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Original Article

Choroid and Retina: A potential guide for glycemic control of diabetes mellitus without diabetic retinopathy

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Abstract

Aim: A considerable amount of evidence is pointing out the possible contribution of neurodegeneration in the etiopathogenesis of diabetic retinopathy (DR).

Materials and Methods: The evaluation and correlation of the neurodegenerative findings in the inner retinal layers with the choroid of patients with type 2 diabetes mellitus (DM) without DR via optic coherence tomography (OCT) are aimed in this study.

Results: Neuronal and axonal loss seems to occur early in DM before the ophthalmoscopic vascular changes.

Conclusion: Strict glycemic control, in addition to neuroprotective measures in this pre-retinopathy phase may offer a promising treatment choice by decelerating the progression to overt retinopathy.

Keywords: Choroidal thickness; diabetic retinopathy; glycemic control; neurodegeneration; retinal nerve fiber layer

INTRODUCTION

Diabetic retinopathy (DR), the most common complication of diabetes mellitus (DM), is unfortunately the leading cause of blindness among working-age population all over the world (1).

Traditionally, DR has been taught as a primary retinal vascular disease for years, since the microvascular theory has been the most accepted mechanism in the DR pathophysiology. However, today, it is known that DM induces apoptosis in ganglion, horizontal, amacrine and Müller cells in accordance with activation of microglia (2,3), evidenced with the functional abnormalities in electroretinograms and perimetries (4), visual evoked potentials (5), dark adaptation (6), contrast sensitivity (7) and colour vision (8) before the clinical appearance of DR. Thus, all these experimental and clinical functional findings evolved the possible role of neuroretinal degeneration to the pathogenesis of DR (9). However, whether neuropathy or vasculopathy precedes or coincides and whether there is a contributory effect neuropathy over visual functions have not been enlightened conclusively.

Since optical coherence tomography (OCT) has allowed high resolution in vivo visualization of retinal layers with automated segmentation, analysis of neurodegenerative alterations in diabetic retinas in a non-invasive, precise and reproducible way is possible (10). While most studies have reported reduced thickness measurements, especially in the retinal nerve fiber layer (RNFL) and ganglion cell complex and interplexiform layer (GC-IPL) of both type 1 and type 2 DM patients (11-13) in addition to choroidal thickness changes (14) even in the early phase before the vascular changes take place, there is no report in the literature regarding the possible association of inner retinal layer and choroidal alterations. In this context, the evaluation of the neurodegenerative findings in the inner retinal layers and choroid of type 2 diabetic patients without diabetic retinopathy via OCT and their correlation are aimed in this current study.

MATERIALS and METHODS

Study Population and Design

This observational cross-sectional controlled study conducted in Ophthalmology Department in Ordu University Training and Research Hospital between August 2017-February 2018, was performed in accordance with the Declaration of Helsinki and was approved by the local ethics commitee. All participants provided written informed consent.

The inclusion criteria were defined as diagnosis of Type 2 DM patients without DR, based on ETDRS classification.

The exclusion criteria were defined as: history of significant ocular disease, ocular surgery other than cataract surgery,

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cataract surgery within the last 6 months, trauma or tumour; refractive measurement of more than 3.0 diopters; best corrected visual acuity (BCVA) <8/10, axial length (AL) > 25 mm, intraocular pressure (IOP) >21 mm Hg, with a cup-to-disc ratio ≥0.5, any kind of amblyopia; history of glaucoma; uveitis; any retinal diseases; any opacities leading to poor image quality (signal strength<7/10); chronic obstructive pulmonary diseases, smoking, sleep apnea, optic disc anomaly, history of optic neuritis, peripapillary atrophy, body mass index of >25 and history of neurogenerative diseases.

A total of 110 eyes were enrolled in the study. Group DM included 55 type 2 DM patients without DR and Group HC included 55 healthy control subjects. Group HC had no history of ocular or systemic diseases. Diabetes was defined according to physician diagnosis, and all patients with diabetes were under oral hypoglycemic treatment for at least six months. Group HC were recruited from the general population or from relatives of patients with diabetes.

Examination Protocol and Study Measurements

A detailed medical history was obtained from each patient with the diagnosis of type 2 DM and duration of the disease; present medications with glycemic control (blood levels of HbA1c) were recorded. Only the right eyes of the patients were included in the study. Patients underwent a full ophthalmologic examination including BCVA assessment, slit-lamp examination, hand-held tonometry (i-Care TA01i, Tiolat Oy, Helsinki, Finland) and fundus examination. All of the measurements and ocular examination were executed by a single physician. AL was measured with combined biometric pachymeter (PacScan 300AP Digital Biometric Ruler; SonoMed, Lake Success, NY). After pupil dilation with tropicamide 1% (w/v) central macular thickness (CMT), RNFL thickness, GCIPL and subfoveal choroidal thickness (SCT) was assessed with OCT (Cirrus HD-OCT, Carl Zeiss Ophthalmic System Inc, Zeiss-Humphrey, Dublin, California, USA).

An experienced single physcian manually measured the SCT by EDI-OCT perpendicularly over the thinnest foveal zone from the basal edge of the retinal pigment epithelium (RPE) to the inner scleral border, between 10 AM and 11 AM to avoid diurnal variations. The macular cube scan 512 × 128 protocol was used to evaluate CMT. The peripapillary RNFL thickness was measured by an optic disc cube 200x200 scan protocol centered on the optic disc. GCA software was used to evaluate the average thickness of the GC-IPL in an elliptical annulus with a vertical inner and outer radius of 0.5 and 2.0 mm, respectively; a horizontal inner and outer radius of 0.6 and 2.4 mm, respectively around the fovea.

Statistical Analysis

Data analyses were performed by using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, United States). Whether the distributions of continuous variables were normal or not was determined by Kolmogorov Smirnov test. Levene test was used for the evaluation of homogeneity of variances. Unless specified otherwise, continuous data were described as mean ± SD. Categorical data were described as number (%) of cases. Qualitative variables were compared using Pearson's Chi-square test, quantitative data were analyzed by Spearman's correlation test, the qualitative data was compared with parametric quantitative data using Student's t test, Anova and Tukey tests and the qualitative data was compared with nonparametric quantitative data using Mann Whitney U and Kruskal Wallis tests. It was accepted p-value < 0.05 as significant level on all statistical analysis.

RESULTS

The Group DM included 28 (50.9%) females and 27 (49.1%) with a mean age of 56.56±6.83 years. Group HC included 25 (45.5%) females and 30 (54.5%) with a mean age of 56.31±5.82 years. Mean disease duration at the beginning of the study was 52.49±34.33 months in Group DM. Mean HbA1c was 6.74±0.48% in DM group and 5.02±0.41% in HC group (p<0.001). There were no statistically significant differences in respect to age, gender, BCVA, IOP, and AL between the groups (p=0.834, 0.733, 0.800, 0.126 and 0.630 respectively). Lens status demonstrated significant difference between groups (p=0.004). The characteristics of patients in groups are demonstrated in Table1.

Table 1. Characteristics of patients

	Group DM	Group HC	p
Age (years)	56.56±6.83	56.31±5.82	0.834*
Gender			
Male (%)	27 (49.1)	30 (54.5)	0.733 β
Female (%)	28 (50.9)	25 (45.5)	
Duration (months)	52.49±34.33	-	-
HbA1c (%)	6.74±0.48	5.02±0.41	**<0.001** μ
BCVA (snellen)	0.92±0.15	0.91±0.19	0.800 μ
IOP (mmHg)	15.15±3.72	14.18±4.03	0.126 μ
AL (mm)	22.13±1.18	22.22±1.25	0.630 μ
Lens status			
Clear phakic (%)	31 (56.4)	43 (78.2)	
Cataractous (%)	15 (27.3)	12 (21.8)	**0.004** β
Pseudophakic (%)	9 (16.4)	0	

*student t test, β: Pearson's chi-square test, μ: Mann whitney U test
Continuous variables are expressed as either the mean ± standard deviation (SD) and categorical variables are expressed as either frequency or percentage. Abbreviations: BCVA: Best corrected visual acuity; IOP: Intraocular pressure; AL: Axial length
P<0.05 values are expressed in bold

Macular thickness parameters provided by the ETDRS protocol are demonstrated in Table 2. Macular thickness in the inner nasal (N3), inner superior (S3) and inner inferior (I3) sectors were measured significantly thinner in Group DM (p= 0.001, p=0.009 and p=0.031 respectively).

Table 2. Macular thickness parameters

Macular thickness	Group DM	Group HC	p
CMT	254.13±12.3	255.36±11.01	0.580*
N3	309.95±16.11	318.2±12.25	**0.001**μ
N6	296.93±18.18	297.71±23.89	0.847*
T3	305±12.52	305.73±12.72	0.724μ
T6	260.93±13.86	261.76±17.13	0.779*
S3	313.49±20.92	323.53±17.36	**0.009**μ
S6	275.6±17.51	277.53±24.42	0.486μ
I3	308.73±22.43	317.73±20.7	**0.031***
I6	267.71±23.92	267.55±19.35	0.765μ

*student t test, μ: Mann whitney U testi
CMT = central macular thickness, N3 = nasal inner macula, N6 = nasal outer macula, T3 = temporal inner macula, T6= temporal outer macula, S3 = superior inner macula, S6 = superior outer macula, I3 = inferior inner macula, I6 = inferior outer macula. P<0.05 values are expressed in bold

Mean SCT in Group DM and Group HC was measured as 272.62±11.12 and 277.4±10.33 respectively. Mean SCT in Group DM was found to be statistically lower than Group HC (p=0.003). Measurements of the thickness of the RNFL in all quadrants and GC-IPL of the macular area in all sectors demonstrated thinning in Group DM compared to Group HC.However significant differences yielded RNFL thickness in the superior (p=0.019) and nasal (p=0.018) quadrants, in addition to minimum (p<0.001), average GCIPL (p<0.001) thicknesses and in superonasal (p=0.002) and in inferonasal (p=0.003) sectors (Table 3).

Table 3. SCT, peripapillary RNFL thicknesses and GC-IPL thicknesses in the groups

	Group DM	Group HC	p
SCT	272.62±11.12	277.4±10.33	**0.003**μ
RNFL			
Average	92.96±7.57	95.64±7.15	0.060*
Superior	111.96±12.01	116.93±9.73	**0.019***
Inferior	118.04±10.75	121.18±9.95	0.093μ
Nasal	68.42±7.76	72.27±9.02	**0.018***
Temporal	61.2±7.23	61.69±9	0.952μ
GCIPL			
Minimum	78.36±2.05	79.73±1.62	**<0.00**μ
Average	81.22±1.99	82.69±1.89	**<0.001**μ
Superior	83.11±2.2	83.53±1.78	0.246μ
Inferior	80.36±1.93	80.51±1.88	0.619μ
Superonasal	84.11±2.22	85.35±1.77	**0.002**μ
Inferonasal	82.78±1.78	83.89±1.73	**0.003**μ
Superotemporal	81.58±1.76	81.73±1.3	0.218μ
Inferotemporal	82.42±1.98	82.62±2.01	0.863μ

*student t test, μ: Mann whitney U testi
p<0.05 values are expressed in bold.
Abbreviations: SCT: Subfoveal choroidal thickness; RNFL: retinal nerve fiber layer; GC-IPL:ganglion cell-inner plexiform layer

Table 4. Correlation analysis

	duration		hba1c		sct	
	r	p	r	p	r	p
duration	-		.385**	**0.004**	0.133	0.333
HbA1c	.385**	**0.004**	-		-0.219*	**0.022**
BCVA	-0.191	0.162	-0.120	0.213	-0.070	0.470
IOP	0.162	0.239	0.121	0.209	-0.041	0.673
AL	0.044	0.750	-0.061	0.525	0.002	0.983
SCT	0.133	0.333	-.219*	**0.022**	-	
RNFL						
Average	-0.101	0.463	-.234*	**0.014**	0.049	0.613
Superior	-0.071	0.607	-0.157	0.103	0.123	0.199
Inferior	-.271*	0.046	-.237*	**0.013**	-0.122	0.203
Nasal	-0.063	0.649	-.216*	**0.023**	-0.017	0.864
Temporal	-0.263	0.052	-0.055	0.567	<0.001	0.999
CMT	0.053	0.701	-0.122	0.202	-0.040	0.679
N3	0.100	0.470	-.231*	**0.015**	0.132	0.169
N6	0.016	0.906	-0.077	0.426	-.239*	**0.012**
T3	0.105	0.447	-0.006	0.952	0.017	0.858
T6	0.057	0.678	0.094	0.326	-0.098	0.308
S3	-0.009	0.946	-.216*	**0.023**	0.048	0.619
S6	-0.099	0.470	-0.008	0.930	-0.044	0.651
I3	0.217	0.112	-0.102	0.288	0.181	0.058
I6	-0.060	0.665	0.031	0.749	-0.054	0.574

GCIPL						
Minimum	0.053	0.702	-.274**	**0.004**	0.017	0.862
Average	-0.025	0.858	-.403**	**<0.001**	0.061	0.524
Superior	-0.165	0.229	-0.139	0.149	0.001	0.989
Inferior	0.166	0.226	-0.102	0.289	0.071	0.459
Superonasal	0.209	0.125	-.210*	**0.028**	-0.024	0.800
Inferonasal	0.083	0.545	-.229*	**0.016**	0.121	0.208
Superotemporal	0.162	0.237	-0.027	0.782	0.017	0.861
Inferotemporal	0.152	0.270	0.043	0.657	0.115	0.233

Spearman's correlation test
Abbreviations: BCVA: Best corrected visual acuity; IOP. Intraocular pressure; AL: Axial length. SCT: Subfoveal choroidal thickness; RNFL: retinal nerve fiber layer; CMT = central macular thickness. N3 = nasal inner macula. N6 = nasal outer macula. T3 = temporal inner macula. T6= temporal outer macula. S3 = superior inner macula. S6 = superior outer macula. I3 = inferior inner macula. I6 = inferior outer macula.
GC-IPL: ganglion cell-inner plexiform layer
P<0.05 values are expressed in bold

Correlation analysis for duration of DM revealed a positive correlation with HbA1c and an inverse correlation with RNFL thickness in the inferior quadrant. Correlation analysis for HbA1c revealed an inverse correlation with SCT, average RNFL thickness with inferior and nasal quadrants, macular thickness in n3 and s3 sectors, GCIPL thickness in the superonasal and inferonasal sectors together with minimum and average GCIPL thicknesses. Correlation analysis for SCT revealed an inverse relation with macular thickness in the n6 sector (Table 4)

DISCUSSION

DR has long been recognized as a microvasculopathy, however, considerable amount of clinical and experimental evidences indicate that retinal neurodegeneration may precede microvascular changes in DM (2,15). Thinning of the inner retina, especially RNFL, was considered occurring at the earliest stage of DR (16). It seems that retinal ganglion cell (RGC) loss and axonal degeneration increases with the increasing DM duration, causing a progressive reduction of the retinal thickness (17). This may be the reason why some patients are poor-responders to current treatment modalities directed towards vascular remodelling such as intravitreal anti-vascular endothelial growth factor. Thus, the early identification and management of preclinical changes might prevent neurodegenerative process.

In this study macular retinal thickness in all sectors, however significant only in S3, N3 and I3 sectors were found to be thinner, inversely related with HbA1c levels in the S3 and N3 sectors, in type 2 DM patients without DR, compared to controls, in relation with previous studies with relatively shorter duration of disease (18-20). Studies with longer duration of diabetes, studies involving patients with DR and studies involving patients with type 1 DM reported different results. In a longitudinal study, conducted over 125 type 2 DM patients without DR, significant reduction was observed in overall retinal thickness independent of the development of DR (17), in relation with our study. In addition, CMT has been shown to be gradually increased with the duration of DM, probably due to an increased vascular permeability. Thus, shorter duration of DM, leads to thinner retinal thickness due to neurodegeneration which is not obscured by the increase in macular thickness due to the vascular leakage (18).

In this study significant reductions in RNFL thickness in the superior and nasal quadrants and average, minimum, superonasal and inferonasal GCIPL thickness, in type 2 DM patients without DR were observed. Thicknesses of average RNFL along with inferior and nasal quadrants, minimum and average GCIPL together with superonasal and inferonasal sectors were found to be inversely correlated with HbA1c. In addition, duration was inversely correlated with RNFL in the inferior quadrant. Thinning of the ganglion cell layer (GCL) in type 1 (12,21) and RNFL in type 2 DM (11,13,16) has been documented via OCT studies in the literature, even before clinical vascular findings of DR. Significant negative correlations of GCC thickness and RNFL thickness with duration of type 1 DM and hemoglobin A1c levels, respectively were reported (22). Over a 4-year period, Sohn et al. (23) notified progressive thinning of the NFL (0.25 µm/year) and of the GCL-IPL (at 0.29 µm/year), comparable with glaucoma, significantly higher than age-related reduction (0.1 µm/year per layer) (24), concluding that neuroretinal loss progresses with increasing duration of DM, preceeding DR. The relevance of this loss in the inner retina to visual functions also determined by few studies (20). However, there is no consensus about where the retinal impairment originates from, whether the GCIPL (25,26) or the RNFL (27). A recent study comparing GCIPL, between controls and type 2 DM patients with and without RNFL defects (28), showed that diabetic eyes with RNFL defects had a significantly thinner average GCIPL thickness than those without RNFL defects more pronounced with cardiovascular autonomic neuropathy involvement. Moreover Netto et al. detected a more pronounced thinning of the macular inner retinal retinal layer in the superior hemisphere (even though not significant) (26). A recent review by Barber and Baccouche suggests that thinning of RNFL is most likely due to loss of RGC axons, which presumably includes the loss of cell bodies and dendrites (9). The RNFL thinning prominent in the superior and nasal quadrants and GCIPL involvement

prominent in the nasal sectors together with pericentral macular retinal thinning, inversely correlated with metabolic control, in this current study, also locates DR in neurodegenerative disease spectrum occuring before the development of vascular pathology with the more prominent loss in RGC leading to RNFL defects.

Histopathologic studies revealed choroidal findings in DM, including increased tortuosity, dilation and narrowing, hypercellularity, vascular loop and microaneurysm formation, choriocapillaris drop-out and sinüs-like structures between choroidal lobules (29,30). In addition, laser doppler flowmetry have shown a decrease in subfoveal choroidal blood flow in patients with DM even in the absence of DR (31). Clinical studies evaluating the choroidal thickness in DM eyes (32-36) exhibited controversial results, especially in patients without DR. Imaging the choroid using different OCT devices, the manual measurement of the choroid, number of the population, inadequate control of factors influencing choroidal thickness, such as age, spherical equivalent, AL, and diurnal variation, in addition to the presence of systemic factors such as microalbuminuria (37), treatments such as anti-VEGF injections and panretinal photocoagulation may explain these inconsistent results. Most studies demonstrating choroidal thinning may be explained by loss of choroidal capillaries causing decrease in blood flow. The consensus from the reviews is that the choroid gets thinner in diabetic eyes without macular edema (38,39), regardless of the DR stage. In addition, optical coherence tomography angiography clearly revealed the reduction in the density choriocapillaris (40) in diabetic patients without DR, in relation with duration of diabetes (37). Recently it was hypothetized that the early reduction in subfoveal CT before the DR takes place is specific for type 2 DM only (14). These findings may point out the potential role of choroidal circulation in the early-phase pathophysiology of DR. However, a recent longitudinal study showed a thicker choroid in diabetic patients without DR after 1 year of follow-up, probably due to choroidal edema or impaired vessel autoregulation; the choroid began to thin as DR developed (18). In conjunction with most of the previous studies (32,34,35) but in contrast to some others (14,17,33), the findings of this current study where potential confounders are avoided as much as possible, such as age, spherical equivalent, AL, and diurnal variation, demonstrated a reduction in choroidal thickness in type 2 DM patients without DR, inversely correlated with metabolic control, suggesting an early decrease in ocular perfusion status in DM, before the retinopathy takes place.

The correlation analysis of retinal and choroidal findings did not reveal an association except negative relation between macular thickness in the outer nasal sector suggesting independent pathogenetic mechanisms play role in retinal and choroidal involvement in DM. However, an inverse correlation between HbA1c levels and the RNFL thinning and GCIPL thinning prominent in the nasal sectors together with pericentral macular retinal thinning and SCT thinning were yielded. This may propound that the strict metabolic control along with neuroprotective strategies in the preretinopathy phase may decelerate the progression to DR. Moreover, retina and choroid may potentially serve as a guide for clinicians in the metabolic control of DM patients before the progression of neurodegeneration and evolvement of microvasculopathy.

LIMITATION

Limitations of this study are the manual measurement of the choroidal thickness and the small size of the population. Another drawback is the uncertainity of the duration of diabetes, as the recognition and diagnosis of type 2 DM patients may be long after the beginning of the disease process.

CONCLUSION

The decrease in thickness of diabetic choroids in the pre-retinopathy phase may suggest the contributary effect in DR pathogenesis. Early RNFL thinning prominent in the superior and nasal quadrants with global GCIPL loss prominent in the nasal sectors and overall RT reduction in the pericentral area before the vascular signs of DR, supports the neurodegenerative theory in the pathophysiology, suggesting the in-time detection and intervention with neuroprotective measures may prevent functional losses in long term. Furthermore, since hyperglycemia is considered in the center of pathogenesis of the diabetic microvasculopathy, strict glycemic control in this preretinopathy phase, when neurodegeneration appears, may decelerate the progression to overt retinopathy. Thus, retinal and choroidal alterations may guide in ensurance of sufficient glycemic control in DM before the development of DR.

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REFERENCES

1. Yau JW, Rogers SL, Kawasaki R, et al. Meta-Analysis for Eye Disease (META-EYE) Study Group. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care 2012;35:556-64.
2. Barber AJ, Lieth E, Khin SA, et al. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. J Clin Invest 1998;102:783-91.
3. Barber AJ. A new view of diabetic retinopathy: A neurodegenerative disease of the eye. Prog. Neuropsychopharmacol Biol Psychiatry 2003;27:283-90.
4. Han Y, Adams AJ, Bearse MA, Jr, et al. Multifocal electroretinogram and short-wavelength automated perimetry measures in diabetic eyes with little or no retinopathy. Arch Ophthalmol 2004;122:1809-15.

5. Balta O, Sungur G, Yakin M, et al. Pattern visual evoked potential changes in diabetic patients without retinopathy. J Ophthalmol 2017;2017:8597629.
6. Drasdo N, Chiti Z, Owens DR, et al. Effect of darkness on inner retinal hypoxia in diabetes. Lancet 2002;359:2251-3.
7. Sokol S, Moskowitz A, Skarf B, et al. Contrast sensitivity in diabetics with and without background retinopathy. Arch Ophthalmol 1985;103:51-4.
8. Roy MS, Gunkel RD, Podgor MJ. Color Vision Defects in Early Diabetic Retinopathy. Arch Ophthalmol. 1986;104:225-8.
9. Barber AJ, Baccouche B. Neurodegeneration in diabetic retinopathy: Potential for novel therapies. Vision Res 2017;139:82-92.
10. Massin P, Vicaut E, Haouchine B, et al. Reproducibility of retinal mapping using optical coherence tomography. Arch Ophthalmol 2001;119:1135-42.
11. van Dijk HW, Verbraak FD, Kok PH, et al. Early neurodegeneration in the retina of type 2 diabetic patients. Invest Ophthalmol Vis Sci 2012;53:2715-9.
12. van Dijk HW, Verbraak FD, Kok PHB, et al. Decreased retinal ganglion cell layer thickness in patients with type 1 diabetes. Invest Ophthalmol Vis Sci 2010;513660-5.
13. Pierro L, Iuliano L, Cicinelli MV, et al. Retinal neurovascular changes appear earlier in type 2 diabetic patients. Eur J Ophthalmol 2017;27:346-51.
14. Ferreira JT, Vicente A, Proença R, et al. Choroidal thickness in diabetic patients without diabetic retinopathy. Retina 2018;38:795-804.
15. Kadlubowska J, Malaguarnera L, Waz P, et al. Neurodegeneration and neuroinflammation in diabetic retinopathy: potential approaches to delay neuronal loss. Curr Neuropharmacol 2016;14:831-9.
16. Chhablani J, Sharma A, Goud A, et al. Neurodegeneration in type 2 diabetes: evidence from spectral-domain optical coherence tomography. Invest Ophthalmol Vis Sci 2015;56:6333-8.
17. Ferreira JT, Proença R, Alves M, et al. Retina and Choroid of Diabetic Patients Without Observed Retinal Vascular Changes: A Longitudinal Study, Am J Ophthalmol 2017;176:15-25
18. Shawky SS, Elagouz MH, Ismail AM, et al. Macular thickness in healthy controls and diabetics without diabetic macular edema. Egyptian Retina J 2018;5:1-5.
19. Verma A, Raman R, Vaitheeswaran K, et al. Does neuronal damage precede vascular damage in subjects with type 2 diabetes mellitus and having no clinical diabetic retinopathy? Ophthalmic Res 2012;47:202-7.
20. Bronson-Castain KW, Bearse MA Jr, Neuville J, et al. Adolescents with type 2 diabetes: early indications of focal retinal neuropathy, retinal thinning, and venular dilation. Retina 2009;29:618-26.
21. Van Dijk HW, Verbraak FD, Stehouwer M et al. Association of visual function and ganglion cell layer thickness in patients with diabetes mellitus type 1 and no or minimal diabetic retinopathy. Vision Res 2011:51:224-8.
22. Gundogan FC, Akay F, Uzun S, et al. Early neurodegeneration of the inner retinal layers in type 1 diabetes mellitus. Ophthalmologica 2016;235:125–32.
23. Sohn EH, van Dijk HW, Jiao C, et al. Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. Proc Natl Acad Sci USA 2016;113:2655-64.
24. Demirkaya N, van Dijk HW, van Schuppen SM, et al. Effect of age on individual retinal layer thickness in normal eyes as measured with spectral-domain optical coherence tomography. Invest Ophthalmol Vis Sci 2013;54:4934-40.
25. Hegazy AI, Zedan RH, Macky TA, et al. Retinal ganglion cell complex changes using spectral domain optical coherence tomography in diabetic patients without retinopathy. Int J Ophthalmol 2017;10:42733.
26. Pincelli Netto M, Castro Lima V, Pacheco MA, et al. Macular Inner Retinal Layer Thinning in Diabetic Patients without Retinopathy Measured by Spectral Domain Optical Coherence Tomography. Med Hypothesis Discov Innov Ophthalmol 2018;7:133-9.
27. Vujosevic S Midena E. Retinal layers changes in human preclinical and early clinical diabetic retinopathy support early retinal neuronal and Müller cells alterations. J Diabetes Res 2013;2013:905058.
28. Choi JA, Kim HW, Kwon JW, et al. Early inner retinal thinning and cardiovascular autonomic dysfunction in type 2 diabetes. PLoS One 2017;12:e0174377.
29. Fryczkowski AW, Sato SE, Hodes BL. Changes in the diabetic choroidal vasculature: scanning electron microscopy findings. Ann Ophthalmol 1988;20:299-305.
30. Fryczkowski AW, Hodes BL, Walker J. Diabetic choroidal and iris vasculature scanning electron microscopy findings. Int Ophthalmol 1989;13:269–79.
31. Nagaoka T , Kitaya N, Sugawara R, et al. Alteration of choroidal circulation in the foveal region in patients with type 2 diabetes. Br J Ophthalmol 2004;88:1060-3.
32. Esmaeelpour M, Považay B, Hermann B, et al. Mapping choroidal and retinal thickness variation in type 2 diabetes using three-dimensional 1,060 nm optical coherence tomography. Invest Ophthalmol Vis Sci 2011;52:5311-6.
33. Xu J, Xu L, Du KF, et al. Subfoveal choroidal thickness in diabetes and diabetic retinopathy. Ophthalmology 2013;120:2023-8.
34. Querques G, Lattanzio R, Querques L, et al. Enhanced depth imaging optical coherence tomography in type 2 diabetes. Invest Ophthalmol Vis Sci 2012;53:6017-24
35. Vujosevic S, Martini F, Cavarzeran F, et al. Macular and peripapillary choroidal thickness in diabetic patients. Retina 2012;32:1781-90.

36. Endo H, Kase S, Ito Y, et al. Relationship between choroidal structure and duration of diabetes. Graefes Arch Clin Exp Ophthalmol 2019;257:1133-40.
37. Farias LB, Lavinsky D, Benfica CZ, et al. Changes in choroidal thickness and volume are related to urinary albumin excretion in type 2 diabetic patients without retinopathy. Clin Ophthalmol 2018;12:1405-11.
38. Melancia D, Vicente A, Cunha JP, et al. Diabetic choroidopathy: A review of the current literature. Graefes Arch Clin Exp Ophthalmol 2016;254:1453-61.
39. Campos A, Campos EJ, Martins J, et al. Viewing the choroid: Where we stand, challenges and contradictions in diabetic retinopathy and diabetic macular oedema. Acta Ophthalmol 2017;95:446-59.
40. Cao D, Yang D, Huang Z et al. Optical coherence tomography angiography discerns preclinical diabetic retinopathy in eyes of patients with type 2 diabetes without clinical diabetic retinopathy. Acta Diabetol 2018;55:469-77.