDR – non-proliferative DR without macular edema; NPDR+DME – NPDR with macular edema; and PDR – active proliferative DR or any previous treatment with panretinal photocoagulation (PRP).

In the same visit, all study subjects were imaged with a 3D horizontal volume (12 x 9mm, 512 x 256 resolution) and a 5-line 12 mm macular cross protocol (resolution 1024 x 12, overlapping scan count 32) using Topcon® DRI OCT-1 Atlantis. All images were acquired in the morning to account for the diurnal CT variation (32).

For the purposes of this study, all medical charts were reviewed and we systematically collected: age; gender; systemic and ophthalmologic comorbidities; current medication, including eye drops; for diabetic patients - year of diagnosis of diabetes, type of diabetes, current medication for this disease, most recent HbA1c value, history of past laser treatments (focal and PRP, including number of spots and sessions, and date of last treatment), and history of any intravitreal injections (including number and date of last injection).

SS-OCT imaging analysis

Macular retinal thickness and CT were obtained with the automatic software incorporated in the SS-OCT device (Topcon® FastMap, version 9.12.003.04). User-independent thickness maps were created according to the ETDRS grid, which consists of three circles, sized according to a 1.5 mm reference disc: the central circle has a radius of 0.5 mm (1/3 disc), the inner circle of 1.5 mm (1 disc) and the outermost circle of 3 mm (2 discs). For all subjects, the position of the grid was confirmed. If it was not automatically properly centered in the fovea, manual reposition was performed with the function GRID -> REPOSITION, using the corresponding B-Scan image as reference. Figure 1 presents an example.

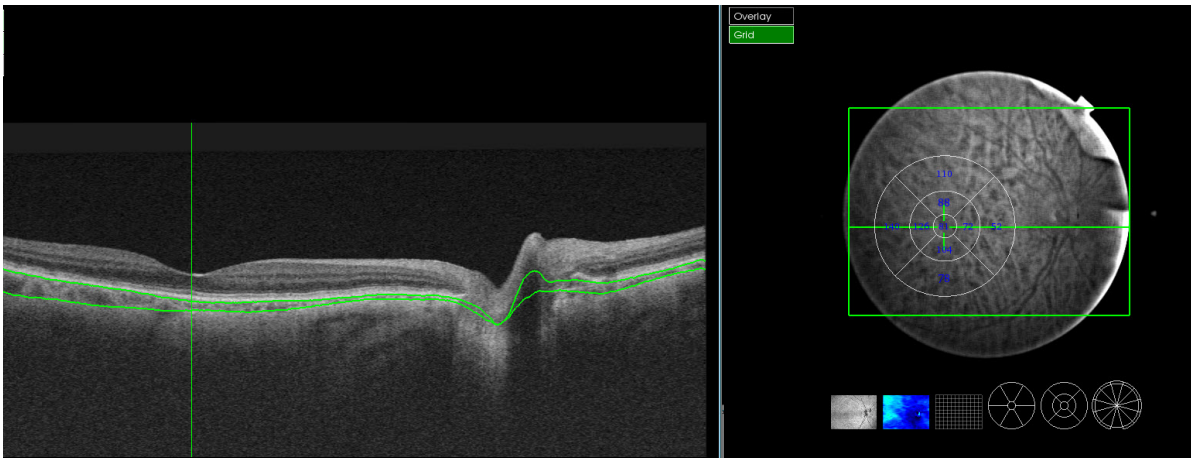

Figure 1. Manual reposition of the ETDRS grid (right) using a subfoveal B-scan as reference (left).

Similarly, retinal and choroidal automatic segmentation were also confirmed for all the obtained volume scans. Retina automatic segmentation was viewed using the function RETINA, which delineates the inner limiting membrane (ILM) and the RPE. Choroidal segmentation was viewed using the CSI function that delineates the inner and outer limits of this vascular layer - Bruch's membrane (BM) and choroidal-scleral interface (CSI), respectively. When these limits were not considered accurately placed (an example is shown in Figure 2), they were manually corrected by an experienced investigator using the functions ILM -> MODIFY, RPE -> MODIFY, BM -> MODIFY and CSI -> MODIFY and drawn accordingly to the International Nomenclature for Optical Coherence Tomography (33). The POINTING MODE in the LAYER MODIFICATION settings was the selected mode for this manual modification.

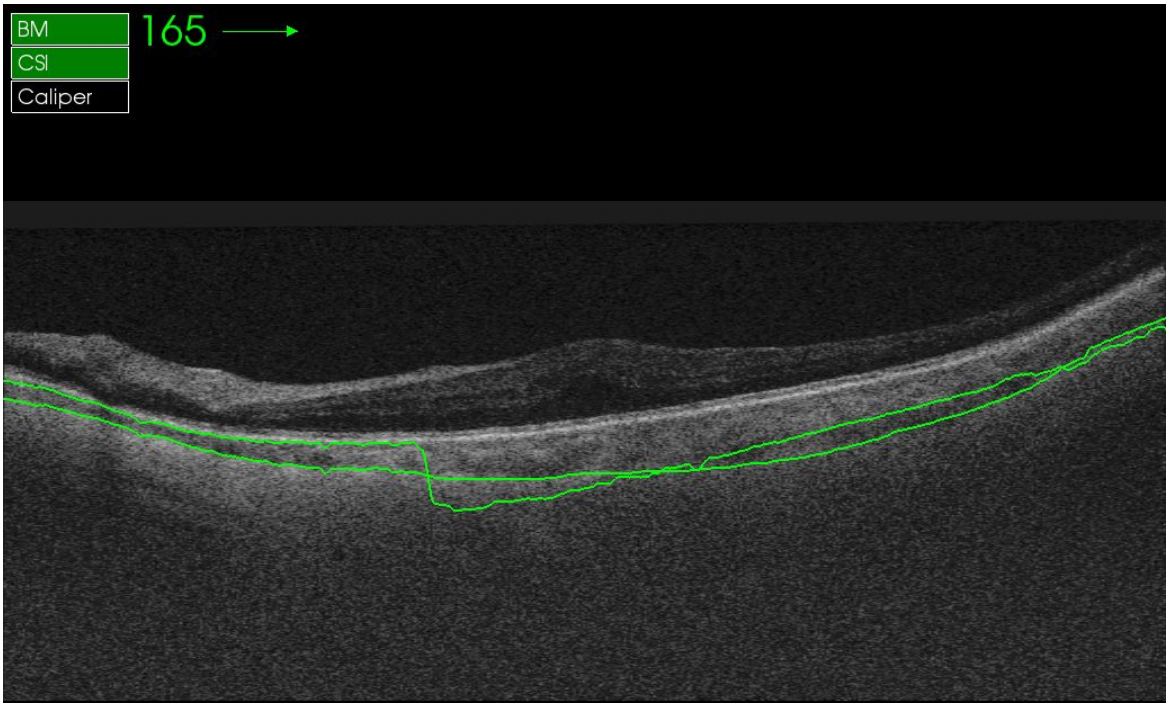

Figure 2. Example of an erroneous automatic segmentation of the choroid, which required manual correction.

Finally, the obtained retinal thickness and choroidal thickness values in the 9 ETDRS different fields were registered (central, inner superior, inner nasal, inner inferior, inner temporal, outer superior, outer nasal, outer inferior, outer temporal). For analysis, mean retinal thickness (RT) and mean CT (CT) were calculated as the mean retinal and choroidal thickness in all the ETDRS grid fields, respectively. Central choroidal thickness (CCT) was defined as the thickness in the central cell of the grid.

Statistical analysis

The study population demographics, clinical and structural characteristics were summarized with traditional descriptive methods. Considering the inclusion of 2 eyes of the same patients, to assess the influence of DR and its severity stages on CT we used multilevel mixed effect linear models. By definition, these models are appropriate for research designs where data for participants are organized at more than one level (i.e., nested data). In this study, the units of analysis were considered the eyes (at a lower level), who are nested within patients - contextual/aggregate units (at a higher level) (34). Using these models, our statistical approach was centered in two outcomes, selected due to their higher clinical relevance: mean CT and CCT. We started by univariate analyses for all the potential confounders (for example, age, gender, spherical equivalent, intraocular pressure, HbA1c value, duration of diabetes mellitus, type of diabetes, presence of high blood pressure and/or dyslipidemia, history of previous treatment with panretinal photocoagulation and/ or focal laser and their total number of spots, history of previous treatment with anti-VEGF and its total number of injections and retinal thickness) and then all the variables with a p-value $\leq$0.250 were included in the initial multivariate model. Other parameters with known clinical relevance for CT, such as gender and spherical equivalent (35–38), were also considered. A backward (step-down) elimination procedure was then used to achieve the final multivariate models presented.

All statistics were performed using Stata version 12.1 (StataCorp LP, College Station, TX, USA) and p-values < 0.05 were considered statistical significant. For the analyses comparing the different DR groups, Bonferroni corrections for multiple comparisons were performed and a p-value < 0.01 was considered significant.

Results

Subjects' demographic and clinical characteristics

We analyzed data and imaged a total of 242 eyes of 121 subjects. Seventy-three percent of them were included at CHUC (n= 178 eyes, 89 subjects) and 27% at MEEI (n= 64 eyes, 32 subjects). Considering our eligibility criteria detailed above, we excluded 32 eyes and thus considered a total of 210 for analysis. Of these, 160 (76%, 90 patients) were diabetic and 50 were control eyes (24%, 25 subjects). Figure 3 presents the flow-chart of the study.

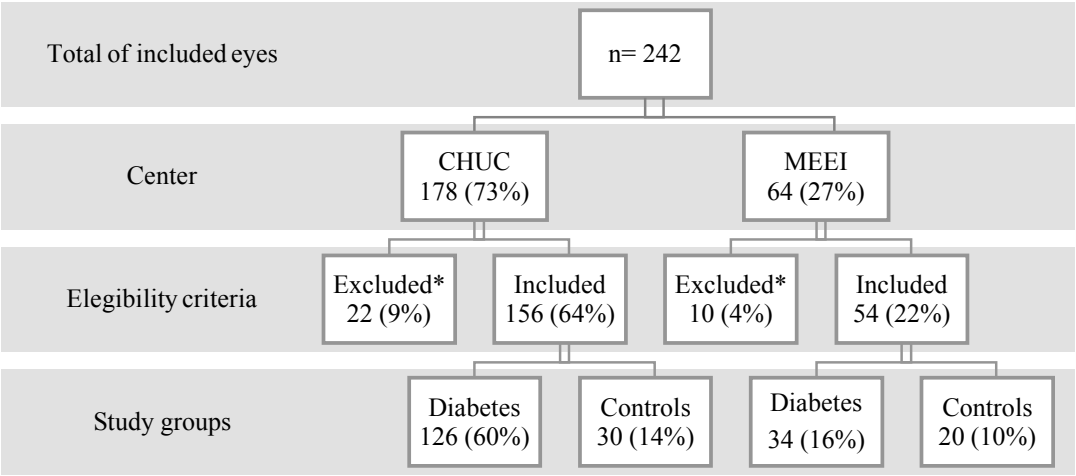

Figure 3. Flow chart of the study. *According to our exclusion criteria, at CHUC 22 eyes were excluded due to: epiretinal membrane (n=1), macular telangiectasia (n=1), amblyopia (n=1), hemianopsia (n=2), retinal vein occlusion (n=1), retinal detachment (n=3), concomitant diagnosis of age-related macular degeneration (n=2), glaucoma or ocular hypertension with a optic nerve cup to disc ratio superior to 0.6 (n=4), previous retinal surgery (n=1) or history of treatment with anti-VEGF in the last 90 days (n=6). At MEEI, 10 eyes were excluded due to: retinal detachment (n=1), previous retinal surgery (n=2), glaucoma or ocular hypertension with a optic nerve cupping ratio superior to 0.6 (n=4), choroidal detachment (n=1) or history of treatment with anti-VEGF in the last 90 days (n=2).

From now on, all the described results refer only to the final included sample size (n= 210 eyes). The mean age of our study population was 65.4 ± 9.9 years. Sixty-one percent of the included subjects were male (70 subjects) and 39% female (45 subjects).

As described in the Methods section, diabetic eyes were divided in 4 groups: 17 % (n=27) in NDR, 32% (n=51) in NPDR, 38% (n=61) in NPDR+DME and 13% (n=21) in PDR. Table 1 presents the demographic and clinical data of the entire sample size and the defined study groups.

Group	**Control**	**NDR**	**NPDR**	**NPDR+DME**	**PDR**	**Total**
Eyes, n (%)	50 (24%)	27 (13%)	51 (24%)	61 (29%)	21 (10%)	210 (100%)
Age, years mean ± SD range	 64.3 ± 12.9 43 - 95	 68.3 ± 10.0 43 - 85	 65.0 ± 9.6 40-79	 65.0 ±6.2 53-79	 65.9 ± 10.9 43-83	 65.4 ±9.9 40-95
Gender						
Female, n (%)	34 (16%)	5 (2%)	16 (8%)	22 (10%)	8 (4%)	85 (40%)
Male, n (%)	16 (8%)	22 (10%)	35 (17%)	39 (19%)	13 (6%)	125 (60%)
BCVA, logMAR mean ± SD	 0.10 ± 0.17	 0.04 ± 0.15	 0.08 ± 0.16	 0.34 ± 0.31	 0.33 ± 0.24	 0.18 ± 0.25
SE, diopters mean ± SD	 -0.38 ±1.77	 0.66 ± 1.17	 0.28 ± 1.16	 -0.15 ± 1.79	 0.10 ± 1.24	 0.03 ± 1.55
IOP, mmHG mean ± SD	 15.4 ± 2.4	 17.3 ± 3.5	 16.6 ± 2.6	 17.0 ± 2.7	 16.3 ± 3.4	 16.5 ± 2.8
HbA1c, % mean ± SD	 -	 6.38 ± 0.86	 7.90 ± 1.73	 8.09 ± 2.00	 8.55 ± 2.61	 7.93 ± 2.00
Years with DM, mean ± SD	 -	 14.8 ± 9.9	 19.9 ± 7.6	 16.9 ± 8.2	 22.3 ± 12.8	 18.2 ± 9.2
Previous treatments						
Focal laser, n (%)	-	-	3 (1%)	23 (11%)	10 (5%)	36 (17%)
Sessions, n ± SD	-	-	1.33 ± 0.58	1.89 ± 1.88	2.11 ± 0.60	1.90 ± 1.51
Total spots, n ±SD	-	-	117.5 ± 6.4	118.4 ± 105.9	126.0 ± 61.1	120.9 ± 86.6
Last, months ± SD	-	-	6.0 ± 5.0	32.6 ± 31.2	39.6 ± 54.8	32.1 ± 38.1
PRP, n (%)	-	-	-	-	15 (7%)	15 (7%)
Sessions, n ± SD	-	-	-	-	4.75 ± 3.67	4.75 ± 3.67
Total spots, n ±SD	-	-	-	-	5434 ± 3820	5434 ± 3820
Last, months ± SD	-	-	-	-	25.5 ± 26.2	25.5 ± 26.2
Injections, n (%)	-	-	-	12 (6%)	7 (3%)	19 (9%)
Injections, n ± SD	-	-	-	6.0 ± 6.0	2.57 ± 1.61	4.59 ± 4.91
Last, months ± SD	-	-	-	15.7 ± 18.9	22.4 ± 19.0	18.9 ± 18.7

Table 1. Demographic and clinical characteristics of the study population included for analysis (n= 210), organized by study group. SD - standard deviation, BCVA - best-corrected visual acuity, SE - spherical equivalent, IOP - intraocular pressure, HbA1c - glycosylated hemoglobin, DM - diabetes mellitus, PRP - panretinal photocoagulation.

A total of 19 eyes (9%) had previously received intravitreal injections, namely ranibizumab (n= 10, 53%), bevacizumab (n= 4, 21%), transeptal kenalog (n= 2, 1%) or unknown drug (n= 3, 16%). Twelve (6%) of these eyes were in the NPDR+DME group and 7 (3%) in the PDR group. In the NPDR+DME group, the mean time since the last injection was 15.7 ± 18.9, range 6-68 months. In the PDR, this interval was 22.4 ± 19.0, range 3-60 months.

Choroidal thickness measurements

Considering all the analyzed eyes, the mean CT was 190.42 ± 69.80 μm and the CCT was 200.56 ± 73.48 μm. Table 2 details the mean retinal and choroidal thickness in the different ETDRS grid sectors, according to study groups.

Grid sector	Control	NDR	NPDR	NPDR + DME	PDR
Retinal thickness					
Central	246.8 ± 29.9	257.7 ± 26.9	260.8 ± 41.1	323.0 ± 95.1	323.0 ± 77.8
Inner superior	303.9 ± 16.5	302.0 ± 24.2	306.1 ± 33.0	334.6 ± 70.4	340.5 ± 63.6
Inner nasal	301.9 ± 36.2	306.8 ± 21.7	306.2 ± 32.6	326.3 ± 62.5	345.0 ± 61.4
Inner inferior	301.0 ± 16.7	298.0 ± 23.1	305.8 ± 32.0	341.6 ± 70.8	330.1 ± 56.0
Inner temporal	293.1 ± 16.7	291.2 ± 24.6	296.7 ± 35.7	349.5 ± 98.3	338.3 ± 68.2
Outer superior	265.4 ± 15.2	267.4 ± 19.1	268.5 ± 24.7	292.3 ± 53.4	298.8 ± 65.7
Outer nasal	277.9 ± 17.1	276.9 ± 20.8	280.8 ± 21.9	298.8 ± 38.2	310.2 ± 64.1
Outer inferior	250.5 ± 36.7	253.0 ± 16.6	264.1 ± 28.6	294.3 ± 66.7	284.3 ± 49.7
Outer temporal	251.4 ± 14.8	251.4 ± 15.5	256.1 ± 32.5	309.9 ± 83.0	294.4 ± 58.1
Mean	276.9 ± 17.2	278.3 ± 18.2	282.8 ± 27.2	318.9 ± 59.5	328.9 ± 62.6
Choroidal thickness					
CCT	210.7 ± 87.4	234.4 ± 49.9	192.6 ± 75.4	194.8 ± 67.0	169.0 ± 60.4
Inner superior	225.6 ± 94.4	234.3 ± 50.0	200.0 ± 80.0	202.1 ± 71.6	167.5 ± 48.2
Inner nasal	203.8 ± 96.5	222.5 ± 58.4	184.3 ± 82.8	182.0 ± 68.6	155.1 ± 54.8
Inner inferior	192.4 ± 89.7	230.9 ± 55.4	184.9 ± 82.3	191.0 ± 66.5	157.0 ± 61.9
Inner temporal	212.7 ± 85.2	232.3 ± 47.1	192.8 ± 71.7	199.6 ± 66.5	166.0 ± 60.0
Outer superior	223.1 ± 88.9	226.4 ± 56.3	186.8 ± 74.3	196.8 ± 73.1	169.2 ± 49.3
Outer nasal	162.0 ± 91.1	176.4 ± 58.6	145.0 ± 76.9	142.2 ± 64.9	123.5 ± 45.6
Outer inferior	181.1 ± 84.5	203.2 ± 58.9	167.6 ± 77.2	181.4 ± 68.2	145.8 ± 53.0
Outer temporal	194.1 ± 78.0	211.3 ± 49.0	176.0 ± 67.8	187.3 ± 56.3	164.9 ± 53.2
Mean	200.6 ± 84.2	223.9 ± 52.4	181.1 ± 73.1	186.3 ± 61.1	157.6 ± 47.5

Table 2. Mean retinal and choroidal thickness in the different ETDRS grid sectors and mean thickness in the grid. Presented values correspond to thickness in μm and standard deviation.

As shown in Table 2, eyes of the NDR group presented the highest CT values in all sectors analyzed. Conversely, compared to the control group, all eyes with DR seem to present a lower CT. The lowest values were observed in the PDR group.

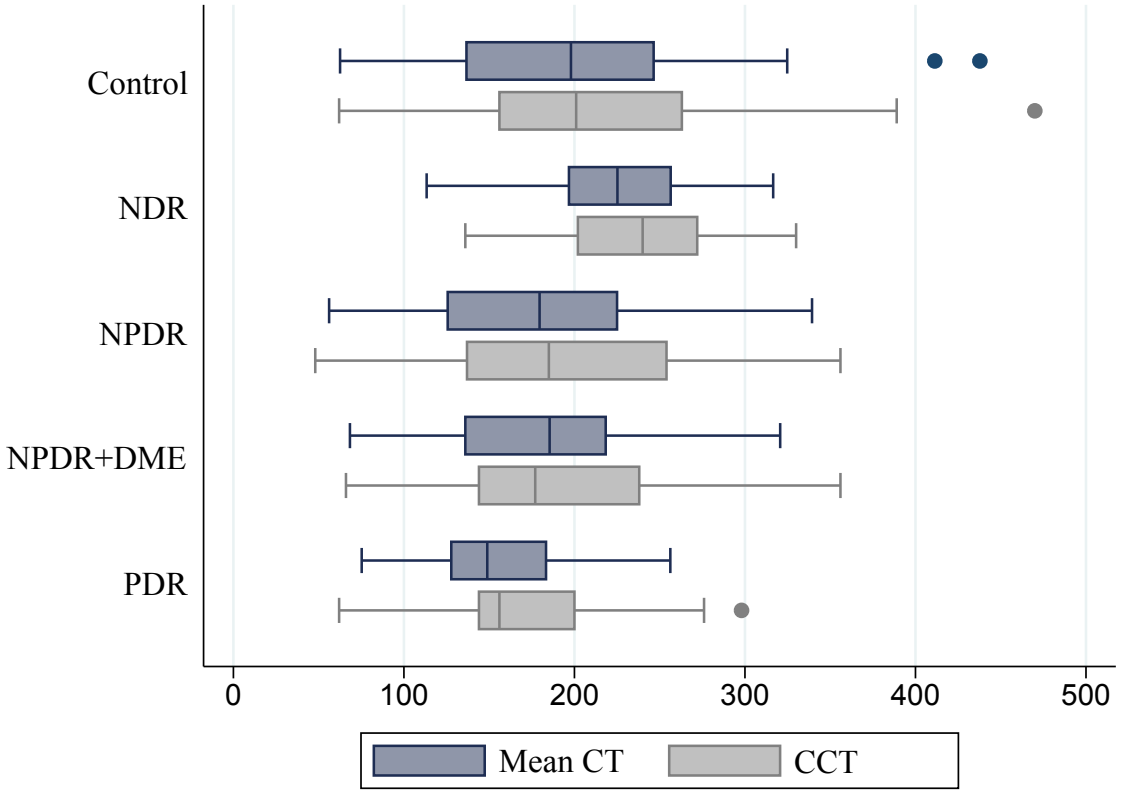

Figure 4. Box plot graph showing the two main outcomes (mean CT and CCT), organized by study group. Thickness values are presented in µm.

Univariate statistical analysis

In the univariate assessments on mean CT, the only statistically significant difference was observed when comparing the PDR group with the control group (β= -40.9, p= 0.046) – a reduction of 40.9 μm in the mean CT in the PDR group compared to the control group. In the remaining diabetic groups (NDR, NPDR and NPDR+DME) no differences were found as compared to the control group. Table 3 details these results. No statistically significant differences were found among all groups (p= 0.234, ANOVA test).

		Mean CT		**Central CT**	
		β	p-value	β	p-value
Group	NDR	0.6	0.975	11.35	0.580
	NPDR	-13.3	0.411	-13.3	0.442
	NPDR+DME	-12.5	0.432	-13.5	0.422
	PDR	-40.9	**0.046**	-50.0	**0.026**

Table 3. Univariate analysis of mean CT and CCT in the different study groups compared to the control group. Multilevel mixed linear models were used for these analyses. β – Regression coeficient. Significant p-values ($p<0.05$) are highlighted as bold.

Accounting for multiple comparisons, we did not observe any significant differences between NDR and PDR groups (p= 0.030, not significant after correcting for multiple comparisons, as described in the Methods section), NDR and NPDR+DME groups (p= 0.345), NDR and NPDR groups (p= 0.296), NPDR and NPDR+DME groups (p= 0.926) or NPDR+DME and PDR groups (p= 0.11).

Regarding central CT (CCT) similar results were found. We did not observe any statistically significant differences between the control group and NDR, NPDR and NPDR+DME groups. CCT was statistically significantly decreased in the PDR group compared to the control group (β= -50.0, p= 0.026). In the comparison among all groups, we found no significant differences (p= 0.096, ANOVA test). After accounting for multiple

comparisons, we additionally observed a statistically significant difference in CCT in the PDR group compared to the NDR group ($p= 0.008$). No significant differences were found between the other evaluated pairs.

Considering the described results, as well as the inclusion of eyes in the PDR group that had been previously submitted to PRP, we also assessed if this treatment was associated with mean CT and CCT. Our analyses (comparing PDR eyes with and without PRP) did not find any statistically significant association between PRP and CT in the PDR group ($p\geq0.200$).

Potential confounders and multivariate analysis

We further analyzed other demographic and clinical variables that could represent potential confounders. As presented in Table 4 and detailed in our Statistical Methods Section, the following variables were considered: age, gender, spherical equivalent, intraocular pressure, HbA1c value, duration of diabetes mellitus, type of diabetes, presence of high blood pressure and/or dyslipidemia, history of previous treatment with panretinal photocoagulation and/ or focal laser and their total number of spots, history of previous treatment with anti-VEGF, total number of injections and retinal thickness.

Variable	Mean CT		CCT	
	β	p-value	β	p-value
Age	-3.12	**<0.001**	-3.21	**<0.001**
Gender	-12.86	0.324	-15.11	0.260
HBP	-24.48	0.080	-22.22	0.126
Dyslipidemia	-20.04	0.150	-17.10	0.236
SE	2.55	0.334	3.55	0.284
Mean RT	0.06	0.430	-	-
Central RT	-	-	-0.02	0.784
Previous treatments				
Focal laser	2.85	0.785	-5.09	0.699
PRP	-38.63	**0.020**	-44.66	**0.027**
Anti-VEGF	-13.01	0.261	-14.35	0.349

Table 4. Univariate analysis of variables potentially related with mean CT and CCT. HBP – high blood pressure, SE – spherical equivalent, RT – Retinal thickness, PRP – panretinal photocoagulation. Multilevel mixed linear models were used for these analyses. β – Regression coeficient. Reference term for gender – male. Significant p-values ($p<0.05$) are highlighted as bold.

After assessing several multivariate models, we verified that the best, from a statistical and clinical point of view, included DR stage, adjusted for age, gender and spherical equivalent. Even after accounting for these covariates, mean CT in the PDR group remained statistically significant inferior to the control group (β= -40.0, p= 0.029), thus confirming the initial univariate results. The remaining DR stages did not present any statistically significant reductions.

Considering CCT, similar results were found. CCT was decreased in the PDR group compared to the control group (β= -50.2, p= 0.013). Additionally, significant differences were found among all the study groups for this outcome (p= 0.028, ANOVA test). Moreover, even after accounting for multiple comparisons, there was a significant difference comparing CCT in the PDR group with NDR group (p= 0.003).

		Mean CT		**Central CT**	
		β	p-value	β	p-value
Group	NDR	0.6	0.877	13.1	0.500
	NPDR	-15.3	0.311	-17.9	0.261
	NPDR+DME	-12.7	0.384	-14.7	0.334
	PDR	-42.9	**0.022**	-50.2	**0.013**
Age		-3.34	**<0.001**	-3.56	**<0.001**
Gender		-9.13	0.441	-9.34	0.440
SE		5.25	**0.047**	7.42	**0.020**

Table 5. Multivariate analysis of mean CT and CCT in the different study groups compared with control group and adjusted for age, gender and spherical equivalent. Multilevel mixed linear models were used for these analyses. β – Regression coeficient, SE – spherical equivalent. Significant p-values (p<0.05) are highlighted as bold

Discussion

We present a cross-sectional multicenter study of 210 eyes. To our knowledge, we explored for the first time the advantages of SS-OCT to analyze the choroidal thickness on diabetic retinopathy. Our results revealed that, even after correcting for confounding, only eyes with PDR presented a statistically significant reduced mean CT as compared to normal eyes without diabetes. Similar results were observed for CT in the central macular region (1mm diameter circle – CCT). In this location, PDR eyes also presented a significant reduction compared to diabetic eyes without DR lesions. All these results were confirmed on our multivariate assessments, adjusting for relevant confounders. The best statistical model included age, gender and spherical equivalent as covariates.

Our results showing a reduction of CT, as assessed by SS-OCT, in the advanced stages of DR are in agreement with most of the previous literature using the EDI protocol for SD-OCT (10–18). Unsal E et al. and Regatieri C et al. (14,16) described a reduced CT in eyes with DME or PDR when compared to controls. Gerendas B et al. (15) used an automated algorithm to analyze CT in the area of the ETDRS grid and found a thinner choroid in diabetic patients with DME compared with controls. Sudhalkar A et al. (18) described a progressive thinning of CT with increasing severity of DR. Altogether, these results suggest that a diabetic choroidopathy probably coexists with diabetic retinopathy. However, whether choroidal abnormalities are a cause or a consequence of the characteristic retinal pathology of diabetes remains unclear. In our study, we did not find any statistically significant decrease of CT in diabetic eyes without DR or in the early stages of the disease, as compared to normal controls. However, previous groups described significant reductions even in these stages (10,11,18). This could suggest that choroidal pathology might even emerge before retinal vessels' abnormalities are apparent and that OCT could allow an earlier diagnosis than the

currently performed using the conventional methods (fundus exam and fluorescein angiography). The lack of significant differences between diabetic eyes without DR and eyes with the non-proliferative stage compared to controls observed in our study may be related to our relatively limited sample size (27 and 51 eyes, respectively) or to a true absence of reduction.

Despite the described agreement of most literature, some groups (19–21) reported opposite results, arguing that diabetic patients present increased choroidal thickness as compared to normal healthy controls. The observed differences among studies are probably linked with the different study designs (for example, some studies included a real control group without diabetes but others (21) did not), the lack of power of some studies (for example, Xu J et al. (20) only included 26 DR eyes, despite the total sample size of 2041 eyes) and also the methods used to analyze choroidal thickness. Most of the previous reports (14,16) only evaluated choroidal thickness in the subfoveal region or in regular intervals of 500 μm within specific B-scans. In our study, we were able to explore the advantages of the software provided by Topcon® DRI SS-OCT and thus to obtain choroidal thickness maps of the entire macular region, according to the ETDRS grid subfields. Despite the need for manual correction in some cases, these maps are much more informative than the evaluation of single arbitrary points. Actually, we were able not only to consider as an outcome the CT in the central macular 1mm diameter circle (CCT), but also the mean CT, which corresponds to the mean of all the choroidal thicknesses within the ETDRS grid. To our knowledge, none of the previous studies on diabetic retinopathy has done such evaluation.

Moreover, in our study we were also able to benefit of the advantages of SS-OCT compared to SD-OCT, namely its higher resolution and better penetration in the RPE due to the higher wavelength, and thus a clearer visualization of the choroidal boundaries (23,24).

Also of note is the statistical approach used in this study. We were able to include data of two eyes of the same patients, as we used multilevel mixed models (34) able to account for nested data, as described in our methods section. These models also enabled us to correct for confounding, and therefore to present more robust conclusions. As presented, and according to the previous literature (35–38), age and spherical equivalent were significantly related with choroidal thickness. Even when the different study groups present similar parameters and we apply exclusion criteria to account for potential confounders (such as the limit for spherical equivalent), including these covariates in the assessed models increases the confidence of the provided results.

Limitations and future directions

Despite the multisite nature of this study and the attempt to include the highest possible sample size, one of its main limitations is the limited number of eyes included in some of the groups. This might have affected our results, namely in what concerns the PDR group and the assessment of a potential influence of previous panretinal photocoagulation. Of note, is that, despite not being significant, our analysis of PRP eyes showed that those that received this treatment presented a trend to an even lower macular CT (β= -30.8, p= 0.200). However, the aim of this study was not to analyze the effects of treatment on CT and its design is not appropriate for this purpose. Our group is already developing a prospective study using SS-OCT to explore these questions, including the effect of anti-VEGF injections on CT of diabetic eyes.

Conclusion

Using swept-source OCT we observed that macular choroidal thickness is decreased in the proliferative stages of diabetic retinopathy, as compared to controls and diabetic eyes without diabetic retinal abnormalities. Understanding choroidal abnormalities can improve our knowledge on the mechanisms behind DR and, in the future, might improve our assessment and staging of this condition.

Acknowledgements

I would like to thank to my thesis' advisor and tutor - Rufino Silva, MD PhD - for opening this door to me and for his guidance. Without Prof. Rufino Silva this work would not had been possible at all.

I would also like to thank my mentor and friend Inês Laíns, MD for her great availability and patience to teach me and for her invaluable contribution to this essay. She consistently allowed me to grow up and to make this thesis my own work, but always lead me in the right direction whenever we felt I needed her support and advice. Ines also allowed me to work with the Retina Service of Massachusetts Eye and Ear Infirmary (MEEI) and the inclusion of their data, which I would also like to thank.

I thank also the Association for Innovation and Biomedical Research on Light and Image (AIBILI), Coimbra, Portugal and all their team for supporting me in every difficulty I faced and for creating the synergy that this work required. My special gratitude is to Dr.ª Patrícia Barreto and Ana Rita Santos for their essential time and collaboration.

I sincerely hope that we can keep working together.

Finally, I must express my profound gratitude to my parents, Fernando Alegre Marques and Ana Maria Peixoto, to the rest of my family and to my girlfriend Carolina Figueiredo for providing me with unfailing support and continuous encouragement throughout my studies and through the process of researching and writing this essay.

João Heitor Marques

References

1. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract. 2014;
2. Yau JWY, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care. 2012;
3. Antonetti DA, Klein R, Gardner TW. Diabetic retinopathy. N Engl J Med. 2012 Mar;
4. Curtis TM, Gardiner T a, Stitt a W. Microvascular lesions of diabetic retinopathy: clues towards understanding pathogenesis? Eye (Lond). 2009;
5. Cunha-Vaz J, Faria de Abreu JR, Campos AJ. Early breakdown of the blood-retinal barrier in diabetes. Br J Ophthalmol. 1975;
6. Nickla DL, Wallman J. The multifunctional choroid. Prog Retin Eye Res. 2010;
7. Hidayat AA, Fine BS. Diabetic choroidopathy. Light and electron microscopic observations of seven cases. Ophthalmology. 1985;
8. Nagaoka T, Kitaya N, Sugawara R, Yokota H, Mori F, Hikichi T, et al. Alteration of choroidal circulation in the foveal region in patients with type 2 diabetes. Br J Ophthalmol. 2004;
9. Spaide RF, Koizumi H, Pozonni MC. Enhanced Depth Imaging Spectral-Domain Optical Coherence Tomography. Am J Ophthalmol. 2008;
10. Querques G, Lattanzio R, Querques L, Turco C Del, Forte R, Pierro L, et al. Enhanced Depth Imaging Optical Coherence Tomography in Type 2 Diabetes. Invest Ophthalmol Vis Sci. 2012;
11. Galgauskas S, Laurinavičiūtė G, Norvydaitė D, Stech S, Ašoklis R. Changes in choroidal thickness and corneal parameters in diabetic eyes. Eur J Ophthalmol. 2015 Sep 14;

12. Lee HK, Lim JW, Shin MC. Comparison of choroidal thickness in patients with diabetes by spectral-domain optical coherence tomography. Korean J Ophthalmol. 2013;
13. Vujosevic S, Martini F, Cavarzeran F, Pilotto E, Midena E. Macular and Peripapillary Choroidal Thickness in Diabetic Patients. Retina. 2012;
14. Regatieri C V, Branchini L, Carmody J, Fujimoto JG, Duker JS. Choroidal thickness in patients with diabetic retinopathy analyzed by spectral-domain optical coherence tomography. Retina. 2012;
15. Gerendas BS, Waldstein SM, Simader C, Deak G, Hajnajeeb B, Zhang L, et al. Three-dimensional automated choroidal volume assessment on standard spectral-domain optical coherence tomography and correlation with the level of diabetic macular edema. Am J Ophthalmol. 2014 Nov;
16. Unsal E, Eltutar K, Zirtiloğlu S, Dinçer N, Ozdoğan Erkul S, Güngel H. Choroidal thickness in patients with diabetic retinopathy. Clin Ophthalmol. 2014;
17. Wang S, Lin S, Zheng Y, Di F, Cao X, Liu C, et al. [Association of choroidal thickness with diabetic retinopathy at different stages]. Zhonghua Yi Xue Za Zhi. 2015 Aug;
18. Sudhalkar A, Chhablani JK, Venkata A, Raman R, Rao PS, Jonnadula GB. Choroidal thickness in diabetic patients of Indian ethnicity. Indian J Ophthalmol. 2015 Dec;
19. Ferreira J, Vicente A, Anjos R, Costa L, Santos A, Cunha J-P, et al. Choroidal thickness in diabetic patients without retinopathy. Invest Ophthalmol Vis Sci. 2015;
20. Xu J, Xu L, Du KF, Shao L, Chen CX, Zhou JQ, et al. Subfoveal choroidal thickness in diabetes and diabetic retinopathy. Ophthalmology. 2013;
21. Kim JT, Lee DH, Joe SG, Kim JG, Yoon YH. Changes in choroidal thickness in relation to the severity of retinopathy and macular edema in type 2 diabetic patients. Investig Ophthalmol Vis Sci. 2013;

22. Michalewski J, Michalewska Z, Nawrocka Z, Bednarski M, Nawrocki J. Correlation of Choroidal Thickness and Volume Measurements with Axial Length and Age Using Swept Source Optical Coherence Tomography and Optical Low-Coherence Reflectometry. Biomed Res Int. 2014;
23. Hirata M, Tsujikawa A, Matsumoto A, Hangai M, Ooto S, Yamashiro K, et al. Macular choroidal thickness and volume in normal subjects measured by swept-source optical coherence tomography. Investig Ophthalmol Vis Sci. 2011;
24. Copete S, Flores-Moreno I, Montero J a, Duker JS, Ruiz-Moreno JM. Direct comparison of spectral-domain and swept-source OCT in the measurement of choroidal thickness in normal eyes. Br J Ophthalmol. 2014;
25. Capuano V, Souied EH, Miere A, Jung C, Costanzo E, Querques G. Choroidal maps in non-exudative age-related macular degeneration. Br J Ophthalmol. 2015 Sep 7;
26. Jirarattanasopa P, Ooto S, Tsujikawa A, Yamashiro K, Hangai M, Hirata M, et al. Assessment of macular choroidal thickness by optical coherence tomography and angiographic changes in central serous chorioretinopathy. Ophthalmology. 2012;
27. Ellabban AA, Tsujikawa A, Matsumoto A, Ogino K, Hangai M, Ooto S, et al. Macular choroidal thickness and volume in eyes with angioid streaks measured by swept source optical coherence tomography. Am J Ophthalmol. 2012;
28. Park HYL, Shin HY, Park CK. Imaging the posterior segment of the eye using swept-source optical coherence tomography in myopic glaucoma eyes: Comparison with enhanced-depth imaging. Am J Ophthalmol. 2014;
29. Tan CSH, Ngo WK, Cheong KX. Comparison of choroidal thicknesses using swept source and spectral domain optical coherence tomography in diseased and normal eyes. Br J Ophthalmol. 2014;
30. Ueda-Arakawa N, Ooto S, Ellabban AA, Takahashi A, Oishi A, Tamura H, et al.

Macular choroidal thickness and volume of eyes with reticular pseudodrusen using swept-source optical coherence tomography. Am J Ophthalmol. 2014;

31. Early Treatment Diabetic Retinopathy Study Research Group. Early photocoagulation for diabetic retinopathy. ETDRS report number 9. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology. 1991;
32. Han YS, Lim H Bin, Lee SH, Kim JY. Diurnal Variation in Choroidal and Retinal Thickness of the Early Treatment of Diabetic Retinopathy Study Macular Subfields Determined Using Swept-Source Optical Coherence Tomography. Ophthalmologica. 2015;
33. Staurenghi G, Sadda S, Chakravarthy U, Spaide RF. Proposed lexicon for anatomic landmarks in normal posterior segment spectral-domain optical coherence tomography: The INOCT consensus. Ophthalmology. 2014;
34. Burton P, Gurrin L, Sly P. Extending the simple linear regression model to account for correlated responses: an introduction to generalized estimating equations and multi-level mixed modelling. Stat Med. 1998 Jun;
35. Wang J, Gao X, Huang W, Wang W, Chen S, Du S, et al. Swept-source optical coherence tomography imaging of macular retinal and choroidal structures in healthy eyes. BMC Ophthalmol. 2015 Jan;
36. Wakatsuki Y, Shinojima A, Kawamura A, Yuzawa M. Correlation of Aging and Segmental Choroidal Thickness Measurement using Swept Source Optical Coherence Tomography in Healthy Eyes. PLoS One. 2015 Jan;
37. Fujiwara T, Imamura Y, Margolis R, Slakter JS, Spaide RF. Enhanced depth imaging optical coherence tomography of the choroid in highly myopic eyes. Am J Ophthalmol. 2009 Sep;
38. Sanchez-Cano A, Orduna E, Segura F, Lopez C, Cuenca N, Abecia E, et al. Choroidal

thickness and volume in healthy young white adults and the relationships between them and axial length, ammetropy and sex. Am J Ophthalmol. 2014 Sep.

Annexes

Abstract

Title: Choroidal thickness in diabetic retinopathy assessed with swept-source optical coherence tomography.

Background and purpose

Diabetic retinopathy (DR) is the leading cause of blindness in the working-age populations. Recent evidence suggests an involvement of the choroidal vessels, suppliers of the outer retina, in the pathogenesis of DR. Enhanced depth imaging protocol (EDI) for spectral-domain optical coherence tomography (OCT) has been used to evaluate choroidal thickness (CT) in DR with controversial results. Swept-source OCT (SS-OCT) is a new device with properties that allow an enhanced visualization of the choroid. This study aimed to compare the CT of diabetic eyes in different stages with normal controls using SS-OCT.

Methods

Multicenter, prospective, cross-sectional study. Diabetic eyes with and without DR were compared with healthy age-matched controls. Exclusion criteria included other retinal diseases, spherical equivalent ≥ 6 diopters and recent intra-vitreal injections or laser treatments. Diabetic eyes were stratified into 4 groups: diabetes without DR (NDR), non-proliferative DR (NPDR), NPDR with diabetic macular edema (NPDR+DME) and proliferative DR (PDR). All participants underwent complete ophthalmologic exam and bilateral SS-OCT imaging with Topcon® DRI OCT. CT in the ETDRS grid was obtained with the software available in the device. For the purposes of this study, mean CT was calculated as the mean value within the ETDRS grid and central CT (CCT) as the value for the central 1mm. Univariate and multivariate multilevel mixed linear models (including correlated measures between 2 eyes) were performed.

Results

Fifty eyes of 25 healthy subjects and 160 eyes of 90 diabetic patients were included (no-DR n= 27; NPDR n= 51; NPDR + DME n= 61; PDR n= 21). Mean age was 64.4±12.9 years for the control group and 65.7±8.7 years for the diabetic group. Mean CT in the control group was 200.6±84.2 μm and in the diabetic groups: no-DR 223.9±52.4 μm; NPDR 181.1±73.1 μm; NPDR+DME 186.4±61.1 μm; PDR 151.6±47.5 μm. Mean CT (β=-40.9, p=0.046) and CCT (β=-50.0, p=0.026) were significantly reduced in PDR as compared to control eyes in the univariate analysis. After adjusting for age, gender and spherical equivalent, mean CT (β=-42.9, p=0.022) and CCT (β=-50.2, p=0.013) were significantly lower compared to the control group. The same was observed for CCT (p=0.003) when comparing PDR eyes to diabetic eyes without DR after adjusting for the same confounders.

Conclusions

SS-OCT demonstrates a statistically significant reduction in choroidal thickness in eyes with proliferative diabetic retinopathy compared with eyes of diabetics without retinopathy as well as age-matched controls.

Keywords: diabetic retinopathy, choroid, optical coherence tomography