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Evaluation of choroidal thickness in different stages of diabetic retinopathy

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Abstract

Purpose: to assess changes of choroidal thickness (CT) in diabetic patients in different stages of diabetic retinopathy and diabetic macular edema using spectral domain optical coherence tomography.

Patients and methods: One hundred and sixty three subjects were enrolled: 113 diabetic patients (186 eyes) and 50 normal individuals as controls. Eyes were divided into two groups according to age; group A from 35 to 50 years and group B from 51 to 65 years. Both groups classified according to retinopathy grade: DR1 (no DR), DR2 (mild- moderate nonproliferative diabetic retinopathy (NPDR)), DR3 (severe NPDR), DR4 (untreated proliferative diabetic retinopathy (PDR)) and DR5 (previously treated PDR).

All participants underwent full ophthalmic examination, stereoscopic color fundus photography, and spectral domain optical coherence tomography (RS-3000; Nidek). Spectral domain optical coherence tomography examination consisted of linear scans, 6 mm in length, centered onto the fovea, and macula multi scans. Choroidal thickness was measured manually at the fovea and at 500, 1000, and 1500 um distance along all scans in the macula.

Results: Mean age was not significantly different between patients with diabetes and controls. In the macular area, CT was significantly lower in the nasal quadrant versus all other quadrants (P, 0.0001), in both groups. No significant CT difference was found between controls and diabetic eyes without detectable DR. Diabetic macular edema did not influence CT. In early NPDR (mild and moderate) and treated PDR the mean choroidal thickness was significantly decreased than control. In severe NPDR and treated PDR, the mean subfoveal choroidal thickness was not different than control. There was a statistically significant increase in choroidal thickness in PDR when compared with the mild NPDR group (P=0.027). DME was associated with a non-statistically significant increase in choroidal thickness compared with eyes without DME (P=0.13).

Conclusion: Choroidal thickness is reduced in diabetic eyes. Subfoveal choroidal thickness increased with the severity of diabetic retinopathy but showed no statistically significant association with the presence of DME. Spectral domain optical coherence tomography clearly confirms in vivo previously reported histopathologic observations. The role of choroid in the pathophysiology of DR needs to be adequately investigated.

Key words: diabetic retinopathy, choroid, choroidal thickness, OCT.

Introduction

Diabetic retinopathy (DR) is the leading cause of blindness among working age adults.[1] Retinal vascular and neural alterations are considered the main pathologic phenomena of DR[1]. Increased retinal vascular permeability because of alterations of the blood– retina barriers caused by tight junction disassembly and endothelial cell mediated leukostasis are considered as major mechanisms for retinal edema and ischemia [(2,3)].

Clinical and histopathologic findings suggest that vascular changes may also affect the choroid in patients with diabetes [(4)]. These findings include obstruction of the choriocapillaris, vascular remodeling, choroidal aneurysms, and choroidal neovascularization [(5–8)]. Because the choroid provides oxygen and nutrients to the outer retina, and it maintains the highly metabolically active photoreceptor cells,

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choroidal hypoperfusion could result in outer retina dysfunction [9].

Clinical evaluation of choroid is usually performed by means of indocyanine green angiography, an invasive procedure, or contact B-scan ultrasonography, but neither allows for an accurate cross-sectional imaging [4, 10, 11]. Recently, with the advent of spectral domain optical coherence tomography (SD-OCT), cross-sectional imaging of the choroid has been obtained [12–17] . Different methods have been proposed to obtain an adequate visualization of the choroid with SD-OCT, in normal and pathologic eyes. These include placing SD-OCT closer to the eye to obtain inverted images or the use of research SD-OCT devices, which use a broadband light source in a deeper infrared region compared with conventional devices [12–15, 17–19]. Significant variability of choroidal thickness (CT) has been reported in controls, mainly depending on age and refractive error [13, 16, 17]. In diabetic patients, macular CT was measured with 3-dimensional 1,060-nm SD-OCT and correlated to the degree of diabetic maculopathy [19].

The aim of this study was to evaluate CT in diabetic subjects, and to correlate it to the presence and severity of DR.

Subjects and Methods

One hundred and eighty six eyes of 113 diabetic participants were included in this observational case series study. Fifty normal volunteers (100 eyes) served as controls. All participants were recruited from outpatient Clinic at the Department of Ophthalmology, Al-Zahraa University Hospital, from September 2015 to September 2017. The inclusion criteria were men or women with Type 1 or 2 diabetes mellitus, any stage of DR (from absent to proliferative DR), refractive error within ±5 diopters (D), and intraocular pressure within normal limits. The exclusion criteria were any type of previous retinal treatment (macular or peripheral laser photocoagulation less than 1 year, vitrectomy, intravitreal steroids, and/or antiangiogenic drugs), significant media opacities that precluded fundus examination or imaging, and treated or untreated ocular hypertension and glaucoma. Hemoglobin A1c was done for all diabetic participants for detection of diabetes control. A written consent form was obtained from all patients as well as the approval from our institutional ethics committee. The study was conducted in accordance with the tenets of the Declaration of Helsinki. Each subject underwent a complete ophthalmic examination, with determination of best-corrected visual acuity, anterior segment examination, Goldmann applanation tonometry, indirect ophthalmoscopy, and slit lamp fundus biomicroscopy. Then, SD-OCT and fundus photography were obtained. Fluorescein angiography was performed in patients with diabetic macular edema or suspected wide ischemic areas.

Study Procedures

Visual acuity: Best-corrected distance visual acuity for each eye was measured using Landlots broken rings and converted to log MAR acuity.

Stereoscopic fundus photography and fluorescein angiography: Color stereoscopic fundus photographs (seven Early Treatment Diabetic Retinopathy Study fields) and fluorescein angiography (when required) were taken using the same TOPCON TRC 50IA 50° fundus camera (Topcon,Tokyo, Japan). Diabetic retinopathy was graded as no DR, nonproliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR).

Spectral domain optical coherence tomography: All eyes were examined with SD-OCT (Retinascan RS-3000; NIDEK, Gamagori, Japan). Each eye was examined, after pupillary dilation, macula line and maculamulti scans, 9 mm in length, were centered onto the fovea. The scans consisted of 1,024 A-scan with high-definition (50 HD) frame enhancement software. This instrument has a light source of 880-nm wavelength. To improve choroidal visualization, each image consisted of 50 averaged B-scans in a single raster line scan, and the optical coherence tomographic device was positioned close to the eye in

order to visualize the image on the top of the monitor (to be in closer proximity to the zero-delay line) in a standard manner (un inverted image). This instrument does not generate inverted images like other SD-OCT machines. Each high definition line image needed to have at least 6 of 10 (maximum) intensity score. Moreover, increasing the luminosity of the images and the luminosity of the monitor and decreasing the contrast of the monitor allowed the graders to better visualize choroidal details. It differs from the recently developed enhanced depth imaging method for choroidal visualization (Heidelberg Spectralis; Heidelberg Engineering, Heidelberg, Germany), which uses an inverted representation of the fundus of each patient and a 100 average B-scans. In this study, we used RS- 3000 SD-OCT with direct fundus representation and 50 averaged B scans.

CT was measured as the perpendicular distance between the hyperreflective outer border of the retinal pigment epithelial layer (automatically detected by the instrument) and the sclero–choroidal interface, manually drawn, independently (Figure 1).

CT was measured at 13 points for each patient: the foveal center (CCT), at 500, 1000, and 1500 um temporal (T) (T500, T1000, and T1500), nasal (N) (N500, N1000, and N1500), superior (S) (S500, S1000, and S1500), inferior (I) (I500, I1500, and I1500) using the image j software. Foveal retinal thickness was determined automatically in all eyes.

Statistical Methods

All data were collected and analyzed statistically using SPSS for Windows, version 22. Qualitative data were expressed as mean and SD. One-way analysis of variance was used to compare the mean values of CT between different groups.

Pearson's correlation coefficient (r) was performed to test the correlation between CT and age. Linear regression analysis was used to assess regression of CT with age. The significance of the data was determined using the probability (P). P value greater than 0.05 was considered non-significant, P value of at least 0.05 was considered significant, and P value of at least 0.01 was considered highly significant.

For each subject, CT of different parts of the macula was computed as follows: the individual value of each one of the 13quantified points; the mean value of subfoveal CT (SFCT), the mean perifoveal CT (PFCT) each determined as the average of 12 points; and overall mean value of the measurements from all points mean CT.

Results

Of 163 enrolled subjects, 110 were women and 53 men. Mean age of patients with diabetes was 56.13 ± 6.93 years (range 35–65 years); mean age of controls was 51.07 ± 10.37 years (range 35–65 years). Seventy five patients (66.37%) were insulin dependent diabetes mellitus and 38 (33.62%) were non-insulin dependent diabetes mellitus. Fifty patients with mean hemoglobin A1c was 5.93% ± 1.5 %(controlled DM) and 63 patients with mean hemoglobin A1c was 12.8% ± 1.3% (uncontrolled DM). Thirty nine eyes were graded as no DR, 59 eyes as mild/moderate NPDR, 33 eyes as severe NPDR , 30 eyes as untreated PDR and 25 eyes as treated PDR.

There was no significant difference in age between controls and patients with diabetes (P = 0.22). All demographic characteristics of the, included patients (eyes) are shown in Table 1.

There was a significant decrease of CT with increasing age of patients both in the control group and patients with diabetes (P=0.001).

Choroid was thinner in the nasal macula when compared with the S, T, and I macula in all groups (controls, no DR, NPDR, and PDR; P, 0.0001).

For all study cases there was statistically significant decrease of mean choroidal thickness (CCT+PFCT) measurements at mild/moderate NPDR (291.63um, P =.005) and treated PDR (281.82um,P=0.002) than control (318.01um).

In cases with age ≤ 50 years there was statistically significant decrease of mean central choroidal thickness measurements in diabetic patients with no retinopathy, diabetic

patients with mild/moderate NPDR and treated PDR stages than control (Table 2/ Figure 2).

In cases with age ≥51 years there was statistically significant decrease of mean central choroidal thickness measurements at mild/moderate NPDR and treated PDR than control. For all study cases there was statistically significant decrease of mean central choroidal thickness measurements at mild/moderate NPDR and treated PDR stages in comparison with control (Table 3/ Figure 3).

For all study cases there was statistically significant decrease of mean perifoveal choroidal thickness measurements at mild/moderate NPDR and treated PDR in comparison with control.

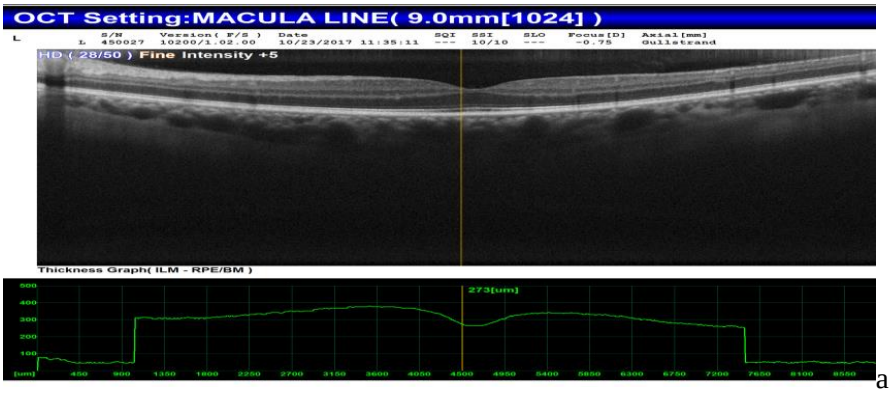
Mean retinal thickness in the fovea was 217.03 ± 16.03um in the control group, 257 ± 82.6 um in no DR group, 252.77± 95.6 um in mild/moderate NPDR group, 374.52 ±157.95um in severe NPDR group, 338.39 ± 118.37um in untreated PDR group and 292.69 ± 14.8 um in treated PDR group.

There was no statistically significant difference of mean CT & CCT between diabetic patients with and without DME (P> 0.05) (Table 4/ Figure 4).

There was statistically no significant difference of mean CT and both controlled/uncontrolled DM (P> 0.05) and presence or absence of hypertension (P> 0.05). There was no significant correlation between mean foveal choroidal and retinal thickness in patients with diabetes (R = 0.36, P = 0.068). There was no significant correlation between mean foveal choroidal and both duration of DM (R = -0.148, P = 0.165).

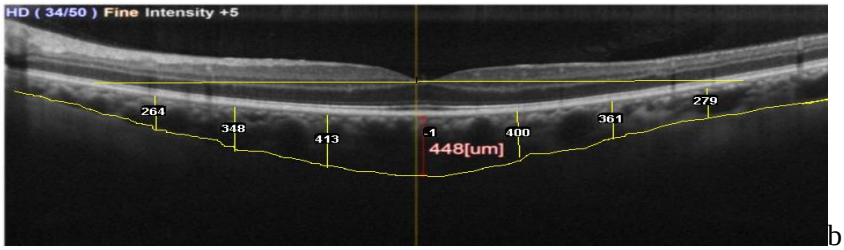
Table 1 : Demographic classification of the study group (n= 163)

Variables	Cases	Controls
Number of participants	113	50
Sex Male Female	 30 83	 23 27
Age (mean±SD) (years)	56.13±6.93	51.07±10.37
Eye (OD vs. OS) (%)	47.84 OD vs. 52.2 OS	50 OD vs. 50 OS
Control of DM (Patients, n %) Controlled Uncontrolled	 63(55.75%) 50(44.24%)	 - -
Hypertension (Patients, n %) Hypertensive No hypertension	 44(38.93%) 69(61.06%)	 - -



a

Figure 1: a) SD-OCT. A macula line scans in the macula showing choroidal structure. b) Demonstration of normal choroidal thickness measured at 500 µm intervals, up to1500 µm temporal and nasal to the fovea.



b

Table 2: Comparison of mean OCT measurements between normal controls ≤ 50 years and cases at different stages of DR by ANOVA test.

Mean	Normal ≤ 50y	DR 1	DR 2	DR 3	DR 4	DR 5
CFT	217.72	220	229.53	278.5	354.85	209
I 1000	318.52	294.87	295.06	306.9	294.14	289.5
I 1500	302.68	278.81	281.06	288.2	282.7	270.5
I 500	334.00	317.12	315.93	327.8	307.42	301.5
N 1000	321.12	297.43	300.2	318.2	300	246.5
N 1500	304.24	283.62	285.2	302	278.57	241
N 500	334.84	314.81	319.26	339.6	307.28	278.5
CCT	374.56	352.25	324.45	360.8	365.06	319.5
S 1000	327.84	304.5	310.7	325.7	292.28	306.5
S 1500	313.16	290.43	297.8	308.2	277	277
S 500	342.12	324.13	329.2	345.2	316.57	313.5
T 1000	327.76	307.25	310.8	331.3	314	282
T 1500	314.32	292.56	297	310.9	298	262.5
T 500	327.64	327	328.86	348.2	324.14	293
P.value	<0.0001		0.0002	1.0000	1.0000	0.0132

Table 3: Comparison of mean OCT measurements between normal controls ≥ 51 years and cases at different stages of DR by ANOVA test.

Mean	Normal ≥51y	DR 1	DR 2	DR 3	DR 4	DR 5
CFT	214.98	213.38	258.11	420.23	331.18	298.4
I 1000	283.04	295.55	268.70	278.48	282.83	268.69
I 1500	300.08	277.86	249.74	261.84	266.22	251.88
I 500	321.44	316.10	289.81	303.13	300.61	289.56
N 1000	300.40	296.00	268.29	287.74	283.48	263.88
N 1500	284.20	279.86	248.92	267.58	264.39	247.19
N 500	323.24	315.62	290.41	311.29	306.04	288.31
CCT	341.8	348.07	324.35	339.48	337.44	328.13
S 1000	307.84	305.35	282.49	294.00	293.78	289.56
S 1500	292.68	290.69	267.22	280.48	279.52	272.38
S 500	333.24	324.97	301.52	314.87	315.04	309.19
T 1000	306.64	306.62	283.46	300.00	304.44	275.69
T 1500	298.96	290.03	267.81	283.84	290.96	261.19
T 500	332.06	324.61	296.32	307.57	311.82	300.4
P.value	1.0000		0.0066	1.0000	1.0000	0.0127

Table 4: Relation between presence of DME and CCT measurement by one way ANOVA test in all cases (n=133 cases, 186 eyes).

CCT	**No DME** (n=80 eyes)	**DME** (n=106 eyes)	**P.value**
Mean	332.300	332.670	= 0.950
SD	41.5294	38.4910	

Discussion

In this study, we found, by means of SD-OCT, that CT decreases in diabetic eyes compared with controls. In diabetic eyes with no clinical signs of DR, CT does not significantly differ from controls. In mild/moderate NPDR the CT significantly

decreased. The mean choroidal thickness increased with the severity of diabetic retinopathy from severe NPDR to untreated PDR but showed no statistically significant association with the presence of DME. The mean CT decreased in PRP-treated eyes.

The choroid comprises blood vessels, melanocytes, fibroblasts, resident immunocompetent cells, and supporting collagenous and elastic connective tissue.

Thus, its main structure is vascular with some connective tissues [21] .Therefore; its thickness is probably related to the diameter of the choroidal vessels, the number of large choroidal vessels, and the amount of connective tissue. Hence, relative vasoconstriction or reduced perfusion pressure may account for reduced endoluminal volume and apparent thinning.

Our findings might reflect an increased production of VEGF or other cytokines mediating choroidal vasodilation and elevation in choroidal blood flow, which could explain relative increase in the thickness of the choroidal vascular layer, especially in patients with severe NPDR or PDR as compared with early stages of diabetic retinopathy. The choroidal thinning after laser PRP, possibly due to the down regulation of VEGF and decreased blood flow with subsequent ischemic atrophic change.

Kim et al. [20] found that choroidal thickness is closely correlated with the stage of DR, and with the degree or type of DME. Progressive thickening of the choroidal layer with the progression of DR, or the development of DME, may reflect the concurrent progression of diabetic choroidopathy. Likewise, choroidal thinning was observed as the regression of advanced DR following PRP.

We found that the choroid is thinner in the nasal portion of the macula, especially with increasing distance from the fovea, both in controls and in patients with diabetes. This confirms recently published data about CT in the macular area in normal and diabetic subjects using different SD-OCT devices [13, 16, 18, 22]. Margolis and Spaide [13] showed, by means of inverted images using Heidelberg Spectralis SD-OCT (Heidelberg Engineering), that CT decreases rapidly in the nasal direction, up to 3 mm from the fovea.

Different authors have also reported that CT is an age-dependent parameter [13, 16, 17, 23]. A significant reduction in CT with increasing age is also confirmed by this study, both in normal and diabetic subjects. Margolis and Spaide [13] found a 16 um decrease of CT per decade of life. Furthermore, Spaide [24] described an entity called age-related choroidal atrophy, consisting in significant decrease in CT because of small vessel disease affecting the choroid in the elderly.

In this study, we found no significant correlation between choroidal and retinal thickness in the macular area. In a recent article, Esmaeelpour et al. [19] showed decreased macular CT in diabetic patients with or without diabetic maculopathy with no difference in foveal CT among different maculopathy groups.

Moreover, when we separately evaluated patients with diabetic macular edema (with increased retinal thickness on SD-OCT) versus patients without edema, CT was not significantly different. This might be explained by the fact that macular edema is primarily caused by altered retinal circulation, which leads to increased retinal thickness, with no concurrent specific alterations in CT.

A structurally and functionally normal choroid is essential for the normal function of the retina. Choriocapillaris degeneration, previously demonstrated in histopathologic studies, may be responsible for photoreceptor dysfunction and death by insufficient removal of waste products generated by the retinal pigment epithelial cells and consequent accumulation of such waste in the Bruch membrane [25].

These can ultimately lead to choroidal autoinfarction and atrophy (indirectly visualized as reduced CT) determining visual loss.

All measurements performed in this study were obtained using direct uninverted scans, a methodology that allows to simply

obtaining and managing SD-OCT images. This method differs from the enhanced depth imaging method, described recently by ***Spaide et al***. [(12)] using the Heidelberg Spectralis SD-OCT. With enhanced depth imaging, an inverted image positioned near the top of the display and a100 average B-scans were used for choroidal visualization. Although measurements of CT are performed in similar ways using "layer editing" function in both instruments, direct visualization of images is more straightforward and natural in optical coherence tomography image analysis. According to our experience, to better visualize the choroid with RS-3000, well-dilated pupils are needed, even in normal and young subjects. On the contrary, with enhanced depth imaging method, good quality choroidal images are obtained even in undilated controls [(12)].

Whereas the choroid may be investigated using B-scan ultrasonography and/or indocyanine green angiography, both these techniques have major limitations.The former allows for limited resolution and suffers from limited reproducibility. The latter is an invasive technique with more qualitative than quantitative results.

In conclusion, spectral domain optical coherence tomography has the advantage of clear and repeatable images, with reproducible quantification of choroidal parameters. Moreover, this technique is undergoing impressive technologic improvement, with the future possibility of fully automatic measurement of CT. Choroidal thinning as documented in this study, and when confirmed in a larger population, should stimulate to reconsider the pathophysiologic role of choroidal circulation in the natural history of DR, mainly in outer retina impairment.

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