Analysis of Retinal Nerve Fibre Layer Thickness with HbA1c and Blood sugar levels in Type 2 Diabetes Mellitus patients without clinical diabetic retinopathy

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Abstract

Objectives:

The purpose of this study is to determine the correlation of retinal nerve fibre layer (RNFL) thickness with glycosylated haemoglobin (HbA1c) and blood sugar levels among Type 2 diabetic patients without clinical diabetic retinopathy. **Methodology:** This is a cross-sectional study involving 40 patients- Type 2 Diabetic patients without any evidence of diabetic retinopathy. A total of 80 eyes were observed. Evaluation of thickness of retinal nerve fibre layer along a 3.4mm diameter circle centred on the optic nerve head using SD-OCT in diabetic patients without clinical retinopathy. Comparison of the RNFL between patients with HbA1c <6, 6-7, >7 & with fasting blood sugar & post prandial blood sugar levels & which quadrant is maximally affected. **Results:** The decrease in the thickness in the average RNFL thickness is affected by raised HbA1c levels, raised Fasting Blood Sugar (FBS) & Post Prandial Blood Sugar (PPBS) levels (poor glycemic control). **Conclusion:** Non proliferative Diabetic Retinopathy in type 2 Diabetes mellitus patients appears to have thinner RNFL thickness and is significantly correlated with high level of fasting & post prandial blood sugar levels & glycosylated haemoglobin levels.

Abbreviation:

RNFL: Retinal Nerve Fibre Layer; HbA1c: Glycosylated Haemoglobin; LDL: Low Density Lipoprotein; NPDR: Non-Proliferative Diabetic Retinopathy; HRT: Heidelberg Retina Tomograph; OCT: Optical Coherence Tomography; ELISA: Enzyme- Linked Immunosorbent Assay; Hb-AGE: Haemoglobin Advanced Glycation End-Products; ApoB: Apolipoprotein B

INTRODUCTION-

Diabetes mellitus is the leading cause of new cases of blindness among adults aged 20 to 74 years. Diabetic retinopathy (DR) is a vascular disorder affecting the microvasculature of the retina. It is estimated that diabetes mellitus affects 4% of the world's population, almost half of whom have some degree of DR at any given time. DR occurs both in type 1 & type 2 Diabetes mellitus and has been shown that nearly all type 1 and 75% of type 2 DM will develop DR after 15 years of duration of diabetes[1,2].

Prevalence of DR in Winconsin epidemiological study of diabetic retinopathy (WESDR)[3] was 99% in Insulin dependent diabetes mellitus (IDDM) & 60% in Non Insulin dependent diabetes mellitus (NIDDM) Prevalence of DR was 54.2% in the Diabetes Control and Complication Trial (DCCT) study in IDDM[4] and 35-39% in United Kingdom Prospective Diabetes study in NIIDM[5]. Majority of the patients have NIIDM or type 2 diabetes. In two studies from south India, done in 2004 the prevalence rate of DR in NIIDM patients were 34.1% and 37% [6,7] . India has more than 62 million diabetic subjects at present as per WHO estimates[8]. In the Andhra Pradesh Eye Disease Study (APEDS) of self reported diabetics the

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prevalence of DR was 22.4%. in the Chennai Urban Rural Study (CURES), done in 2005 they evaluated the urban sample of diabetic patients and estimate the overall prevalence of DR as 17.6% [9].

Particularly vision loss in diabetes mellitus is seen in uncontrolled glycemic levels. Moreover, once set in, requires frequent ophthalmic examination and high cost drugs (eg. Bevacizumab) for treatment. However it is preventable by achieving the glycemic control and reducing the disease duration.

Normal vision depends on the normal function of the retinal neurons to produce a good quality of vision. The quality of vision starts to deteriorate early in diabetes, before the clinical retinopathy becomes evident, probably indicating the early signs of neuronal dysfunction. Retinal nerve fibre layer (RNFL) is an important structural neuron in the retina layer which is often shown to affect in the early pathogenesis of diabetic retinopathy. Several studies have reported RNFL thinning or defects in people with diabetes[10,11,12,13,14]. Histological studies of neural components of the retina have revealed that diabetesinduced biochemical mechanisms can potentially cause neural cell degeneration [15-16]. An in-depth understanding of the vascular changes in the retina during diabetes has given cause for the treatment of diabetic retinopathy. Indeed, the only proven treatment for diabetic retinopathy apart from intensive insulin therapy is laser photocoagulation, which involves the destruction of the retinal regions which contains overt vascular abnormalities [17]. Subsequently, early detection of RNFL thinning may help ophthalmologists to provide effective treatment of diabetic retinopathy and with early prevention, thus reducing vision loss.

HbA1c is glycosylated haemoglobin. It is formed due to non enzymatic glycation pathway by hemoglobins exposure to plasma glucose and reflects the blood glucose over the last 8 to 12 weeks. In diabetes mellitus, higher amount of glycated haemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, and retinopathy. Monitoring HbA1c levels may improve outcome[18].

Spectral domain OCT allows for non invasive in vivo cross sectional image of ocular structure such as retina, RNFL and optic nerve head. Spectral domain OCT applies the principle of interferometry to determine the interface between different ocular tissue. Using automated segmentation algorithms based on reflectivity changes between adjacent retinal layers, the RNFL thickness can be calculated[19,20,21,22,23].

"Thus the purpose of study is to evaluate if poorly controlled diabetes –as reflected by high recent HbA1c levels causes thinning of the nerve fibres & to asses if there is a significant correlation between raised fasting & post prandial blood sugar level with the retinal nerve fiber layer thickness."

METHODS-

Study profile-

Department of Ophthalmology, Sir Sundar Lal Hospital, Institute of Medical Science, Banaras Hindu University, Varanasi & Department of Endocrinology Sir Sundar Lal Hospital, Institute of Medical Science, Banaras Hindu University, Varanasi. Patients attending Department of Ophthalmology OPD & Department of Endocrinology OPD Sir Sundar Lal Hospital, BHU. Study period between 01-09-2015 to 30-06-2017. 40 patients- Type 2 Diabetic patients without any evidence of diabetic retinopathy. A total of 80 eyes were observed. Cross sectional, hospital based study.

INCLUSION CRITERIA:- Cases-

- Type 2 Diabetic patients of both sexes without retinopathy receiving treatment at OPD clinic of S. S. Hospital BHU, Varanasi.
- Only adults (>18years of age)

EXCLUSION CRITERIA:- Patients having ophthalmoscopic conditions where evaluation of fundus by

+90D or +78D lenses, indirect ophthalmoscopy and spectral domain OCT procedures cannot be possible (like Nuclear sclerosis grade 3 & onward cataracts, complicated cataracts, cortical cataracts & dense media opacities which hinders the evaluation)

STUDY PROCEDURE-

Fundus photograph was taken for grading of non-proliferative diabetic retinopathy based on proposed International Diabetic Retinopathy Severity Scales . The subject underwent the RNFL thickness measurement using Spectral-Domain OCT (Cirrus HD-OCT, Carl Zeiss Meditec, Dublin, CA).

STATISTICAL ANALYSIS-

Statistical analysis was performed using **IBM® SPSS® Statistics 19.0.0 software. Master chart** was prepared by Microsoft excel & then loaded onto the SPSS software. Descriptive statistical analysis was performed to prepare different frequency tables and to calculate the means with corresponding standard errors. Pearson Chi Square test was applied as measure of association. $P<0.05$ was taken to be statistically significant.

RESULTS-

Total number of patients studied is 40. Both eyes of the patient were studied (80 eyes)

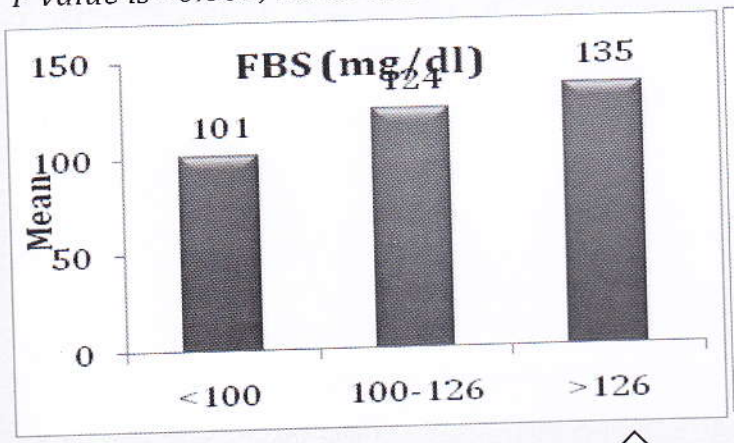
Table 1 - Showing age distribution among the study group

Age in years	Count	Percentage
31-40	6	7.5
41-50	20	25
51-60	44	55
61-70	10	12.5

Table 2 - Showing statistical correl ation between Fasting blood suga r (FBS) with Retinal Nerve Fibre Layer Thickness (RNFL) in all quadrants

FBS (mg/dl)	—	f-value	p-value
<100	101.00±12.336	32.827	<0.001
100-126	124.00±13.543		
>126	135.00±11.015		

P value is <0.001, hence the result is statistically significant



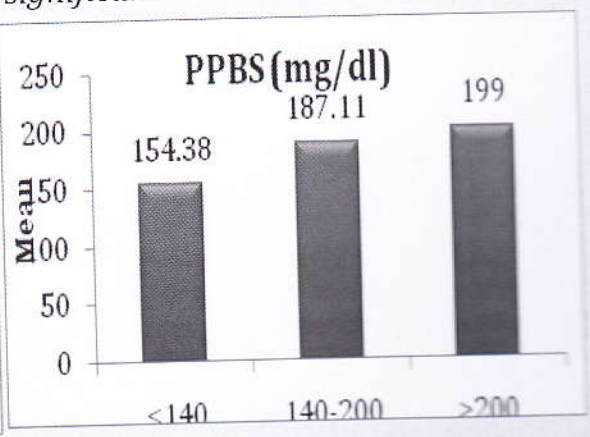


Table-3 Showing statistical correlation between post-prandial blood sugar (PPBS) with Retinal Nerve Fibre Layer Thickness in all quadrants

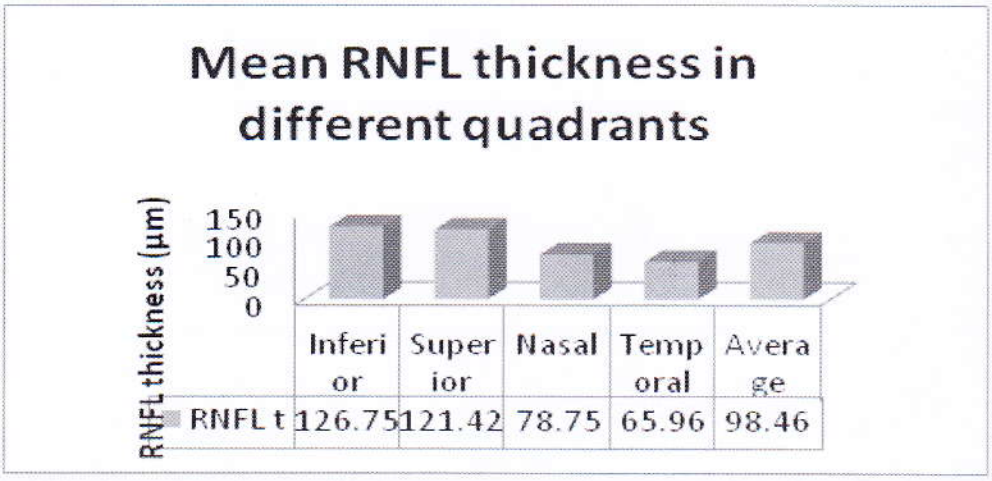
PPBS (mg/dl)	Mean±SD	f-value	p-value
<140	154.38±20.827	21.184	<0.001
140-200	187.11±24.523		
>200	199.00±19.900		

P value is <0.001, hence the result is statistically significant

Table 4- Showing statistical correlation between various age groups with HbA1c

	Value	Df	Asymp. Sig. (2- Sided)
Pearson Chi-Square	13.342[a]	6	0.038
Likelihood Ratio	14.241	6	0.027
Linear-by-Linear Association	0.003		
N of valid Cases	80		

P value is 0.038, hence the result is statistically significant.



Showing Mean Retinal Nerve Fibre Layer Thickness in all quadrants (in µm)

Retinal Nerve Fibre Layer Thickness in each quadrants:

The average RNFL thickness in each quadrant was available as continuous data. As Heidelberg tomogram machine has age matched data fed in its database a normal range of RNFL cannot be defined for comparison between the different age groups. Hence the data is converted into ordinal form.

70% of the 80 eyes have RNFL thickness within 2 SD (Green-G)

24% of the 80 eyes have Borderline RNFL (Yellow-**Y**)
6% of the 80 eyes have decreased RNFL (Red-**R**)
P value is <0.001, hence the result is statistically significant.

Table 6- Showing comparison of Average RNFL thickness with HbA1c

AVERAGE RNFL thickness	HbA1c			Total
	<6%	6-7%	>7%	
G	40	11	5	56
Y	3	8	8	19
R	1	1	3	5
Total	44	20	16	80

Table- 7 Showing statistical correlation between Average RNFL thickness with HbA1c

	Value	Df	Asymp. Sig. (2- Sided)
Pearson Chi-Square	35.14	4	<0.001
No. of valid Cases	80		

P value is <0.001, hence the result is statistically significant.

DISCUSSION-

Advances in ocular imaging technology have made it possible to evaluate the RNFL thickness in an objective, quantifiable, and reproducible fashion, Optical Coherence Tomography (OCT), which uses short coherence length interferometer, has a fine resolution (up to 2 microns) and reflects the histologic characteristics of the tissue. Because OCT is based on the cross sectional image of the retina, the instrument measures the nerve fibre layer directly, has no need for a reference plane, and is known to be unaffected by the refractive status, axial length of the subject, sclerosis of the lens, or pupillary dilation. The only limitations of OCT imaging are the uncertainty of the assumed group refractive index of tissue, the effect of eye movements during the B-scan location, and the interface detection artifacts. The Cirrus HD-OCT, Carl Zeiss Meditec, Dublin, CA has eye tracking system and it negate the effect of the eye movements. To avoid any influence of the eye movements , we observed the scanned eye software interface detection artifacts, we inspected every B-scan and repeated the scan if we noticed any eye movements. 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.

Previous studies have shown that in patients with diabetes mellitus, poor glycemic control leads to infarction in the nerve fibre layer leading to axonal degeneration and decrease in the number of optic nerve axons and the number of retro-bulbar optic nerve fibres[100-103].

In vivo studies of the retinal nerve fibre layer have shown both broad and slit like defects, suggesting that retinal nerve fibre loss and optic nerve fibre loss are related to subclinical vision loss in diabetic patients without any clinical retinopathy.

The purpose of our study was to measure-

The retinal nerve fibre layer (RNFL) thickness in diabetic patients without retinopathy and in relation to the glycemic levels. During study period 80 eyes of 40 patients with type 2 diabetes mellitus without any diabetic retinopathy changes were evaluated. Fasting blood sugar (FBS), post prandial blood sugar (PPBS) & HbA1c of each patient were considered as glycemic status markers. All the three parameters were correlated with average RNFL thickness.

Using the spectral domain OCT in this study, we were able to detect significant decrease in average RNFL thickness measurement in Type 2 diabetic patients without any clinical evidence of diabetic retinopathy.

The final results are-

I. Statistical correlation between FBS and RNFL thickness in different quadrants- P value is <0.001. Hence the result is statistically significant. (Table 2)

II. Statistical correlation between PPBS and RNFL thickness in different quadrants- P value is <0.001. Hence the result is statistically significant. (Table 3)

III. Statistical correlation between Average RNFL thickness with HbA1c- P value is <0.001, hence the result is statistically significant. (Table 6 & 7)

Our findings were in parallel to other studies done by Takahashi et al. [24] and Tekeli et al.[25]. In study done by Tekeli et al., HRT was used to evaluate optic nerve head parameter in diabetes mellitus with and without retinopathy. Whereas, Takahashi et al. used the stratus OCT which is a different tool compared with our study. Both studies did not find any significant reduction in the RNFL thickness among subjects of mild to moderate NPDR compared with age-matched healthy subjects.

Our results were fairly similar to studies done by Lopes de Faria et al. [27] and Takahashi et al. [26], which disclosed that RNFL was thinner in the superior quadrant. This finding corroborates with previous study by Kern[28] showing that the early events of diabetic retinal disease (micro aneurysms and acellular capillaries) occur preferentially in the superior temporal quadrant rather than in inferior areas[28]. Among other studies, Chung et al. demonstrated that blood flow in the superior temporal retina increased in response to hypercapnia, but did not decrease in response to hyperoxia. In contrast, hyperoxia led to a decrease in blood flow to the inferior retina, whereas hypercapnia did not result in an increased blood flow within this area[28]. The lack of normal vasoconstrictor response in this superior quadrant could explain why this region is more susceptible to micro aneurysms and acellular capillaries in diabetes mellitus and also why the retinal fibres are preferentially lost in this region even before clinically detectable diabetic retinopathy[27]. Sugimoto postulated that the superior quadrant was more susceptible to undergoing damage compared with other areas and may have a tendency for higher rates of cell death, which results in RNFL thinning[29]. Besides this, we also noticed that the least affected RNFL in nasal quadrant might be due to the lack of micro aneurysm presence in this area and therefore less retinal nerve fibre layer damage occurred in this quadrant.

HbA1c is known as an index of mean blood glucose in fasting and the postprandial state[30], and is well established and widely used as a clinical measure of chronic glycemia[31]. HbA1c of 6.5% has now been seen as sufficiently sensitive and specific to identify individuals who are at risk of developing diabetic retinopathy[32]. From our findings, we noted that the majority of our subjects in diabetic patients without retinopathy changes had poor glycemic control. The majority of them had HbA1c ≥ 6.5%. Our results of mean HbA1c were fairly consistent with other studies[28,32].

We find out significant decrease in average RNFL thickness measurement in relation to HbA1c level in Type 2 diabetic patients without any clinical evidence of diabetic retinopathy. Our findings were in

contrast to other studies by Chihara et al. [36] and Peng et al. [35]. However our findings were fairly similar to study conducted by Ozdek et al. [37] who compared diabetic patients whose blood glucose was well regulated and with those who were not well regulated according to the levels of blood glucose, HbA1c, fructosamine and triglyceride. They found that the average RNFL thickness value obtained by scanning laser polarimetry was reduced in patients without diabetic retinopathy who had poor blood glucose control but not for those with good control.

CONCLUSION-

Using the spectral domain OCT in this study, we were able to detect significant decrease average RNFL thickness measurement in Type 2 diabetic patients without any clinical evidence of diabetic retinopathy. The decrease in the thickness in the average RNFL thickness is affected by raised HbA1c levels, raised Fasting Blood Sugar & Post Prandial Blood Sugar levels (poor glycemic control).

A large population cohort study is needed to establish the correlation between HbA1c & oxidised LDL and RNFL thickness & central macular thickness in the management of blood sugar levels & lipids in diabetic patients respectively.

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