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COMPARISON OF RETINAL NERVE FIBRE LAYER THICKNESS BETWEEN DIABETIC RETINOPATHY AND NON-DIABETIC PATIENTS USING OPTICAL COHERENCE TOMOGRAPHY AT REGIONAL INSTITUTE OF OPHTHALMOLOGY, KOLKATA

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Abstract

Background and objective: Diabetes mellitus is the leading cause of blindness in the working age population world- wide. Retinopathy is the most common microvascular complication of diabetes, resulting in blindness. The emerging evidence positively shows that much before the onset of micro-vascular changes, the neuronal tissue loss occurs which results to a decrease in RNFL thickness. Here is a study to compare the retinal nerve fiber thickness in diabetic retinopathy and non diabetic patients using optcal coherence tomography (OCT) at Regional institute of ophthalmology, Kolkata. ***Methods:*** Hospital based observational crossectional study of 95 patients (190 eyes) with diabetic retinopathy and 95 non- diabetic (190 eyes) patients presenting to outpatient department of RIO, Medical College, Kolkata was included in our study. Patient was selected by systematic random sampling method. Best corrected visual acuity and RNFL thickness was measured by OCT in central 3.4 mm area around the optic disc. ***Results and Observations:*** The inferior retinal nerve fiber layer (RNFL) thickness in the diabetic group was 122.00 ± 8.08 microns and in the non-diabetic group was 128.84 ± 2.26 microns. The p-value was 0.000, which is statistically significant. The superior retinal nerve fiber layer thickness in the diabetic group was 118.00 ± 8.08 microns and in the non-diabetic group was 124.00 ± 2.31 microns. The p-value was 0.000, which is statistically significant. The nasal retinal nerve fiber layer thickness in the diabetic group was 72.00 ± 9.44 microns and in the non-diabetic group was 76.03 ± 2.34 microns. The p-value was 0.000, which is statistically significant. The temporal retinal nerve fiber layer thickness in the diabetic group was 68.00 ± 9.22 microns and in the non-diabetic group was 72.03 ± 2.34 microns. The p-value was 0.000, which is statistically significant. Average retinal nerve fiber layer thicknes in the diabetic group was 95.00 ± 8.53 microns and in the non-diabetic group was 100.22 ± 2.27 microns. The p-value was 0.000, which is statistically significant. ***Conclusions:*** There is significant decrease in the RNFL thickness in Superior, Inferior, nasal, temporal quadrant as well as the average RNFL thickness in diabetic retinopathy patients. This is suggests that there is neurodegeneration in DR. this study suggests aggressive management of diabetes along with neuroprotective drug to prevent or reduce further damage to RNFL and also prevent progression of DR and its complication. Thus the burden of vision loss due to DR can be prevented.

Keywords: Diabetic retinopathy, Retinal nerve fiber layer thickness, optical coherence tomogram.

INTRODUCTION

Diabetes mellitus is the leading cause of blindness in the working age population world- wide. Retinopathy is the most common microvascular complication of diabetes, resulting in blindness for over 10,000 people with diabetes per year.1

Diabetes mellitus (DM) is a metabolic disease, involving inappropriately elevated blood glucose levels. DM has several categories, including type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary causes due to endocrinopathies, steroid use, etc. The main subtypes of DM are Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM), which classically result from defective insulin secretion (T1DM) and/or action (T2DM).

Epidemiological studies have described the natural history of and treatment for diabetic retinopathy. There is evidence that retinopathy begins to develop at least 7

years before the clinical diagnosis of type 2 diabetes mellitus.2

By 2030 an estimated 191.0 million people globally will have Diabetic Retinopathy (DR), an approximately 56.3 million will have vision threating DR. 3

The Wisconsin epidemiologic study of Diabetic Retinopathy (WESDR) cohort showed that after 20 years of DM, 99% of patients with typel and 60% of patients with type2 have some degree of diabetic retinopathy.4

Specifically vision loss in diabetes mellitus is seen in uncontrolled glycemic level and the risk of DR progression increases once retinal lesions become clinically visible. However this progression is preventable by achieving the glycemic control and reducing the disease duration.

HbAlc is glycosylated haemoglobin. It is formed due to non- enzymatic glycation pathway by haemoglobins exposure to plasma glucose and reflects the blood glucose over the last 8 to 12 weeks. In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, neuropathy, nephropathy, and retinopathy Monitoring HbA1c levels may improve outcome.5

There are various theories that describe the pathogenesis of DR, of which the most common accepted theory is micro-vascular theory. However the emerging evidence positively shows that much before the onset of micro-vascular changes, the neuronal tissue loss occurs which results to a decrease in RNFL thickness.6

Therefore non- glaucomatous optic nerve atrophy may be associated with DM patients. So, it is essential to study the neuro- degenerative component of DR so that at a later date a preventive method instead of interventional method can be adapted in its treatment.

Due to the asymptomatic nature of diabetic retinopathy early detection of diabetic retinopathy is more effective preventive strategy and optical coherence tomography has become an indispensable imaging technique in Diabetic retinopathy.Spectral Domain OCT allows for non -invasive in vivo cross-sectional image of ocular structure such as the retina, RNFL and optic nerve head. Spectral Domain OCT applies the principle of interferometry to determine the interface between different ocular tissues. Using automated segmentation algorithms based on reflectivity changes between adjacent retinal layers, the RNFL thickness can be calculated. 7-10

Our study aims to investigateretinal layer changes in patients with DR and their role in determining DR severity using spectral domain optical coherence tomography. Moreover, we also evaluated the possible association between HbA1c levels with retina layer changes in patients with DR.

MATERIALS AND METHODS

STUDY DESIGN: Hospital based observational crossectional study

STUDY SETTINGS: Regional Institute of ophthalmology,Kolkata, West Bengal.

STUDY PERIOD: 1 year 6 months

SAMPLE SIZE: Sample size was calculated using this formula:

$$n = 15.7\ (\sigma_1 \times \sigma_2)/d^2$$

Where, σ_1 and σ_2 are the standard deviations of the 2 study group (σ_1=10.07 and σ_2 =5.41) d is the smallest meaningful differences that can be measured (d = 3)

by using this formula 95 patients (190 eyes) with diabetic retinopathy and 95 non- diabetic (190 eyes) patients presenting to outpatient department of RIO, Medical College, Kolkata will be included in our study.

SAMPLING TECHNIQUE:

Systematic random sampling was done to select the cases.

PARAMETERS STUDIED:

i) Best corrected visual acuity.

ii) Retinal nerve fibre layer thickness along a 3.4mm diameter circle centering the optic nerve head. RNFL thickness defined as the number of pixels between the anterior and posterior edge of the RNFL detected using the attached automatic boundary detection software

iii) Fasting blood sugar [FBS] and post prandial blood sugar [PPBS] at first contact with patient. HbAlc at first contact with patient.

STUDY TOOLS:

1. Snellen's visual acuity chart.
2. Slit lamp biomicroscopy with +90D and+78D convex lenses
3. Autorefractometer
4. Streak retinoscope.
5. Indirect ophthalmoscope
6. Spectral domain hra+ OCT, HEIDELBERG ENGINEERING

INCLUSION CRITERIA:

- Patients aged 40 years and above newly diagnosed with diabetic retinopathy did not receive any treatment.
- Sex both male and female.
- Willingness to provide signed informed Consent Form by patients.

- Ability and willingness to return for all scheduled visits and assessments

EXCLUSION CRITERIA:

- Any ophthalmoscopic conditions where evaluation by indirect ophthalmoscopy and SD OCT procedures cannot be possible eg- NS grade 3 and onwards cataracts , complicated cataracts , cortical cataracts , corneal opacities.
- Any retinal vessel occlusive disease (BRVO, CRVO,CRAOetc), vitreoshaemorrhage, macular edema.
- High myopia.
- Neural ophthalmic anomalies.
- Glaucoma
- Hypertension.
- Pregnancy.
- Patients with history of other eye disease or surgery or photocoagulation.

DATA COLLECTION,CALCULATION AND INTERPRETATION:

This clinical cross sectional comparative study was conducted in a tertiary care hospital and throughout the period of study a total of 190 patients were taken up for study, fulfilling inclusion and exclusion criteria.

The study was conducted after taking clearance from Institutional Ethical committee.

A comprehensive ophthalmic examination was performed on all study participants after taking informed consent, which included a thorough medical history, assessing best corrected visual acuity (BCVA) using Snellen'schart , examining the anterior segment using Slit lamp biomicroscope, dilated fundus examination using +20D volk lens and indirect ophthalmoscope. Both groups were age matched. Average RNFL thickness, along with RNFL of each quadrant of individuals was noted using SD OCT, and compared between two groups.

STASTICAL ANALYSIS :

The data of all enrolled patients were collected and entered in Microsoft excel software spreadsheet. Data were analysed and the results were presented as means, standard deviations, frequencies and percentages along with various tables, bar diagrams, pie charts. The data were tabulated in a Microsoft excel and were analysed using SPSS V.24software . P value of less than 0.05 is considered statistically significant.

ETHICAL CLEARENCE:

The study protocol, patient information sheet and the Informed Consent Form (ICF) in Bengali, English and Hindi versions is submitted to the Institutional Ethics Committee of Medical College Kolkata for approval. Subject recruitment was commenced once written approval was obtained from IEC (Ref. No. MC/KOL/IEC/NON-SPON/2252/03/2024 dated 07/03/2024).

RESULT

The comparison of age group distribution between diabetic and non-diabetic patients (Table 1) shows highest proportion of study population in the diabetic group was in the 50-60 years category (35.79%), while in the non-diabetic group it was also 50-60 years (49.47%). The p-value was 0.054, which is not statistically significant.

Table 1: showing age distribution between Diabetic and Non-diabetic patients

Parameter		Group				P value
		Diabetic		Non-diabetic		
		N	%	N	%	
Age group	40-50 years	29	30.53%	24	25.26%	0.054
	50-60 years	34	35.79%	47	49.47%	
	60-70 years	27	28.42%	21	22.11%	
	70-80 years	5	5.26%	3	3.16%	

The comparison of sex distribution (Table 2) between diabetic and non-diabetic patients showed females constituted 49.47% of the diabetic group and 45.26% of the non-diabetic group, while males constituted 50.53% and 54.74% respectively. The p-value was 0.561, which is not statistically significant.

Table 2: Comparison of sex distribution among diabetic and non-diabetic patients

Parameter		Group				P value
		Diabetic		Non-diabetic		
		N	%	N	%	
Sex	Female	47	49.47%	43	45.26%	0.561
	Male	48	50.53%	52	54.74%	

In our study the mean FBS in the diabetic group was 147.73 ± 34.56 mg/dL and in the non-diabetic group was 86.45 ± 7.62 mg/dL. The p-value was 0.000, which is statistically significant.

The mean PPBS in the diabetic group was 229.43 ± 54.15 mg/dL and in the non-diabetic group was 115.48 ± 12.54 mg/dL. The p-value was 0.000, which is statistically significant.

The mean HbA1c in the diabetic group was 6.80 ± 0.44% and in the non-diabetic group was 5.34 ± 0.17%. The p-value was 0.000, which is statistically significant.

Table3: Comparison of HbA1C among diabetic and non-diabetic patients

Parameter	Group	Mean	SD	P value
HbA1C	Diabetic	6.80	0.44	0.000
	Non-diabetic	5.34	0.17	

The comparison of inferior retinal nerve fiber layer thickness (table 4) between diabetic and non-diabetic patients shows, the mean value in the diabetic group was 122.00 ± 8.08 microns and in the non-diabetic group was 128.84 ± 2.26 microns. The p-value was 0.000, which is statistically significant.

Table 4: Comparison of Inferior RNFL thickness

Parameter	Group	Mean	SD	P value
Inferior RNFL thickness	Diabetic	122.00	8.08	0.000
	Non-diabetic	128.84	2.26	

In our study it was shown (table 5) that the mean value of superior RNFL thickness in the diabetic group was 118.00 ± 8.08 microns and in the non-diabetic group it was 124.00 ± 2.31 microns. The p-value was 0.000, which is statistically significant.

Table 5: Comparison of superior RNFL thickness

Parameter	Group	Mean	SD	P value
Superior RNFL thickness	Diabetic	118.00	8.08	0.000
	Non-diabetic	124.00	2.31	

The comparison of nasal retinal nerve fiber layer thickness (table 6) in the study population showed that, the mean value in the diabetic group was 72.00 ± 9.44 microns and in the non-diabetic group was 76.03 ± 2.34 microns. The p-value was 0.000, which is statistically significant.

Table 6: Comparison of nasal RNFL thickness

Parameter	Group	Mean	SD	P value
Nasal RNFL thickness	Diabetic	72.00	9.44	0.000
	Non-diabetic	76.03	2.34	

In our study the mean of temporal RNFL thickness (table 7) in the diabetic group was 68.00 ± 9.22 microns and in the non-diabetic group was 72.03 ± 2.34 microns. The p-value was 0.000, which is statistically significant.

Table 7: Comparison of temporal RNFL thickness

Parameter	Group	Mean	SD	P value
Temporal RNFL thickness	Diabetic	68.00	9.22	0.000
	Non-diabetic	72.03	2.34	

The comparison of average retinal nerve fiber layer thickness (table 8) showed that the mean value in the diabetic group was 95.00 ± 8.53 microns and in the non-diabetic group was 100.22 ± 2.27 microns. The p-value was 0.000, which is statistically significant.

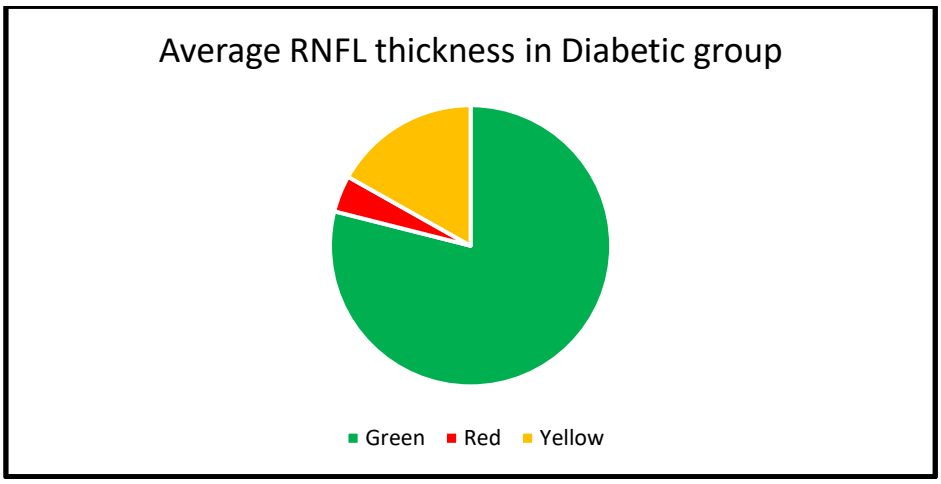
Table 8: Comparison of average RNFL thickness

Parameter	Group	Mean	SD	P value
Average RNFL thickness	Diabetic	95.00	8.53	0.000
	Non-diabetic	100.22	2.27	

The distribution of average RNFL thickness in the diabetic group by colour coding as shown in the table 9.. Green colour (normal) was present in 150 patients (78.95%), Yellow colour (borderline) in 32 patients (16.84%), and Red colour (thinned) in 8 patients (4.21%).

Table 9: Distribution of average RNFL thickness in Diabetic group

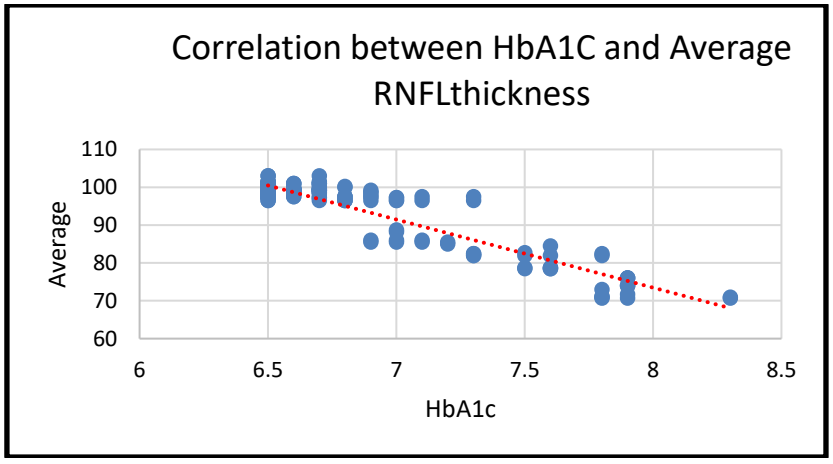
Parameter		N	%
Colour in Diabetic group	Green (Normal)	150	78.95%
	Yellow (Borderline)	32	16.84%
	Red (Thinned)	8	4.21%



The correlation between HbA1c and average retinal nerve fiber layer thickness (table 10) showed that the Pearson correlation coefficient (r) was -0.937 with a p-value of 0.000, which shows significantly negative correlation.

Table 10: Correlation between HbA1C and Average RNFL thickness

Correlation	Pearson Correlation coefficient (r)	P value	Interpretation
HbA1C and Average RNFL thickness	-0.937	0.000	Significantly Negative correlation



DISCUSSION

The assessment of retinal nerve fibre layer thickness in Diabetic patients with retinopathy can demonstrate the health of retina and the thinning of retinal nerve fibre layer thickness suggests the pathology i.e. apoptotic cells in retinal layer , that involves different type of neurons.

OCT, which uses short coherence length interferometer, has a fine resolution (up to 2microns) and reflects the histologic characteristics of the tissue[11,12]. Because OCT is based on cross-sectional images of the retina, the instrument measures the retinal NFL directly, has no need for a reference plane, and is known to be unaffected by the refractive status. axial length of the subject, or pupillary dilation[13,14,15].

The SPECTRALIS HRA+OCTTM, HEIDELBERG ENGINEERINGTM has eye tracking system and it negate the effect of eye movements. To avoid any influence of eye movements, we observed the scanned eye during B-scan and repeated the scan if we noticed any eye movement. To avoid software interface detection artifacts, we inspected every B-scan after acquisition and repeated the scan if the software was unable to detect the RNFL borders.

The purpose of the study was to measure the retinal nerve fibre layer thickness in diabetic patients with retinopathy and compare the same with non-diabetic patients and find out which quadrant has maximal change in thickness in relation to the glycemic levels. 190 eyes of 95 patients with Diabetes Mellitus with diabetic retinopathy and 190 eyes of 95 non-diabetic patients were evaluated.

Using the spectral domain OCT in this study, we were able to detect significant decrease in Inferior, Superior, nasal, temporal and Average RNFL thickness measurements in diabetic patients with retinopathy.

Multiple studies showed that diabetic patients have significantly reduced average peripapillary RNFL thickness compared to age matched non-diabetic controls. In most of the studiesSuperiorRNFL thickness showed a statistically significant difference between nondiabetics and diabetics with retinopathy. Inferior quadrant thinning of RNFL in diabetics with retinopathy was found in only a few studies like Carpineto et al[16] and Salvi et al.[17]Most of the studies have shown that the nasal and temporal quadrants were not affected, except in the studies by Carpineto et al. [18] and Mehboob et al.,[19] who reported a statistically significant difference in ARNFL and in all quadrants. The nasal quadrant was found to be the least affected due to the sparsity of retinal fibers in the region. Chhablani et al.[20] and Ambiya et al.[21]found no difference in ARNFL or the RNFL in all quadrants in people with DR.

Results show significant decrease in the average RNFL thickness with poor glycemic control{hbA1c>7}(P-0.00). According to clinical evidence, there is no doubt that glycemic control is most important for stability of DM and DR.

It should be noted that several reports also have shown interaction between glycemic control and retina change including blood-retina barrier (BRB) or macula edema progression [5-7]. Such vascular breakdown may change the tissue construction and it is reasonable to propose that RNFL damage is followed by this change as an aspect of neurodegenerative change.

LIMITATION OF THE STUDY:

In spite of every sincere effort my study has lacunae. The notable limitations of this study were:

- The main drawback of our study is the sample size. We can improve our study by increasing the sample size.
- The study has been conducted in a single centr, so hospital bias cannot be ruled out.
- Also, the patients are poorly characterized in aspects of several factors such as age, duration of diabetes, type of treatment whether the patient receives no treatment /oral hypoglycemic agents/Insulin .

Participants with DM were included in our study irrespective of the grade of DR.

CONCLUSION

1) There is significant decrease in the RNFL thickness in Superior, Inferior, nasal, temporal quadrant as well as the average RNFL thickness in diabetic retinopathy patients. This is suggests that there is neurodegeneration in DR.
2) There is less significant change in the Nasal and Temporal RNFL thickness noted compare to superior and inferior quadrant.
3) The decrease in the thickness in the superior, inferior, nasal,temporal and average RNFL is affected by raised HbA1c levels (poor glycemic control).
4)The decrease in the thickness in the average RNFL thickness is affected by raisedFasting Blood Sugar and Post Prandial Blood Sugar (poor glycemic control).

Thus this study suggests aggressive management of diabetes along with neuroprotective drugs to prevent or reduce further damage to RNFL and also prevent progression of DR and its complication. Thus the burden of vision loss due to DR can be prevented.

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