Research Article

A study of retinal neurodegeneration using optical coherence tomography in type 2 diabetes mellitus patients without retinopathy: A cross-sectional study

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ABSTRACT

Background: Neurodegeneration of retina (retinal diabetic neuropathy) is the earlier sign of diabetes mellitus before the appearance of any vascular sign of diabetic retinopathy. Hence, through neurodegenerative changes, we can diagnose and monitor the early neuronal damage of the retina. **Objectives:** The objective of the study were to establish whether the retinal neurodegeneration is earlier than vasculopathic changes in patients with diabetes and their relationship with the duration of diabetes. **Materials and Methods:** A total of 180 diabetic patients and 164 healthy controls were collected from a rural-based tertiary care hospital in West Bengal for this cross-sectional study. All have gone through proper history taking, comprehensive ocular examinations and spectral-domain optical coherence tomography imaging to detect the thickness of retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL). **Results:** Our study showed that the RNFL thickness of the right and left eyes for the cases was 37.62 μm and 37.68 μm, respectively, and that for controls were 39.68 μm and 39.70 μm, respectively. The GCL thickness of the right and left eyes was 32.63 μm and 32.43 μm, respectively, in cases and that for controls were 33.73 μm and 33.87 μm, respectively. In respect of the duration of diabetes, mean RNFL thickness of the right and left eyes for <1 year, 1–2 years, and >2 years was 40.20 μm and 40.03 μm; 38.17 μm and 38.31 μm; and 35.48 μm and 35.69 μm, respectively. Mean GCL thickness of the right and left eyes for <1 year, 1–2 years, and >2 years was 34.25 μm and 33.72 μm; 31.98 μm and 31.44 μm; and 30.82 μm and 31.35 μm, respectively. The data were analyzed by appropriate statistical methods. **Conclusion:** This study showed that thinning of RNFL and GCL occurred in diabetes before the appearance of microaneurysm.

KEY WORDS: Diabetic Retinopathy; Retinal Nerve Fiber Layer; Ganglion Cell Layer; Retinal Neurodegeneration; Optical Coherence Tomography

INTRODUCTION

Diabetes mellitus (DM) accounts for about 425 million cases in the world, projected to reach 629 million by 2045. One in every two patients remains undiagnosed. There were 72 million cases in India in 2017. With the prevalence of 8.8, it will reach to 101.2 million by 2030.[1] As diabetes is a fast progressing pandemic and one in every three diabetic patients has diabetic retinopathy (DR), there is also a high load of DR.[2]

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Diabetes affects the eyes in many ways. It can cause early cataract formation, increase adnexal infective disease, corneal wearing out, decrease corneal sensation, frequent change of refractive error, retinal vascular, and neuropathic changes. Of these DR is the most common and serious complication and may result in permanent visual loss which is also preventable blindness.

The complex pathology of DR affects both vascular and neural tissue. Apart from the microvascular complication of diabetes (different stages of DR), retinal neurodegeneration is an important and early component of retinopathy.[3-5] This retinal diabetic neuropathy (RDN) is observed structurally, as neural apoptosis, ganglion cell loss, reactive gliosis, glutamate excitotoxicity, overexpression of renin-angiotensin system, decrease in neuroprotective factor, and impairment of neurovascular coupling.[6-8] These subtle functional changes are not detected by fundus examination.[9] The relationship between RDN and retinal vascular changes is still not established. Previously, it is thought that microaneurysms causes RDN, but recent evidence using optical coherence tomography (OCT) clearly shows that RDN is an early change in DM patients and independent of any vascular abnormalities of the retina.[10]

Most of the studies in India and world have carried out regarding the prevalence, progression, and treatment outcome of various methods of DR. However, there are few studies regarding the neurodegenerative ocular changes due to DM. The purpose of this study is to see the effects on retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL) at large, in a larger sample size using OCT and to see what and how much thinning actually occurs in diabetic patients without any microaneurysm, compared to non-diabetic patients.[8] It is thus an attempt to see retinal changes through neurodegeneration before clinical symptoms and signs and vascular abnormalities of the retina so that we can predict and prevent DR well in advance. The concept of neurodegeneration as an early component of DR explores alternative therapies to prevent the onset of vision loss and preserves the integrity of the neural retina by potential neuroprotective approaches using different neurotrophic factors that rescue damaged retinal ganglion cells (RGCs) and inhibit the progression of RGC loss and axonal degeneration.[11]

Objectives

The purpose of this study was to identify whether the neurodegenerative changes in retina are earlier than vasculopathic changes in patients with diabetes.

To compare the RNFL and GCL thickness obtained by OCT between diabetic patients with no DR and their age- and sex-matched healthy subjects.

The purpose of this study was to assess the relationship between the thickness of RNFL and GCL with the duration of diabetes.

The purpose of this study was to evaluate OCT as a clinical test for early diagnose of retinal neurodegeneration in DM.

MATERIALS AND METHODS

All patients with diabetes above the age of 40 were collected from the outpatient Department of Ophthalmology and in the Diabetic Clinic under Department of Internal Medicine of Burdwan Medical College and Hospital, meeting the inclusion and exclusion criteria and their age- and sex-matched control groups. Diabetes is defined as per the American Diabetes Association (ADA) criteria, i.e. fasting blood glucose (FBS) ≥ 126 mg/dl or postprandial blood sugar (PPBS) ≥ 200 mg/dl or glycated hemoglobin (HbA1c) ≥ 6.5% or random blood sugar (RBS) ≥ 200 mg/dl in patients with symptoms of hyperglycemia or hyperglycemic crisis.

A total of 180 diabetic patients as cases (as per the ADA criteria) and 164 healthy cases as with their age- and sex-matched control population were selected. The study period was 12 months from June 2017 to May 2018. It is a hospital-based cross-sectional observational case–control study. The patients with DM with no DR above the age of 40 years and their age- and sex-matched control subjects giving consent were included in this study. No DR was defined as no microaneurysm conforming to level none of the International Clinical DR Disease Severity Scale. Age and sex match controls were free of any ocular disease, systemic hypertension, diabetes or any other systemic disease. Diabetic patients with refractive error more than +5D or −6D, best-corrected visual acuity below 20/25, significant media opacity, glaucoma, uveitis, history of previous laser treatment, intravitreal injection, previous any intraocular surgery, any retinal or choroidal disease, taking any retinotoxic drugs, and having infectious disease which causes neurodegeneration such as leprosy, neurotrophic viral keratitis, and having systemic neurodegenerative disorders such as dementia, Parkinson's disease, Alzheimer's disease, mono- and polyneuropathies, pregnancy, <40 years of age, and refused to give valid informed consent were excluded from this study.

Laboratory parameters of FBS, RBS, PPBS, and HbA1c were investigated and documented. All patients had gone through detailed medical and ocular history taking, refraction, intraocular pressure checking, external ocular examination, slit-lamp biomicroscopy with 90D examination, indirect ophthalmoscopy on dilated pupil, and visual field testing by Humphrey Field Analyzer and OCT imaging to detect the mean thickness of RNFL and GCL of cases and controls. A spectral-domain OCT (SD-OCT) machine (Topcon 3D OCT-1 Maestro, Japan) was used. A 6 mm × 6 mm wide 3D macular cube (512 A scans by 128 B scans) scan combined with automatic segmentation was performed for measurement and topographical maps of the macula with reference database in one scan. It provides 50,000 A scans each second with a 20 μ lateral and 2 μ in-depth resolution. The mean RNFL thickness was calculated by averaging thickness measurements of nine macular sectors around the fovea within three concentric grids as defined by the Early Treatment DR Study of cases and controls separately. For GCL thickness GCL+ was selected. Our area of interest for the macula was around

the foveola, and the average distance was around 100 µ. The values were averaged for each patient, and the mean value of GCL was considered. Scans were done twice for each subject to assess the repeatability of measurements. The study was conducted with approval by the Institutional Ethical Committee. The statistical analysis was performed using software SPSS version 20. The number of patients and percentage of patients were compared across the groups using Pearson's Chi-square test for independence of attributes/Fisher's exact test as appropriate. Continuous variables were expressed as mean and standard deviation (SD) and compared across the groups using Mann–Whitney U-test/Kruskal–Wallis test as appropriate. For all cases, $P < 0.05$ was considered as statistically significant.

RESULTS

A total of 180 cases and 164 controls were included in our study. Duration of diabetes detected had been divided into three groups, namely, <1 year, 1–2 years, and >2 years. There were 95 males in the cases and 91 in control group. Females were 85 and 73 in number, respectively. Our study showed that the mean age of cases and controls was 56.87 ± 9.26 and 57.29 ± 10.90 years, respectively, which is not significant ($P = 0.964$). Male and female percentage of cases and controls was 52.78 and 47.22 and 55.49 and 44.51, respectively, which is not significant ($P = 0.614$). The percentage of Hindu and Muslim patients of cases and controls was 53.89 and 46.11 and 56.1 and 43.9, respectively, which is also not significant ($P = 0.681$) [Table 1].

The RNFL thickness of the right eye in cases was having a mean of 37.62 µm with SD of 3.10, and in control group was 39.68 µm with SD of 2.45. The mean RNFL thickness for the left eye in cases was 37.68 µm with SD of 2.99 and in control group was 39.70 µm with SD 2.42. The GCL thickness of the right eyes and left eyes was 32.63 µm ± 2.54 and 32.43 µm ± 3.14, respectively, in cases group. The GCL thickness in the non-diabetic control groups was 33.73 µm ± 2.20 and 33.87 µm ± 1.78 for the right and left eyes, respectively. This reduction of GCL thickness was also significant at the level of $P < 0.001$ [Table 2].

In respect of duration of diabetes, mean RNFL thickness of the right and left eyes for <1 year, 1–2 years, and >2 years was 40.20 µm ± 2.48 and 40.03 µm ± 2.52; 38.17 µm ± 2.21 and 38.3 µm 1 ± 2.01; and 35.48 ± 2.2 µm 4 and 35.69 µm ± 2.31, respectively, which was significant ($P < 0.001$). Mean GCL thickness of the right and left eyes for <1 year, 1–2 years, and >2 years was 34.25 µm ± 2.06 and 33.72 µm ± 1.74; 31.98 µm ± 2.09 and 31.44 µm ± 5.27; and 30.82 µm ± 1.96 and 31.35 µm ± 1.73, respectively, which was also significant ($P < 0.001$) [Table 3].

Table 1: Demographic details of cases and controls

Parameter	Cases (*n*=180)	Controls (*n*=164)	*P* value	Significance
Age in years mean (±SD)	56.87±9.26	57.29±10.90	0.964	Not significant
Male:Female	1.12:1.00	1.24:1.00	0.614	Not significant
Hindu:Muslim	1.17:1.00	1.27:1.00	0.681	Not significant

SD: Standard deviation

Table 2: RNFL and GCL thickness in µm of cases (*n*=180) and controls (*n*=164)

Parameter	Cases		Controls		*P* value	Significance
	Mean	SD	Mean	SD		
RNFL thickening; RE	37.62	3.10	39.68	2.45	<0.001	Significant
RNFL thickening; LE	37.68	2.99	39.70	2.42	<0.001	Significant
GCL thickening; RE	32.63	2.54	33.73	2.20	<0.001	Significant
GCL thickening; LE	32.43	3.14	33.87	1.78	<0.001	Significant

RNFL: Retinal nerve fiber layer, GCL: Ganglion cell layer, RE: Right eye, LE: Left eye, SD: Standard deviation

Table 3: RNFL and GCL thickness in µm in respect of the duration of diabetes (*n*=180)

Parameter	Duration of diabetes						*P* value	Significance
	<1 year		1–2 years		>2 year			
	Mean	SD	Mean	SD	Mean	SD		
RNFL thickening; RE	40.20	2.48	38.17	2.21	35.48	2.24	<0.001	Significant
RNFL thickening; LE	40.03	2.52	38.31	2.01	35.69	2.31	<0.001	Significant
GCL thickening; RE	34.25	2.06	31.98	2.09	30.82	1.96	<0.001	Significant
GCL thickening; LE	33.72	1.74	31.44	5.27	31.35	1.73	<0.001	Significant

RNFL: Retinal nerve fiber layer, GCL: Ganglion cell layer, RE: Right eye, LE: Left eye, SD: Standard deviation

DISCUSSION

We have found in our cross-sectional study that the demographic profiles of the cases and controls are statistically insignificant. The mean RNFL thickness for the right eye in a case of DM without DR decreases in comparison with the healthy control subject and that for the left eye is also same. In OCT we also found that the mean GCL thickness in both the eyes of diabetic patients has thinned out even before the development of retinopathic and or vasculopathic changes. In other words, a neuropathic change occurs earlier than a vasculopathy change in patients with type 2 DM. Mean thickness of RNFL of the right eye and left eye progressively decreases with the duration of diabetes. This is also true for the GCL thickness. Hence, the RNFL and GCL thickness in diabetic patients without DR has a statistically significant thinning ($P < 0.05$) compared to age- and sex-matched non-diabetic healthy subjects. As the duration of diabetes increases, the thickness of RNFL and GCL also decreases significantly.

Our findings are in concordance with several studies. Sohn *et al*. reported that in 45 people with DM and no to minimal DR there was significant, progressive loss of the NFL (0.25 μm/y) and the GC/inner plexiform layer (IPL) (0.29 μm/y) on OCT over a 4-year period, independent of age and sex.[12] Our study also has similar results showing significant loss of RNFL and GCL. The decrease in thickness of RNFL and GCL over the years as duration of diabetes progresses is also significant. Vujosevic and Midena examined 30 patients with no DR, 44 patients with non-proliferative DR. They have shown that there was statistically significant thinning of RNFL and GCL thickness in the macula. In the peripapillary area, there were no differences between diabetics and controls.[3] This result is also similar to our result, which shows significant RNFL and GCL thinning ($P < 0.001$) in the macula. Our values are more or less compatible with the study results of Cabrera DeBuc and Somfai, who have found reduced RNFL thickness in the pericentral and peripheral regions (27 ± 2 vs. 18 ± 5 μ and 42 ± 3 vs. 33 ± 9 μ, respectively, $P < 0.001$) and reduced thickness of GCL + IPL complex in the pericentral region of the macula (92 ± 7 μ vs. 80 ± 10 μ, $P < 0.001$) in the mild DR group.[13] Peng *et al.* in their study have shown using Stratus OCT that the mean RNFL thickness in diabetic patients and healthy subjects is significantly different (104.2 [SD 10.4] and 108.6 [SD 9.2] μ, respectively; $P = 0.004$). Compared with the healthy group, the RNFL thickness in the diabetic group is also significantly less in the superior quadrant and at the 5, 11, and 12 o'clock sectors ($P = 0.04$, 0.002, and 0.001, respectively).[14] This is also almost compatible with our study results, which is on average significant at level $P < 0.001$. A study from Eastern India, conducted by Chakrabarty *et al*. examined 500 eyes of 250 patients and concluded that there was low RNFL thickness around the optic nerve in patients with high glucose parameters, especially for HbA1c. In our study, the RNFL thickness is also low at the pericentral and peripheral areas around the fovea.[15] Tavares Ferreira *et al.* have shown that the thickness of the GCL (I3 and N6 sectors), IPL (S6 and N6 sectors), inner nuclear layer (T6 and N6 sectors), and outer plexiform layer (S6 sector) as well as the overall retinal thickness (RT) (S3, N3, I3, S6, and T6 sectors) is decreased at the second visit after 12 months ($P < 0.001$).[16] Our study and the aforesaid study are similar. Carpineto *et al*. using SD-OCT have found in their study that mean GCL-IPL thickness is 85.3 ± 9.9 μm in healthy controls groups and that thickness reduces to 80.6 ± 8.1 μm in no-DR. Average RNFL thickness is 91.2 ± 7.3 μm in controls and was 86.4 ± 10.2 μm in no-DR group.[4] Our study also supports this showing significant decrease in thickness. Our study result is also similar to Sugimoto *et al*. who have found that the overall RT significantly increased ($P = 0.03$) and RNFL thickness significantly decreased ($P = 0.02$) in the superior areas in no DR eyes compared to normal.[17] Our study results are compatible with the study of van Dijk *et al*. In their very famous study they have shown that RNFL, GCL, and IPL are thinner in the pericentral area of the macula in patients with diabetes but no or minimal DR compared to normal controls (respective difference 1.9 μm, 95% confidence interval [CI] 0.3–3.5 μm; 5.2 μm, 95% CI 1.0–9.3 μm; 4.5 μm, 95% CI 2.2–6.7 μm). RNFL and IPL thickness is also reduced in peripheral area of the macula in patients with DM but no or minimal DR compared to controls (respective difference 3.2 μm, 95% CI 0.1–6.4 μm; 3.3 μm, and 95% CI 1.2–5.4 μm).[18]

Strength and Limitations

Most of the previous studies were performed with small sample size. We have performed with a larger number of cases. However, as compared with the prevalence of diabetes and DR, the study population was also relatively small. The study duration and the study period were also short. As the cases and controls were examined only once, the course of the disease and the natural history of the effect due to the disease could not be studied. The actual onset of DM could not be actually predicted as the patient population belonged to different socio-economic group and their lack of awareness. An undiagnosed case of neuropathy was not ruled out, which may have interfered with the OCT results.

CONCLUSION

DM is a fast progressing pandemic in the world. About one-third of diabetic patients have DR which is a serious complication of eye and may result in permanent visual loss. Hence, early diagnosis and prompt treatment of DR are important to save the vision. There are evidences of retinal neurodegeneration without microvascular abnormalities in patients with diabetes.

In this study, it has shown that RNFL and GCL thickness in macula decrease in the diabetic eye even without any

clinical sign of retinopathy than in normal subjects. Reduced RNFL and GCL thickness is due to progressive apoptosis of ganglionic cells and astrocytes with axonal degeneration induced by diabetes. This thinning of RNFL and GCL suggests that retinal neurodegeneration is an early event in DM before the clinical stage of DR, and this neurodegeneration is independent of any vascular abnormalities of retina.

The RNFL and GCL thickness measurement by SD-OCT may be a useful tool to diagnose and monitor the early neuronal damages of the retina in DM. Hence, it is important to identify new therapeutic targets in the early stage of DR which prevent further neurodegenerative and vascular damages.

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