RNFL, GC-IPL and Choroidal Thickness in Non-Proliferative Diabetic Retinopathy

Erol Erkan[1], *Sema Dündar*[2]

ABSTRACT

Purpose: To assess retinal nerve fiber layer (RNFL), ganglion cell-inner plexiform layer (GC-IPL) and subfoveal choroidal thickness (SCT) using spectral-domain optical coherence tomography (SD-OCT) in treatment-naive diabetes mellitus (DM) patients and compare the results according to diabetic retinopathy (DR) severity, DM duration and HbA1c levels

Methods: Two hundred and seventy four eyes 144 DR treatment-naive DM patients were included. Complete ophthalmic examination was performed in each patient by a single examiner. DR was graded according to the International Clinical Diabetic Retinopathy Disease Severity scale. OCT scans were performed by an experienced technician using Cirrus HD-OCT.

Results: As we compared average GC-IPL thicknesses of patients with respect to DR stages; moderate non-proliferative diabetic retinopathy (NPDR) and no apparent DR groups showed statistically significant difference (p = 0,012); and also severe NPDR and no apparent DR groups showed statistically significant difference (p = 0,008). When we selected the patients with HbA1c≤7%; we have found statistically significant difference only between no DR and severe NPDR group (p=0,013).According to DR stages; we did not find any statistically significant differences in terms of RNFL and SCT. DM duration, HbA1C levels and medications used to control DM did not show any correlation with average RNFL and GC-IPL thicknesses.

Conclusions: We found statistically significant differences in GC-IPL thicknesses but RNFL thicknesses were comparable across DR stages. Despite the absence of proliferative disease neuroretinal changes were evident in patients with good metabolic control (HbA1c≤7%). The intricate relationship between neural and vascular structures requires additional insights to understand the initiation of DR.

Keywords: Choroidal thickness. Diabetic retinopathy, Ganglion cell-inner plexiform layer thickness, Optical coherence tomography, Retinal nerve fiber layer thickness

INTRODUCTION

Diabetic retinopathy (DR) is the leading cause of acquired visual impairment and possesses a great risk for public health.[1,2] For many years, DR has been considered a type of microvascular problem in the retinal vessels leading to breakdown of the blood-retina barrier and increased vascular permeability. However, recent research suggested that vasculopathy might not be the sole pathway in progression of DR. In patients without evident DR, retinal functional alterations documented by electrophysiological studies have been reported.[3-5] These data might indicate that retinal functional impairment may be prior to breakdown of blood-retina barrier. Furthermore, retinal neurodegeneration has been found to have a significant role in the pathogenesis of DR, including apoptosis of retinal neuronal cells and peripapillary retinal nerve fiber layer (RNFL) thinning.[6] Vascular nourishment of retinal pigment epithelium (RPE) and photoreceptors are supplied by the choroid. Thus, choroid is a key element in the vast majority of retinal diseases including DR.

RNFL is composed of retinal ganglion cell axons and makes up the innermost neural layer of the retina.[7] With optical coherence tomography, (OCT) accurate imaging and assessment of retinal thicknesses became possible and this enabled early detection of progression in retinal diseases. Spectral domain-OCT (SD-OCT) captures high-resolution images of retinal sections and allows thickness measurements of all individual retinal layers after image procession. Retinal, peripapillary nerve fiber and choroidal thickness measurements using OCT were investigated and

1- MD, Çiğli Training and Research Hospital, Department of Ophthalmology, İzmir, Turkey

2- Prof. MD, Adnan Menderes University Faculty of Medicine, Department of Ophthalmology, Aydın, Turkey

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Correspondence Adress:
Erol Erkan
Çiğli Training and Research Hospital, Department of Ophthalmology, İzmir, Turkey

Phone: +90 505 386 6965
E-mail: erolerkan@gmail.com

statistically significant alterations were reported before.[8-10] The loss of ganglion cells affects retinal ganglion cell layer and RNFL thickness, and reduce thickness of these layers. Enhanced-depth imaging using SD-OCT has been proven a reliable method for evaluating structural situation of the choroid [11, 12].

In this study, we aimed to assess RNFL, ganglion cell-inner plexiform layer (GC-IPL) and subfoveal choroidal thicknesses (SCT) using SD-OCT in treatment-naive diabetes mellitus (DM) patients and compare the results according to DR severity, DM duration and HbA1c levels.

MATERIAL AND METHODS

In this study, 144 DM patients were enrolled from the Endocrinology department of our hospital. Criteria for inclusion were as follows: (1) age ≥18 years old; (2) diagnosis of type 2 diabetes; (3) no sign of proliferative diabetic retinopathy. Exclusion criteria were as follows: (1) evidence of diabetic macular edema on SD-OCT images (central subfield thickness >250 microns); (2) previous DR treatment history of any kind; (3) any kind of vitreo-macular interface disorders; (4) any condition increasing the risk of optic neuropathy (e.g., glaucoma, pseudo-exfoliation syndrome); (5) any neurodegenerative diseases known to influence RNFL thickness. Age, gender, duration of DM, medication used for DM, HbA1C levels within the last three months of enrolment and other systemic diseases were recorded. A complete ophthalmologic examination including best corrected visual acuity (BCVA), intraocular pressure (IOP) anterior segment and dilated fundus examination findings were recorded by one ophthalmologist and one technician performed OCT scans. Diabetic retinopathy was graded according to the International Clinical Diabetic Retinopathy Disease Severity scale; as no apparent retinopathy, mild NPDR (microaneurysm only), moderate NPDR (more than just microaneurysms but less than severe NPDR), severe NPDR (>20 intraretinal hemorrhages in each of the 4 quadrants, definite venous beading in ≥2 quadrants, and prominent intraretinal microvascular abnormalities in ≥1 quadrants).[13] After dilated fundus examination, an experienced technician performed OCT scans with Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA, USA) using macular cube 512x128 with enhanced depth imaging (EDI) mode and Optic Disc Cube 200x200 protocol. The GCA algorithm, incorporated into the Cirrus SD-OCT was used to process and measure the thickness of the macular GC-IPL within a 14.13-mm^2 elliptical annulus area centered on the fovea. The GCA algorithm automatically segmented the GC-IPL based on the three-dimensional data generated from the macular cube 512 × 128 scan protocol. Using the Cirrus linear measurement tool, choroidal thickness was measured perpendicularly from the outer edge of the hyper-reflective RPE to the inner sclera at the fovea. To evaluate RNFL thickness, Cirrus SD-OCT algorithms identify the optic disc and automatically place a calculation circle with a 3.46-mm diameter evenly around it. Layer-seeking algorithms determine the RNFL inner (anterior) boundary and RNFL outer (posterior) boundary for the entire cube, except the optic disc. Scans with signal strength of 7/10 or more were recorded for evaluation. HbA1C levels were obtained from endocrinology records.

For statistical analysis, Statistical Package for the Social Sciences 17 (SPSS) program was used. Quantitative data were analyzed whether they were suitable for normal distribution or not with Kolmogorov Simirnov test. Data suitable for normal distribution were analyzed with parametric test, data unsuitable for normal distribution were analyzed with non-parametric tests. For inter-group comparison of independent paired groups Mann Whitney U test was used. The correlation between quantitative data was analyzed with Spearman RHo Correlation tests. Categorical data were compared with Pearson Chi-Square test. Quantitative data were shown in tables as average ±standard deviation. Categorical values are expressed as numbers (n) and percentage (%). Data were analyzed at 95% trust level and accepted as significant if p value is less than 0.05. Our study adhered to the tenets of the Declaration of Helsinki and was approved by the local Ethics Committee. For every patient included in the study, a written informed consent was obtained.

RESULTS

Two hundred and seventy four eyes of 144 type 2 DM patients (79 female (54.8%), 65 male (45, 2%)) were enrolled. The average age of patients was 61.91 ± 8.97 years. The average duration of DM was 11, 87 ± 7.2 years and average HbA1C levels within the last three months were 6.90%±1.23 percentage. Seventy-six (52%) patients were using only oral anti-diabetic agents, while 68 patients (48%) were on insulin. Mean BCVA of the whole patient group was 0.66±0.30 and mean IOP measured with pneumatic tonometer was 15.41±2.94 mmHg. When the patients were graded according to DR severity; 76 eyes showed no signs of DR, 76 eyes showed mild NPDR, 44 eyes showed moderate NPDR and 78 eyes showed severe NPDR signs (Table 1).

Average RNFL thickness of patients with no apparent DR findings was 90, 96 ± 11, 40 µm, 90, 08 ± 14, 06 µm in mild NPDR, 89, 32 ± 17, 11 µm in moderate NPDR and 89, 08± 15, 68 µm in severe NPDR. As we compared average RNFL thicknesses of patients with respect to DR stages; we did not find any statistically significant differences between any groups (Figure 1). When we selected the

Table 1: *Patient characteristics*

Characteristic	DR Severity				
	No NPDR	Mild NPDR	Moderate NPDR	Severe NPDR	Overall
Age (year)	59,6±7,8	64,4±8,2	63,1±8,0	60,9±10,4	61,9±8,9
BCVA	0,8±0,2	0,6±0,2	0,5±0,2	0,5±0,3	0,6±0,3
IOP (mmHg)	16,0±2,7	15,8±3,4	14,4±2,4	14,9±2,6	15,4±2,9
DM Duration (year)	8,9±7,0	12,5±6,8	11,6±6,7	14,1±7,3	11,8±7,2
HbA1c (%)	6,4±1,2	6,9±1,2	7,2±1,1	7,0±1,1	6,9±1,2
RNFL (µm)	90,9±11,4	90,0±14,3	89,3±17,1	89,0±15,6	89,9±14,4
GC-IPL (µm)	78,1±13,4	75,1±18,3	70,0±20,0	72,8±20,2	74,4±18,1
SCT (µm)	264,3±52,9	255,0±44,7	263,0±46,5	258,1±45,2	259,7±47,4

Abbreviations: DR, diabetic retinopathy; BCVA, best-corrected visual acuity; IOP, intraocular pressure; HbA1c, haemoglobin A1c; RNFL, retinal nerve fiber layer; GC-IPL, ganglion cell-inner plexiform layer; NPDR, non-proliferative diabetic retinopathy; SCT, subfoveal choroidal thickness

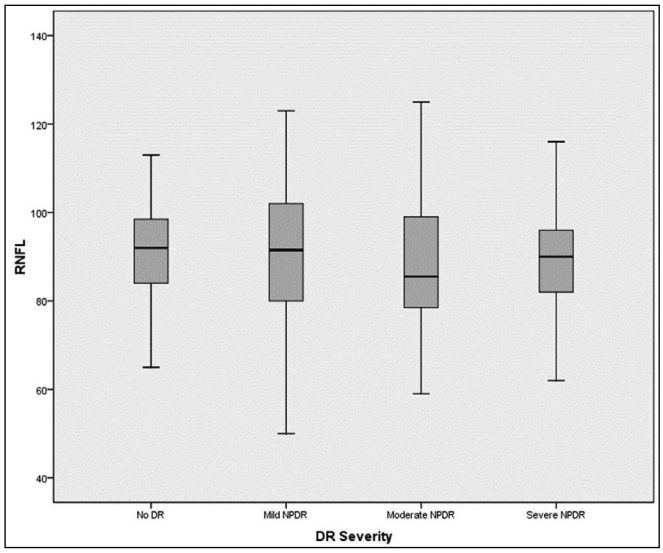

Figure 1: *Boxplot of average RNFL thickness according to DR severity.*

patients with HbA1c≤7%; we have not found statistically significant difference between any groups.

Average GC-IPL thickness of patients with no apparent DR findings was 78, 14 ± 13, 42 µm, 75, 13 ± 19, 39 µm in mild NPDR, 70 ± 20, 00 µm in moderate NPDR and 72, 85± 20, 23 µm in severe NPDR. As we compared average GC-IPL thicknesses of patients with respect to DR stages; moderate NPDR and no apparent DR groups showed statistically significant difference (p = 0,012); and also severe NPDR and no apparent DR groups showed statistically significant difference (p = 0,008), (Figure 2). When we selected the patients with HbA1c≤7%; we have found statistically significant difference only between no DR and severe NPDR group (p=0,013).

Average SCT of patients with no apparent DR findings was 264, 3 ± 52, 9 µm, 255, 0 ± 44, 7 µm in mild NPDR,

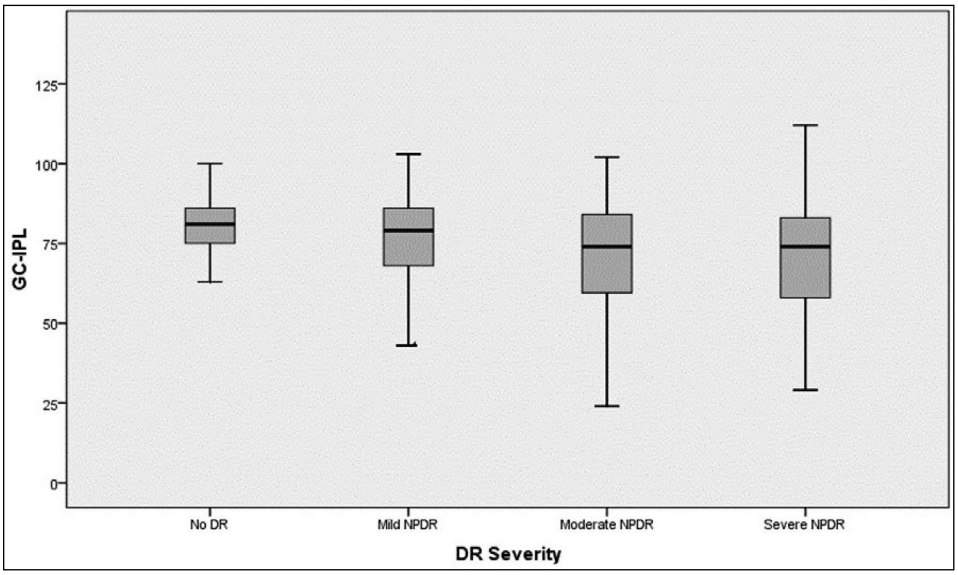

Figure 2: *Boxplot of average GC-IPL thickness according to DR severity.*

263, 0 ± 46, 5 µm in moderate NPDR and 258, 1± 45, 2 µm in severe NPDR. As we compared average SCT thicknesses of patients with respect to DR stages; we did not find any statistically significant differences between any groups (Figure 3). When we selected the patients with HbA1c≤7%; we have not found statistically significant difference between any groups.

We also found statistically significant positive correlation (Figure 4) between average RNFL and average GCL thicknesses (Spearman rho's correlation coefficient: 0,250;

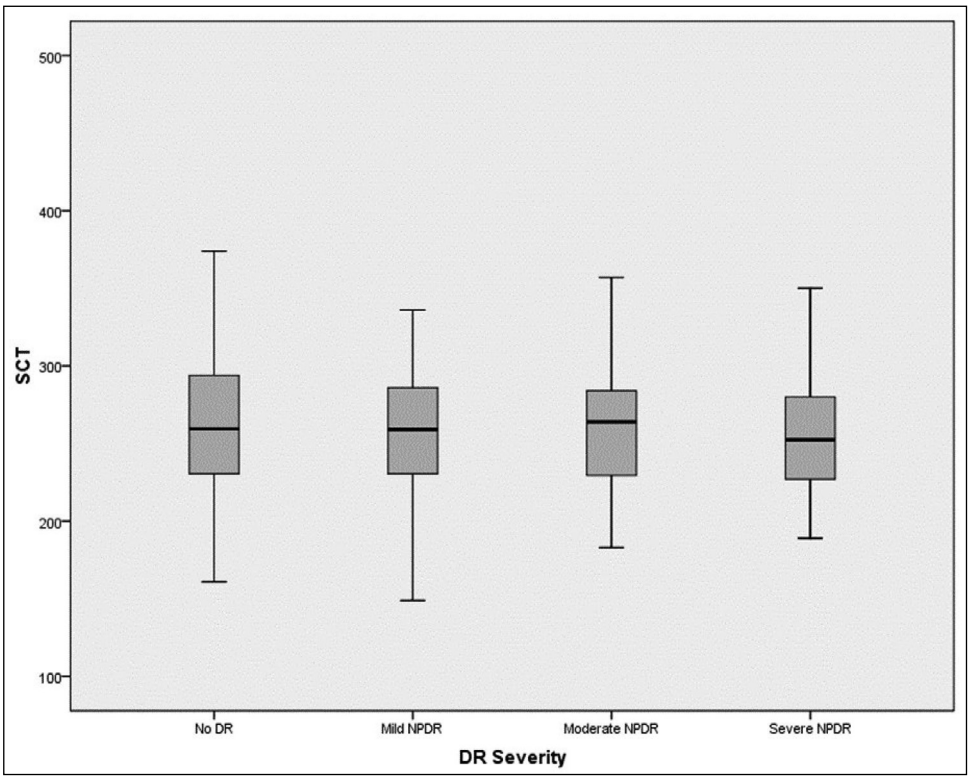

Figure 3: *Boxplot of average SCT according to DR severity.*

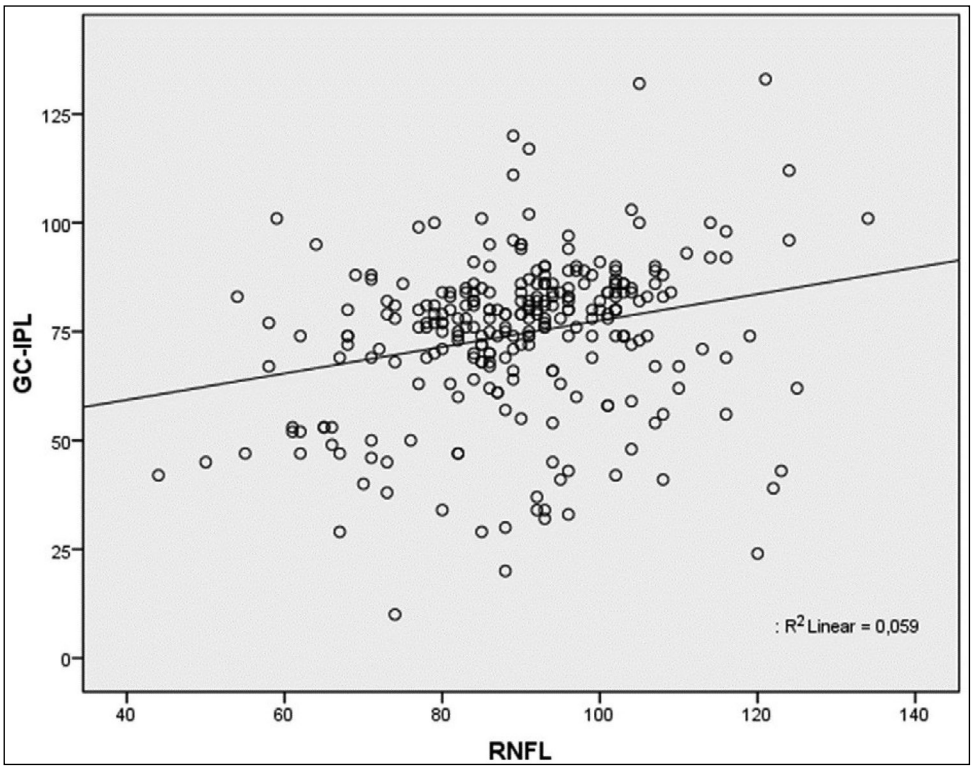

Figure 4: Scatterplot of GC-IPL and RNFL thickness measurements

p=0,001). While patients' age did not show statistically significant correlation with average RNFL thickness, we found statistically significant correlation between age and average GCL thickness (Spearman rho's correlation coefficient: 0,222; p=0,001). Average GCL thickness also showed positive correlation with visual acuity (Spearman rho's correlation coefficient: 0,189; p=0,002) and SCT (Spearman rho's correlation coefficient: 0,137; p=0,023). DM duration, HbA1C levels and medications used to control DM did not show any correlation with average RNFL and GC-IPL thicknesses (Table 2).

DISCUSSION

The neurovascular unit (NVU) is in intimate communication and maintain the integrity of the inner blood-retinal barrier whilst dynamically regulating blood flow in response to metabolic demands. The components of the NVU are diverse neural cell types (i.e. ganglion cells, amacrine cells, horizontal and bipolar cells), glia (Müller cells and astrocytes), professional immune cells (microglia and perivascular macrophages) and vascular cells (endothelial cells and pericytes).[14] In order to maintain the neuroretinal function these cells are closely attached in the neurovascular unit to form the blood-retinal barrier and execute neural signaling.[2, 15] The impairment of the NVU is a primary event in the pathogenesis of diabetic retinopathy. This attachment elucidates the concurrent microvascular and neuronal changes. Neurodegeneration in patients with diabetes both manifests structurally and functionally. Inner retinal thinning, neural apoptosis, and reactive gliosis are structural changes while abnormal electroretinography, loss of dark adaptation and contrast sensitivity, color vision disturbance, and abnormal microperimetry findings compose functional deterioration.[15-19] Previous studies reported that retinal ganglion and amacrine cells were the first neurons in which diabetes-induced apoptosis detected, and this led to a reduced thickness of the inner retinal layers, including the GC-IPL and the RNFL.[14,20] In our study, RNFL thickness values did not show statistically significant difference with respect to DR stage. But GC-IPL thickness values showed statistically significant differences between no DR group and both moderate and severe DR groups. So, GCL may give an earlier sign than RNFL for retinal neurodegeneration.

Carpineto et al. reported significant changes in GC-IPL and RNFL values between controls and diabetic patients. We can assume that detrimental effect of DM on neuroretinal unit might well be the reason for this result. But if a patient has DM, there is no significant difference in GC-IPL and RNFL according to their study.[21] At this point, our study revealed that moderate NPDR stage might be the turning point for detecting progression using GC-IPL measurements as we found statistically significant difference while RNFL measurement were still indecisive.

In DM, the progression of neuronal degeneration is associated with multiple factors, including metabolic and ischemic effects. Hyperglycemia triggers metabolic processes, including protein and lipid glycosylation and the production of oxidative species, which may induce nerve damage.[22]Target value of HbA1c in terms of good metabolic control for a DM is less than %7. In our study group when we selected patients with HbA1c≤7%; GCL thicknesses showed statistically significant difference between no DR and severe DR groups. We can infer that; in spite of good metabolic control, neurodegeneration continues through other mechanisms in earlier stages of DR. Zeng et al. investigated DM patients without clinically detectable retinopathy and healthy controls in terms of function and corresponding neurovascular structures using OCT angiography and electroretinography (ERG). They have found that functional and structural impairments were evident although patients did not show visible retinal lesions. Vessel density (VD) in superficial and deep capillary plexus were significantly decreased in comparison to healthy controls; while ganglion cell complex (GCC)

Table 2: *Cross-tabulation of correlation coefficients between patient variables*

			GC-IPL	RNFL	HbA1c	DM Duration	SCT	Age	BCVA	IOP
Spearman's rho	GC-IPL	Correlation Coefficient	1,000	**,250****	,009	-,053	**,137***	**-,222****	**,189****	**,159****
		Sig. [2-tailed]	.	**,000**	,886	,378	**,023**	**,000**	**,002**	**,008**
	RNFL	Correlation Coefficient	**,250****	1,000	,075	-,070	,064	-,100	,001	,098
		Sig. [2-tailed]	**,000**	.	,213	,245	,289	,100	,988	,106
	SCT	Correlation Coefficient	**,137***	,064	-,078	**-,177****	1,000	**-,299****	**,208****	,038
		Sig. [2-tailed]	**,023**	,289	,199	**,003**	.	**,000**	**,001**	,533

**. Correlation is significant at the 0.01 level [2-tailed], *. Correlation is significant at the 0.05 level [2-tailed] Abbreviations: BCVA, best-corrected visual acuity; IOP, intraocular pressure; HbA1c, haemoglobin A1c; RNFL, retinal nerve fiber layer; GC-IPL, ganglion cell-inner plexiform layer; SCT, subfoveal choroidal thickness.

did not show statistically significant difference between groups. Based on these results, authors concluded that subtle microvascular abnormalities; stemming from disruption of retinal neurovascular autoregulation, may reflect early functional changes in DR better than ganglion cell loss.[23] They have also stated that GCC thickness changes were not concomitant with that of RNFL thickness in patients with DM without clinically detectable retinopathy. In contrast to this result, we have found statistically significant correlation between RNFL and GC-IPL in our no DR group. The conflict of these results may be due to good metabolic control of our patients compared to their group (6, 47% vs 9, 21%). OCT angiography findings of patients with relatively good metabolic control may not reveal the aforementioned results in Zeng et al.'s study; since higher levels of HbA1c is associated with endothelial cell injury leading to microvascular abnormalities.

In the initiation and progression of DR; retinal, glial, neural and microvascular dysfunction is interdependent and essential. Carpineto et al. commented that retinal neurodegeneration is a critical endpoint and has a potential impact on microvascular disease.[23] As stated by Simó et al. interactions between the neurosensory retina and its blood vessels may be a key causative factor in the development of clinically evident diabetic retinopathy.[14] The intra-retinal vasculature lacks autonomic innervation and, therefore, a dynamic autoregulatory response of the NVU to complex circulatory and neural cues is essential to regulate blood flow through the inner retina.[24] Before being a critical endpoint for DM patient; neurodegeneration can give us important clues in progression of DR in earlier stages when microvascular changes are not evident or merely visible.

There are controversial results regarding the association between choroidal thickness and DR severity.[25, 26] In a cross-sectional study conducted in Zhongshan Ophthalmic Centre (ZOC), Sun Yat-sen University; 1347 treatment-naïve patients were evaluated in terms of choroidal thickness and associated factors.[27] They have found that choroidal thickness increased in the early stage of DR, and further decreased with DR progression. However, Weng et al. also did not find statistically significant difference in central region as in our study. We have also observed a decline in SCT as DR progresses, but remained statistically insignificant. Our relatively small sample size compared to Weng et al.'s study might explain this insignificance.

Ohara et al. reported 61 eyes of DM patients to compare choroidal thickness in relation to DR severity and investigate alterations in choroidal thickness after panretinal photocoagulation.[10] The central field choroidal thickness of severe NPDR was significantly thicker than that of normal and mild to moderate NPDR. Moreover, the central field choroidal thickness of PDR was significantly thicker than of mild to moderate NPDR. In our study, we did not find any significant difference in SCT across groups of DR severity. Ohara et al. suggested that increases in choroidal thickness as the severity of retinopathy worsens are probably due to vascular endothelial growth factor secretion (VEGF). The difference between their study and ours can be explained with this suggestion that our patients had a lower average HbA1c levels (6, 9 vs 8, 1) and shorter DM duration (11, 8 vs 12, 8). Moreover, our patient group was composed of non-proliferative DR patients, while Ohara et al. included.

As for the implications about neurovascular unit mentioned above, several factors might have an influence on alterations of choroidal thickness. Along with metabolic impairment of DM in choriocapillaris, choroidal thickening is mediated by increased vascular permeability and cytokine overexpression [(28)];but in later stages hypoxia is proposed to have an important role as choroid becomes thinner.[29]

Our study has some limitations. The variability around the true value is referred to as the test-retest variability. GC-IPL and RNFL thickness measurements obtained with automated segmentation may show test-retest variability.[30] Still, OCT is the best tool for examining the thickness of these layers but OCT angiography might have offered additional insights about microvascular structure. From a cost-effective point of view, OCT still offers valuable information. Another limitation is that we did not measure the axial length and spherical equivalent; both of which may alter the GC-IPL and RNFL measurements. Also the cross-sectional design of our study hinders to put forth the longitudinal relationship between neuronal damage and the appearance of microvascular lesions.

In this study we aimed to evaluate RNFL, GC-IPL and subfoveal choroidal thickness measurements of treatment-naïve type 2 DM patients without proliferative DR. Despite the significant changes in GC-IPL values, we did not find any significant changes in RNFL values across the groups. Average HbA1c of our patient group was 6, 9%±1, 2; indicating a good metabolic control. Average duration of DM was 11, 8±7, 2 years. We also found statistically significant positive correlation between average RNFL and average GCL thicknesses (Spearman rho's correlation coefficient: 0,250; p=0,001) and also between age and average GCL thickness (Spearman rho's correlation coefficient: 0,222; p=0,001). Average GCL thickness also showed positive correlation with visual acuity (Spearman rho's correlation coefficient: 0,189; p=0,002) and choroidal thickness (Spearman rho's correlation coefficient: 0,137;

p=0,023). DM duration, HbA1C levels and medications used to control DM did not show any correlation with average RNFL and GCL thicknesses.

In conclusion, we believe that neuroretinal degeneration may be evident prior to microvascular damages. But, microvascular changes like capillary dropouts may be preceding neuroretinal degeneration. The intricate relationship between neural and vascular structures requires additional insights to understand the initiation of DR. Further studies may contribute to establish the relationship between neuroretinal degeneration and microvascular changes and a new diagnostic and screening parameter may be established for the timely detection of DR.

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