Original Research Article

Evaluation of thickness of retinal nerve fiber layer and ganglion cell layer with inner plexiform layer in patients without diabetic retinopathy and mild diabetic retinopathy in type 2 diabetes mellitus patients using spectral-domain optical coherence tomography

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ABSTRACT

Background: A widely accepted pathogenesis of DR consists of microvascular abnormalities. However recent investigations have demonstrated neurodegenerative alterations before the appearance of microvascular changes in patients with DM. Aim of the study was to evaluate thickness of retinal nerve fiber layer and ganglion cell layer with inner plexiform layer in patent without diabetic retinopathy and mild diabetic retinopathy in type 2 diabetic patients using spectral domain optical coherence tomography.

Methods: Thirty patients with type 2 diabetes mellitus without diabetic retinopathy, 30 with mild diabetic retinopathy and 30 healthy controls are taken considering inclusion and exclusion criteria. GCL-ILM and RNFL thickness was measured in each individual and measurements were compared using one way ANOVA test and Pearson's correlation was performed to evaluate the linear correlation between variables and calculated p value <0.05 was regarded as significant.

Results: The average RNFL thickness was 86.18±8.44μm and 91.79±4.77μm in diabetic patients and controls respectively (p=0.002). Furthermore, for two different groups of diabetic patients, the average RNFL thickness was 86.74±11.18μm in the no DR group and 85.62±11.10μm in the mild DR group (p=0.697). The average GCL-IPL thickness was 79.95±4.32μm and 84.66±3.26μm in diabetic patients and controls, respectively (p=<0.001). Furthermore, for two different groups of diabetic patients, the average GCL-IPL thickness was 80.15±5.78μm in the no DR group and 79.75±5.70μm in the mild DR group (p=0.788).

Conclusions: There was a statistically significant reduction of the mean GCL-IPL and RNFL thickness in type 2 diabetic patients with no or mild DR compared with a homogenous control group indicating neuroretinal changes occur before vascular changes of diabetic retinopathy. But the correlation of average RNFL thickness and GCL-IPL thickness was not statistically significant with the duration of diabetes and HbA1c value.

Keywords: Diabetic retinopathy, Diabetes mellitus, GCL-ILM thickness, RNFL thickness

INTRODUCTION

Diabetic retinopathy (DR) is the leading cause of visual impairment in the working age population.[1] The prevalence of diabetes mellitus is attaining epidemic proportion worldwide with number expected to rise to 592 million by 2035.[2] A widely accepted pathogenesis of DR consists of abnormalities and microvasculopathy and the early clinical signs of DR include microaneurysms and retinal microhemorrhages.[2-5] However, research on the pathogenesis of DR found that neuronal dysfunction and neurodegeneration are closely correlated with

microvascular dysfunction and that neurovascular unit degeneration should be considered as an important component of the pathology of DR.[6-8] Evidence from the retinas of both diabetic donors and diabetic animal models has also shown that retinal neuronal cell degeneration or apoptosis occurs early in the course of diabetes.[9-11]

The integrity of the retina ganglion cell (RGC) is crucial for preserving visual function, and the retinal nerve fiber layer (RNFL), the GCL and the inner plexiform layer (IPL) consist of the axons, nuclei and dendrites of RGCs, respectively. In type 2 diabetes mellitus (T2DM) patients, however, data from several OCT studies have been conflicting. Chhablani J et al, suggested that early thinning of the intra-retinal layer occurs in T2DM even before visible vascular signs of DR, whereas van Dijk et al, reported that GCL thickness was significantly thinner only in patients with apparent microvascular DR lesions.[12-14]

Furthermore, Chen Y et al, found that the ganglion cell inner plexiform layer (GCIPL) complex thickness in T2DM patients was not significantly different from that of controls.[15] Most of the previously published studies have focused on the analysis of macular GCIPL thickness via Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA, USA) using a ganglion cell analysis (GCA) algorithm and intra-retinal layer thickness via 3D OCT-1000 (Topcon Corp, Tokyo, Japan).[12-17] These studies quantified RGC loss in terms of reduction of the peripapillary RNFL or macular GCIPL complex thickness and seldom reported the macular RNFL, GCL or IPL thickness changes separately

The invention of optical coherence tomography (OCT) has allowed imaging and measuring various aspects of retina and optic disc.[18] The high resolution of spectral domain OCT (SDOCT) allows measurement of the thickness of all individual retinal layers, including retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL).[19]

SD-OCT can directly measure and quantify retinal nerve fiber layer (RNFL) thickness by calculating the area between internal limiting membrane and RNFL border.[20] The Cirrus RNFL maps represents a 6x6 mm cube of A-Scan data centered over the optic nerve in which a 3.4mm diameter circle of RNFL data is extracted to create what is referred to as the TSNIT map (Temporal, superior, nasal, inferior, temporal). It is displayed as a false color scale with the thickness values referenced to a normative database. The TSNIT map displays RNFL thickness values by quadrants and clock hours, and the RNFL peaks give a sense of the anatomic distribution of nerve fiber axons represented by the superior and inferior bundles that spread out from the optic nerve.[21]

Ganglion cell complex is studied by OCT which has the maximum density at the macula. Roughly one million ganglion cells are found in the human retina and half of this number is centered on the fovea. This anatomic arrangement suggests that a macula scan with ganglion cell analysis could be early indicator of this disease Segmenting ganglion cell layer alone is very difficult based on reflectivity and thus cirrus chose to measure its ganglion cells analysis consisting of combined ganglion cell layer (GCL) and inner plexiform layer (IPL).[22] The cirrus macular scan map is displayed using a false color scale and divided into various pie sectors around the fovea. Again the calculated sectors are compared to a normative database.[21]

Thus, with the help of SD-OCT we tried to evaluate the early neurodegenerative effects of DM on inner retinal structures and optic disc.

METHODS

The present study was conducted taking up to 60 type 2 diabetic patients more than 40 years of age and of both sex, out of which 30 patients were having mild diabetic retinopathy and 30 without diabetic retinopathy. 30 healthy controls of more than 40 years of age and of both sex was also included. The study was conducted in the Department of Ophthalmology, Assam Medical College and Hospital, Dibrugarh, Assam, India for a duration of 1 year from July 2016 to June 2017. Ethical clearance was taken from institutional ethics committee.

Diabetic patients were recruited from diabetic clinic OPD and retina clinic, Assam Medical College and hospital, Dibrugarh. Healthy controls were randomly recruited from individuals accompanying patients. Each subject was explained the nature of the study and prior written informed consent was taken from every subject before inclusion in study. After obtaining detailed history, a comprehensive ophthalmological examination was done with special emphasis on the fundus examination. The level of severity of retinopathy was determined by indirect ophthalmoscopy for a pan retinal view, and stereoscopic slit lamp biomicroscopy of the disc and macula using +90D lens. Ancillary tests selectively done were stereo fundus photography, fluorescein angiography

All 180 eyes (120 diabetic and 60 control eyes) were tested after pupil dilation using eye drops containing 0.8% tropicamide and 5% phenylephrine hydrochloride using a SD-OCT (Zeiss Cirrus 4000 HD OCT system.), a commercially available device with a scan speed of 27, 000 axial scans per second and an axial resolution of 5μm. All scans were acquired by the same operator. cirrus SD-OCT was used to acquire a macular scan using the macular cube 512×128 scan protocol. The GCA algorithm, incorporated into the cirrus SD-OCT software 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. The average, minimum and six sectoral GC-IPL thickness values supero-temporal (ST), superior (S), supero-nasal (SN), infero-nasal (IN), inferior (I), and infero-temporal (IT)) were measured from the elliptical annulus centered on the fovea. RNFL thickness was measured with the fast RNFL scanning protocol (256 A-scans).

After proper alignment, 200×200 cube optic disc scan were obtained by centering a circle of fixed diameter (3.4mm) on the disc. The system extracts data from the cube 256A-scan samples along the path of the calculation circle. The process results in a T, S, N, I, and T profile map. The TSNIT map displays RNFL thickness values by quadrants and clock hours. Only good-quality scans, defined as scans with signal strength ≥six, were used for the analysis.

Exclusion criteria

- Type 1 diabetes.
- Moderate and severe non-proliferative diabetic retinopathy.
- Proliferative diabetic retinopathy.
- Diabetic macular edema.
- Any type of previous retinal treatment (laser phocoagulation, vitrectomy, intravitreal steroids, and/or antiangiogenic drugs).
- Refractive error >6D.
- Hazy media.
- Other ocular diseases such as cataract, uveitis or macular degeneration.
- Diagnosed cases of glaucoma or those with intraocular pressure (IOP) >21 mm Hg in either eye; those showing evidence of a reproducible Visual field defects in either eye, as detected using the Humphrey Visual Field analyzer.
- Neurodegenerative disease such as Alzheimer's, Parkinson's and dementia.

Statistical analysis

All data are in the form of percentage and/or mean ±SD. RNFL and GC-IPL measurements were compared using one-way ANVA test and calculated p value <0.05 was regarded as significant followed by the Bonferroni post hoc test. Furthermore, categorical variables were compared with the χ^2-test. Finally, Pearson's correlation was performed to evaluate the linear correlation between variables.

RESULTS

Most of the diabetic patients and control were distributed in the age group of 50-59 years (35% diabetic patients and 43.33% controls). The mean age group of diabetic patients was 55.20±6.76 years and controls were 52.33±6.09 respectively. Out of 60 diabetic patients 35 (58.33%) were males and 25 (41.67%) were females and out of 30 controls 16 (53.33%) were males and 14 (46.67%) were females. The mean duration of diabetic patients was 4.78±2.22 years (no DR group, 4.21±3.03 and mild DR group, 5.35±3.32). The mean HbA1c of diabetic patients was 7.95±0.94% (no DR group, 7.72±1.20% and mild DR group, 8.17±1.20%)

The average RNFL thickness was 86.18±8.44μm and 91.79±4.77μm in diabetic patients and controls respectively (p=0.002). Furthermore, for two different groups of diabetic patients, the average RNFL thickness was 86.74±11.18μm in the no DR group and 85.62±11.10μm in the mild DR group (p=0.697) Both the no DR group and mild DR group showed a statistically significant difference in the average RNFL thickness compared with the control group (p=0.027 and p=0.007, respectively).Moreover, the RNFL thickness was significantly different between the diabetic patients and controls in the superior, inferior and temporal quadrants (Table 1).

Table 1: Retinal nerve fiber layer thickness in diabetic patients and control.

RNFL thickness (μM)	DM with no DR		DM with MDR		Overall		Control	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Average	86.74	11.18	85.62	11.10	86.18	8.44	91.79	4.77
Superior	106.93	22.36	104.90	22.43	105.92	17.78	114.77	13.87
Nasal	66.73	14.73	68.25	15.76	67.49	10.53	69.10	9.44
Inferior	111.13	16.70	108.52	16.71	109.83	11.23	116.33	10.97
Temporal	62.17	11.81	60.80	11.35	61.48	8.67	66.95	5.86
RNFL thickness (μM)	DM with No DR Vs. Control		DM with MDR Vs. Control		Overall Vs. Control		DM with No DR Vs. DM with MDR	
Average	0.027		0.007		0.002		0.697	
Superior	0.108		0.045		0.036		0.726	
Nasal	0.462		0.801		0.536		0.702	
Inferior	0.160		0.036		0.027		0.546	
Temporal	0.052		0.010		0.006		0.649	

*Values were compared by One-way ANOVA test followed by Bonferroni post hoc test

Table 2: Ganglion cell layer-inner plexiform layer thickness in diabetic patients and controls.

GCL-IPL thickness (μM)	DM with NO DR		DM with MDR		Overall		Control	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Average	80.15	5.78	79.75	5.70	79.95	4.32	84.66	3.26
Superior nasal	80.83	13.41	79.30	14.44	80.07	9.27	84.05	6.73
Superior	81.28	12.53	79.62	10.72	80.45	8.46	85.35	8.16
Supero temporal	80.00	11.45	79.77	10.26	79.88	6.73	84.98	5.47
Infero nasal	79.58	11.56	80.35	11.32	79.97	7.22	84.18	5.65
Inferior	79.33	12.52	79.65	9.06	79.49	8.08	84.33	5.60
Infero temporal	79.85	8.68	79.80	10.12	79.83	6.03	85.03	4.92
GCL-IPL thickness (μM)	DM with NO DR VS. Control		DM with MDR VS. Control		Overall VS. Control		DM with NO DR VS. DM with MDR	
Average	0.0004		0.0001		<0.001		0.788	
Superior nasal	0.245		0.107		0.062		0.672	
Superior	0.142		0.023		0.026		0.581	
Supero temporal	0.036		0.017		0.002		0.934	
Infero nasal	0.055		0.102		0.015		0.796	
Inferior	0.051		0.019		0.009		0.911	
Infero temporal	0.006		0.014		0.001		0.984	

*Values were compared by One-way ANOVA test followed by Bonferroni post hoc test

Table 3: Correlation among diabetes duration, HbA1c VALUE, RNFL thickness and GCL-IPL thickness in diabetic patients.

		Duration of diabetes mellitus	HbA1c value
RNFL thickness (μm)			
Average	r	-0.2003	-0.122
	p	0.289	0.521
Superior	r	-0.225	-0.0002
	p	0.231	1.00
Nasal	r	0.0137	-0.2039
	p	0.943	0.281
Inferior	r	-0.173	-0.199
	p	0.363	0.292
Temporal	r	-0.109	0.0316
	p	0.566	0.868
GCL-IPL thickness (μm)			
Average	r	-0.130	-0.063
	p	0.493	0.741
Superior Nasal	p	0.050	-0.104
	r	0.792	0.584
Superior	p	-0.075	-0.050
	r	0.697	0.797
Supero Temporal	p	0.056	-0.035
	r	0.771	0.858
Infero Nasal	p	-0.312	0.065
	r	0.093	0.732
Inferior	p	-0.106	-0.039
	r	0.581	0.838
Infero Temporal	p	-0.077	-0.031
	r	0.685	0.875

The average GCL-IPL thickness was 79.95±4.32μm and 84.66±3.26μm in diabetic patients and controls, respectively (p=<0.001). Furthermore, for two different groups of diabetic patients, the average GCL-IPL thickness was 80.15±5.78μm in the no DR group and 79.75±5.70μm in the mild DR group (p=0.788) Both the no DR group and mild DR group showed a statistically significant difference in the average GCL-IPL thickness compared with the control group (p=0.004 and p=0.0001, respectively).Moreover, the GCL-IPL thickness was significantly different between the diabetic patients and controls in the superior, supero-temporal, infero-nasal, inferior and infero- temporal quadrants (Table 2).

Correlation among diabetes duration, HbA1c value, RNFL thickness and GCL-IPL thickness in diabetic patients found to be non-significant (Table 3).

DISCUSSION

In this cross-sectional study using SD-OCT, the GC-IPL and RNFL thickness were evaluated in asymptomatic type 2 diabetic patients with no or mild DR.

Overall, the current analysis revealed a statistically significant reduction of the mean GCL-IPL and RNFL thickness in type 2 diabetic patients with no or mild DR compared with a homogenous control group.

These findings were also present in patients without any sign of DR compared with healthy controls, indicating this alteration occurs early in diabetes.

In our present study, we observed thinning of GCL- IPL in patients with DM, both groups with no DR and those with mild DR. In addition, there was statistically

significant thinning of GCL + IPL between diabetes eyes and control eyes in several sectors (superior, supero temporal, infero nasal, inferior and infero temporal, respectively). In our study we found RNFL thickness was reduced significantly in diabetes group compared with those controls and also thinning was significant in other two groups of diabetes, no DR and mild DR group as compared with controls. In contrast to the previous study done by Lopes de Faria et al, Takahashi et al, Kern et al, our present study showed statistically significant RNFL thinning in superior, temporal and inferior quadrants.[23-25] Nor-Sharina et al, noticed that the thickest RNFL in nasal quadrant might be due to the lack of microaneurysm presence in this area and therefore less retinal nerve fibre layer damage occurred in this quadrant.[26] Similarly, in our present study, thinning was not significant in the nasal quadrant between diabetic patients and controls.

The findings of the present study were consistent with the study of Rodrigues et al and Carpineto et al who observed a significant reduction in thickness of ganglion cell layer and retinal nerve fiber layer in patients with no DR or mild DR, which suggests neuroretinal changes occur before vascular signs of diabetic retinopathy.[27,28]

In the present study, no significant correlation was found between RNFL and GCL-IPL thickness with diabetic duration and HbA1c value in type 2 diabetic patients. These results were also observed in studies done by Chihara et al, Peng et al and Nor-Sharina et al.[29,26]

CONCLUSION

In present study, we have detected changes in the GCL-IPL and the RNFL thickness by the SD-OCT in type 2 diabetic patients with no DR and mild DR. These changes may be related to both neuronal and vascular abnormalities that occur in the early stage of diabetic retinas. By measuring GC-IPL and RNFL thicknesses separately, the present study showed that in subjects with DM, RGC neurons are vulnerable to damage prior to the onset of apparent microvascular DR lesions compared with healthy controls, as reflected by GC-IPL and RNFL thinning, and such RGC damage is likely to progress in subsequent severe forms of DR development. We also confirmed the role of SD-OCT for the evaluation of asymptomatic diabetes patient without any sign of DR and also that neuroretinal degeneration occurred early, preceding microvascular damages. Since early detection of diabetic ocular complications is utmost important to maintain a useful vision, the thinning of the inner retinal layers such as RNFL, GCL, and IPL may indicate initial damage of DM on the posterior pole before the appearance of obvious retinal findings.

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