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RETINAL NERVE FIBER LAYER THICKNESS IN DIABETIC PATIENTS WITH AND WITHOUT DIABETIC RETINOPATHY USING SPECTRAL-DOMAIN OPTICAL COHERENCE TOMOGRAPHY

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ABSTRACT

Background: Diabetes mellitus is associated with retinal neurodegenerative changes that may occur before clinically detectable diabetic retinopathy. Spectral-domain optical coherence tomography (SD-OCT) permits quantitative assessment of retinal nerve fiber layer (RNFL) thickness and may help identify early neuroretinal structural changes.

Methods: A hospital-based comparative study was conducted from September 2024 to September 2025 among 75 participants, with 25 participants in each group. All participants underwent comprehensive ophthalmic examination and SD-OCT assessment of average and quadrant-wise RNFL thickness. Clinical and systemic parameters including diabetes duration and HbA1c were recorded. Data were analysed using SPSS version 26. Continuous variables were compared using appropriate parametric tests, categorical variables using chi-square test, and Pearson correlation was used to assess relationships between RNFL thickness and systemic factors. A p-value <0.05 was considered statistically significant.

Results: Mean average RNFL thickness was 100.3 ± 4.9 µm in controls, 95.1 ± 5.1 µm in diabetes without retinopathy, and 86.7 ± 4.7 µm in NPDR ($p<0.001$). All four quadrants showed significant progressive thinning, with the greatest reduction between controls and NPDR occurring in the inferior quadrant (16.8 µm). RNFL thickness demonstrated significant inverse correlations with diabetes duration ($r=-0.46$, $p<0.001$) and HbA1c ($r=-0.42$, $p<0.001$).

Conclusion: RNFL thickness progressively decreased from healthy controls to diabetes without retinopathy and NPDR. SD-OCT may therefore provide a useful quantitative marker of diabetic neuroretinal structural involvement.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterised by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. It is one of the most important non-communicable diseases worldwide and is associated with progressive microvascular and macrovascular complications. According to the International Diabetes Federation (IDF), approximately 537 million adults aged 20–79 years were living with diabetes in 2021, and this number is projected to increase to 643 million by 2030 and 783 million by 2045 [1]. The increasing prevalence of diabetes represents a major public health challenge because of its substantial contribution to disability, premature mortality and long-term complications affecting multiple organ systems. India carries a particularly large burden of diabetes. The ICMR-INDIAB population-based cross-sectional study conducted across 15 states of India demonstrated a substantial prevalence of diabetes and prediabetes, highlighting the growing burden of diabetes in the country [2]. The World Health Organization (WHO) recognises diabetes as an important cause of morbidity and mortality and emphasises the importance of early detection and prevention of its complications [3].

One of the major complications of diabetes mellitus is diabetic retinopathy (DR), a potentially vision-threatening retinal disorder associated with chronic metabolic and vascular abnormalities. Diabetic retinopathy has traditionally been considered primarily a microvascular complication involving retinal capillary damage, vascular leakage, capillary non-perfusion and subsequent retinal structural changes. The Chennai Urban Rural Epidemiology Study (CURES) Eye Study demonstrated a significant burden of diabetic retinopathy among individuals with diabetes in urban India, emphasising the importance of DR as an ophthalmic and public health problem [4]. However, increasing evidence indicates that diabetic retinal disease is not exclusively a vascular disorder. Retinal neurodegeneration is now recognised as an important component of diabetic retinal pathology and may occur before clinically detectable vascular manifestations of DR. The retinal nerve fibre layer (RNFL), composed predominantly of the axons of retinal ganglion cells, is therefore of particular interest as a structural marker of retinal neuronal integrity. Studies have demonstrated selective loss of inner retinal layers in diabetic patients, including individuals with minimal clinically evident retinopathy [5]. Furthermore,

retinal neurodegeneration may precede characteristic microvascular changes of diabetic retinopathy, supporting the concept that neuronal injury may represent an early event in diabetic retinal disease [6].

The retinal nerve fibre layer thickness provides a quantitative measure of the structural integrity of retinal ganglion cell axons. Reduction in RNFL thickness may therefore reflect neuronal loss or neurodegenerative changes associated with diabetes. In patients with type 2 diabetes mellitus, spectral-domain optical coherence tomography (SD-OCT) has demonstrated neuroretinal structural abnormalities, supporting the presence of retinal neurodegeneration at relatively early stages of diabetes [7]. Optical coherence tomography (OCT) is a non-invasive imaging modality that provides high-resolution cross-sectional images and quantitative measurements of retinal structures. Measurements of retinal thickness and volume in normal eyes have established OCT as a useful and reproducible method for assessing retinal morphology [8]. Spectral-domain OCT provides detailed evaluation of the peripapillary RNFL and its individual quadrants, thereby allowing subtle retinal structural alterations to be identified quantitatively.

An important area of current research is whether RNFL alterations can be detected before the clinical appearance of diabetic retinopathy. Sugimoto et al. investigated early diabetic retinal changes using OCT in patients with type 2 diabetes mellitus without clinically apparent retinopathy, demonstrating the potential utility of OCT for identifying early retinal structural abnormalities [9]. However, findings across individual studies have not always been completely consistent, indicating the need for further evaluation of RNFL changes across different populations and stages of diabetic disease. Indian data are particularly relevant because retinal measurements may vary according to population characteristics and imaging-related factors. The Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study (SN-DREAMS 2) evaluated RNFL thickness in individuals with type 2 diabetes and provided important evidence regarding retinal structural changes in an Indian population [10].

The emerging recognition of retinal neurodegeneration as an early component of diabetic retinal disease creates an important opportunity for identifying structural changes before advanced visual impairment develops. Assessment of RNFL thickness using SD-OCT may provide an objective and quantitative method for detecting early neuroretinal damage in diabetic patients, including those without clinically evident retinopathy. Comparison of RNFL thickness among healthy individuals, diabetic patients without retinopathy and diabetic patients with non-proliferative diabetic retinopathy can help clarify the relationship between diabetes and progressive retinal neuronal changes. Therefore, the present study was undertaken to assess and compare RNFL thickness in diabetic patients with and without diabetic retinopathy with healthy controls using SD-OCT, and to evaluate the relationship between RNFL thickness and important systemic characteristics such as diabetes duration and glycaemic control. Such evidence may contribute to a better understanding of diabetic neuroretinal changes and the potential role of RNFL thickness as an early structural marker of diabetic retinal disease.

METHODOLOGY

A hospital-based comparative case-control study was conducted in the Department of Ophthalmology from September 2024 to September 2025 to evaluate retinal nerve fiber layer (RNFL) thickness in patients with type 2 diabetes mellitus with and without diabetic retinopathy using spectral-domain optical coherence tomography (SD-OCT). A total of 75 participants were included and divided into three groups of 25 participants each: healthy controls, patients with type 2 diabetes mellitus without diabetic retinopathy, and patients with type 2 diabetes mellitus with non-proliferative diabetic retinopathy (NPDR). Participants were recruited using purposive sampling with consecutive enrolment until the required sample size was achieved.

Adults aged ≥18 years with documented type 2 diabetes mellitus were eligible for inclusion. HbA1c ≤10% within the preceding two months was required. Participants in the diabetic retinopathy group had moderately severe or severe NPDR confirmed by fundus examination, while best-corrected visual acuity (BCVA) of at least 6/24 was required in the study eye. Participants with cerebrovascular accident or myocardial infarction within six months, uncontrolled hypertension, active systemic infection, centre-involving diabetic macular edema, proliferative diabetic retinopathy, glaucoma or other optic nerve pathology, vitreous hemorrhage, intraocular inflammation, corneal or media opacity affecting imaging, previous intraocular procedures, or previous intravitreal anti-VEGF/corticosteroid treatment, PRP or laser therapy were excluded.

The sample size was calculated using the formula $n = 2 \times (Z\alpha/2 + Z\beta)^2/d^2$, where $Z\alpha/2 = 1.96$ for a two-sided 5% significance level, $Z\beta = 0.84$ for 80% power, and $d = 0.8$ as the standardized effect size. The formula yielded $n = 24.5$, which was rounded to 25 participants per group, resulting in a total sample size of 75 participants. The approach used for sample-size calculation was based on the standard method described by Charan and Biswas [11].

After obtaining written informed consent, demographic information including age, sex, occupational category and socioeconomic status was recorded. Relevant systemic history included age at onset of diabetes, duration of diabetes, associated comorbidities and medications. Ocular history was also documented. Visual acuity was assessed using the Snellen chart and expressed in logMAR units. A comprehensive ophthalmic examination was performed, including slit-lamp examination, Goldmann applanation tonometry, and dilated fundus examination using a 90-D lens and indirect ophthalmoscopy. Diabetic retinopathy was graded according to the Early Treatment Diabetic Retinopathy Study (ETDRS) classification. HbA1c was recorded as an indicator of glycaemic control.

SD-OCT was performed to assess RNFL thickness. The average RNFL thickness and quadrant-wise RNFL thickness in the superior, inferior, nasal and temporal quadrants were recorded. Scans with poor signal quality or faulty segmentation were excluded from analysis. The collected data were entered into Microsoft Excel, checked for completeness and accuracy, and subsequently analysed using SPSS version 26. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Comparisons of continuous variables were performed using independent t-test or one-way analysis of variance (ANOVA), as appropriate, while

categorical variables were compared using the chi-square test. Pearson correlation analysis was used to assess the relationship between RNFL thickness and duration of diabetes and HbA1c. A p-value <0.05 was considered statistically significant.

Ethical approval was obtained from the Institutional Ethics Committee before commencement of the study. Written informed consent was obtained from all participants. Participant confidentiality was maintained by using coded, de-identified data, and the study procedures were non-invasive with no additional financial burden to the participants.

RESULTS

Table 1. Baseline Demographic, Anthropometric and Diabetes-Related Characteristics of Study Participants

Variable	Healthy Controls (n=25)	Diabetes Mellitus without Retinopathy (n=25)	Diabetes Mellitus with NPDR (n=25)	Total (n=75)
Age (years), Mean ± SD	54.2 ± 7.5	56.1 ± 6.9	57.4 ± 7.1	55.9 ± 7.2
BMI (kg/m²), Mean ± SD	24.8 ± 3.1	26.9 ± 2.9	27.5 ± 3.2	26.4 ± 3.1
Male, n (%)	13 (52%)	14 (56%)	15 (60%)	42 (56%)
Female, n (%)	12 (48%)	11 (44%)	10 (40%)	33 (44%)
Duration of diabetes (years), Mean ± SD	—	6.2 ± 2.1	12.4 ± 4.5	—
HbA1c (%), Mean ± SD	5.4 ± 0.3	7.2 ± 0.6	8.8 ± 0.7	—
Hypertension, n (%)	5 (20%)	11 (44%)	13 (52%)	29 (39%)
Dyslipidemia, n (%)	6 (24%)	12 (48%)	14 (56%)	32 (43%)
Smoking, n (%)	3 (12%)	4 (16%)	5 (20%)	12 (16%)
Oral antidiabetic drugs, n (%)	—	15 (60%)	—	—
Insulin therapy, n (%)	—	10 (40%)	13 (52%)	—
OAD + Insulin, n (%)	—	—	12 (48%)	—

Note: Values are expressed as mean ± standard deviation or number (percentage), as applicable. The study included 75 participants divided equally into three groups of 25 each.

Table 2. Distribution of Gender among the Study Groups

Gender	Healthy Controls (n=25)	Diabetes Mellitus without Retinopathy (n=25)	Diabetes Mellitus with NPDR (n=25)	Total (n=75)
Male, n (%)	13 (52%)	14 (56%)	15 (60%)	42 (56%)
Female, n (%)	12 (48%)	11 (44%)	10 (40%)	33 (44%)
Total, n (%)	25 (100%)	25 (100%)	25 (100%)	75 (100%)
Chi-square (χ^2)	—	—	—	0.42
p-value	—	—	—	0.81

Note: The difference in gender distribution among the three groups was not statistically significant (χ^2=0.42, p=0.81). The reported male percentages were 52%, 56% and 60% in the three groups, respectively.

Table 3. Comparison of Visual, Ocular and Optical Coherence Tomography Parameters among the Study Groups

Parameter	Healthy Controls (n=25)	Diabetes Mellitus without Retinopathy (n=25)	Diabetes Mellitus with NPDR (n=25)	p-value
BCVA (LogMAR), Mean ± SD	0.05 ± 0.03	0.12 ± 0.06	0.21 ± 0.09	<0.001*
ETDRS visual acuity (letters), Mean ± SD	83.6 ± 3.4	78.5 ± 4.1	72.4 ± 5.3	<0.001*
Intraocular pressure	14.9 ± 2.8	15.2 ± 3.1	15.6 ± 2.9	0.62

(mmHg), Mean ± SD				
Axial length (mm), Mean ± SD	23.6 ± 0.8	23.8 ± 0.9	23.9 ± 1.0	0.41
Central macular thickness (µm), Mean ± SD	258.4 ± 17.3	262.1 ± 19.5	268.9 ± 20.2	0.03*

Note: BCVA was expressed in LogMAR units. Higher LogMAR values indicate poorer visual acuity. ETDRS denotes Early Treatment Diabetic Retinopathy Study. CMT denotes central macular thickness. *Statistically significant.
The source data demonstrate progressively poorer BCVA and lower ETDRS scores from controls to diabetic participants without retinopathy and further to NPDR. CMT showed a statistically significant increase, whereas IOP and axial length did not differ significantly between groups.

Table 4. Comparison of Average Retinal Nerve Fiber Layer Thickness among the Study Groups

Study Group	Average RNFL Thickness (µm), Mean ± SD	Difference from Healthy Controls (µm)	p-value
Healthy Controls (n=25)	100.3 ± 4.9	Reference	<0.001*
Diabetes Mellitus without Retinopathy (n=25)	95.1 ± 5.1	−5.2	<0.001*
Diabetes Mellitus with NPDR (n=25)	86.7 ± 4.7	−13.6	<0.001*

Note: Average RNFL thickness demonstrated a progressive reduction across the three study groups, with the highest value among healthy controls and the lowest value among participants with NPDR. The overall difference between groups was statistically significant ($p<0.001$).

Table 5. Quadrant-wise Comparison of Retinal Nerve Fiber Layer Thickness among the Study Groups

RNFL Quadrant	Healthy Controls (n=25), Mean ± SD (µm)	DM without Retinopathy (n=25), Mean ± SD (µm)	DM with NPDR (n=25), Mean ± SD (µm)	p-value
Superior	124.7 ± 6.8	118.9 ± 7.3	111.2 ± 6.7	<0.001*
Inferior	120.5 ± 6.5	114.6 ± 7.2	103.7 ± 7.0	<0.001*
Nasal	75.3 ± 5.8	69.8 ± 6.2	65.1 ± 6.5	<0.001*
Temporal	70.4 ± 5.7	64.7 ± 5.9	60.2 ± 6.3	<0.001*

Absolute Reduction in RNFL Thickness from Healthy Controls to NPDR

RNFL Quadrant	Healthy Controls (µm)	NPDR (µm)	Absolute Reduction (µm)
Superior	124.7	111.2	13.5
Inferior	120.5	103.7	16.8
Nasal	75.3	65.1	10.2
Temporal	70.4	60.2	10.2

Note: All four RNFL quadrants demonstrated statistically significant differences among the three study groups ($p<0.001$). The greatest absolute reduction between healthy controls and NPDR was observed in the inferior quadrant, followed by the superior quadrant.

Table 6. Correlation of Systemic Factors with RNFL Thickness and Association of Categorical Variables with Study Groups

A. Correlation of Continuous Variables with Average RNFL Thickness

Variable	Correlation Coefficient (r)	p-value	Direction of Correlation	Statistical Significance
Duration of diabetes	−0.46	<0.001*	Negative	Significant
HbA1c (%)	−0.42	<0.001*	Negative	Significant
Age (years)	−0.21	0.07	Negative	Not significant

B. Association of Categorical Variables with Study Groups

Categorical Variable	Chi-square (χ^2)	p-value	Statistical Significance
Gender	0.42	0.81	Not significant
Hypertension	6.23	0.045*	Significant
Dyslipidemia	7.18	0.028*	Significant
Smoking	1.34	0.51	Not significant

Note: Duration of diabetes and HbA1c demonstrated significant inverse correlations with average RNFL thickness, indicating lower RNFL thickness with increasing duration of diabetes and poorer glycaemic control. Age showed a weak inverse but statistically non-significant correlation. Among categorical variables, hypertension and dyslipidemia were significantly associated with study-group status, whereas gender and smoking were not.

Table 7. Comparison of Average RNFL Thickness at Baseline and Three-Month Follow-up

Study Group	Baseline RNFL Thickness (µm), Mean ± SD	3-Month RNFL Thickness (µm), Mean ± SD	Mean Change (µm)	Percentage Change*	Interpretation
Healthy Controls (n=25)	98.23 ± 5.20	97.90 ± 5.19	−0.33	−0.34%	Essentially stable
DM without Retinopathy (n=25)	95.26 ± 4.77	94.07 ± 4.74	−1.20	−1.26%	Mild reduction
DM with NPDR (n=25)	86.03 ± 5.81	83.18 ± 5.73	−2.85	−3.31%	Greatest reduction

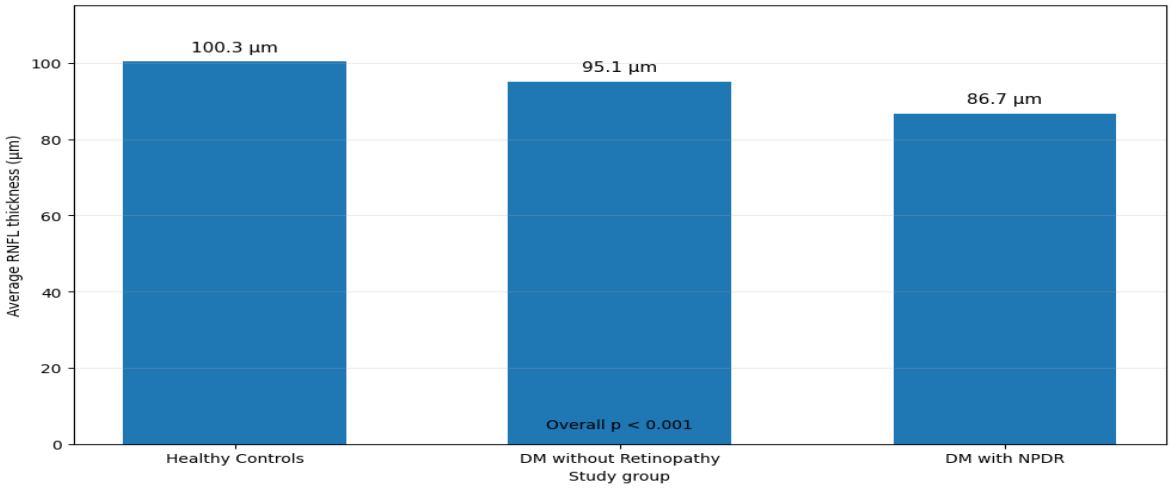
*Percentage change calculated as:
[(3-month value − baseline value) / baseline value] × 100

Comparative Change in Average RNFL Thickness

Comparison	Difference in Mean Change (µm)
DM without Retinopathy vs Healthy Controls	0.87 µm greater reduction
NPDR vs Healthy Controls	2.52 µm greater reduction
NPDR vs DM without Retinopathy	1.65 µm greater reduction

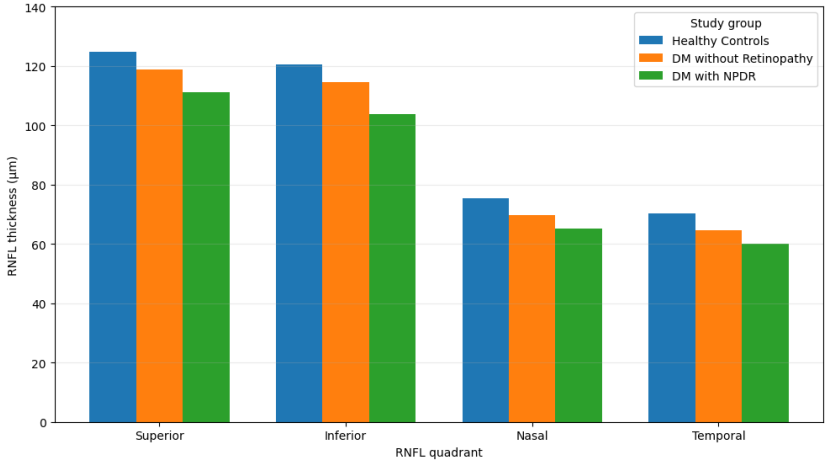
Note: The greatest reduction in average RNFL thickness over three months was observed in the NPDR group (−2.85 µm), followed by the DM without retinopathy group (−1.20 µm), while the healthy control group showed minimal change (−0.33 µm).

Figure 1. Comparison of Average RNFL Thickness among the Study Groups



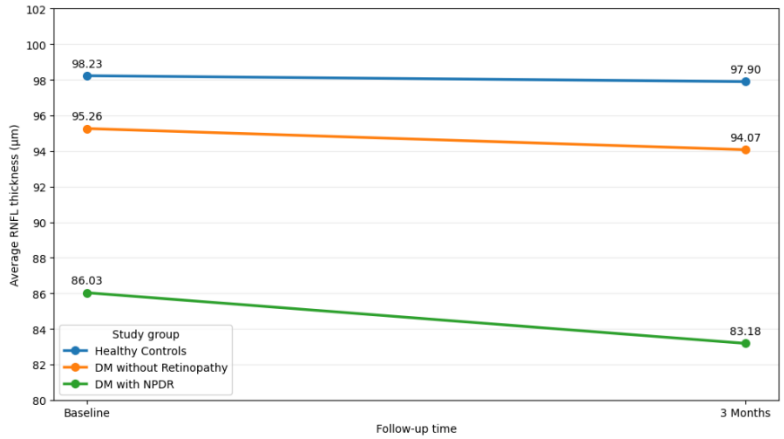
Legend: DM – Diabetes Mellitus; NPDR – Non-Proliferative Diabetic Retinopathy; RNFL – Retinal Nerve Fiber Layer. Values are mean RNFL thickness.

Figure 2. Quadrant-wise Comparison of RNFL Thickness among the Study Groups



Legend: DM – Diabetes Mellitus; NPDR – Non-Proliferative Diabetic Retinopathy; RNFL – Retinal Nerve Fiber Layer. Values are mean RNFL thickness in µm. All quadrant-wise comparisons were statistically significant ($p < 0.001$).

Figure 3. Three-Month Change in Average RNFL Thickness



Legend: DM – Diabetes Mellitus; NPDR – Non-Proliferative Diabetic Retinopathy; RNFL – Retinal Nerve Fiber Layer. Values are mean RNFL thickness in µm. Mean change: Healthy Controls −0.33 µm; DM without Retinopathy −1.20 µm; DM with NPDR −2.85 µm.

DISCUSSION

The present study demonstrated a progressive reduction in retinal nerve fiber layer (RNFL) thickness across the three study groups, with the highest values observed among healthy controls, followed by diabetic patients without retinopathy, and the lowest values among patients with non-proliferative diabetic retinopathy (NPDR). The mean average RNFL thickness was 100.3 ± 4.9 µm in healthy controls, 95.1 ± 5.1 µm in diabetic patients without retinopathy, and 86.7 ± 4.7 µm in patients with NPDR, with the difference between groups being statistically significant ($p<0.001$). Compared with healthy controls, RNFL thickness was reduced by 5.2 µm in diabetic patients without retinopathy and by 13.6 µm in patients with NPDR. A further reduction of 8.4 µm was observed between diabetic patients without retinopathy and those with NPDR.

The clinical relevance of this progressive structural change is supported by the established relationship between diabetes and diabetic retinopathy. A large pooled analysis by Yau et al. [12], involving 22,896 individuals with diabetes from 35 population-based studies, reported an overall prevalence of diabetic retinopathy of 34.6% and demonstrated that the prevalence of retinopathy increased with longer diabetes duration, higher HbA1c and higher blood pressure levels. In the present study, similar systemic gradients were observed, with diabetes duration increasing from 6.2 ± 2.1 years in patients without retinopathy to 12.4 ± 4.5 years in patients with NPDR, while HbA1c increased from 7.2 ± 0.6% to 8.8 ± 0.7%, respectively. Thus, the greater RNFL loss observed in the NPDR group occurred in the setting of longer diabetes duration and poorer glycaemic control.

Importantly, the present study identified RNFL thinning even before clinically evident diabetic retinopathy. The 5.2 µm reduction in average RNFL thickness between healthy controls and diabetic patients without retinopathy suggests that neuroretinal structural alteration may occur before clinically apparent vascular retinopathy. This finding is consistent with the observations of Vujosevic et al. [13], who demonstrated retinal structural alterations in type 2 diabetic patients without retinopathy and in those with mild diabetic retinopathy. Their findings support the concept that retinal neurodegenerative changes may be detectable during the early stages of diabetes. The present study extends this observation by demonstrating a further marked reduction to 86.7 ± 4.7 µm in patients with NPDR.

The findings were also comparable with those reported by Pierro et al. [14], who evaluated RNFL thickness in patients with type 2 diabetes without retinopathy. The present study similarly demonstrated measurable RNFL reduction despite the absence of clinically detectable retinopathy. In the present cohort, the mean RNFL thickness of 95.1 ± 5.1 µm in diabetic patients without retinopathy was lower than the 100.3 ± 4.9 µm observed in healthy controls. This difference of 5.2 µm provides quantitative evidence of early neuroretinal involvement. Differences in absolute RNFL values between studies may be attributable to differences in population characteristics, OCT platforms, scanning protocols, age distribution and ocular anatomical parameters.

The present findings also need to be interpreted in the context of changes occurring in other inner retinal neuronal layers. In this study, the primary outcome was peripapillary RNFL thickness; however, diabetic neurodegeneration may involve multiple retinal neuronal compartments. Kim et al. [15] reported changes in the ganglion cell-inner plexiform layer (GC-IPL) in patients with diabetes using spectral-domain OCT. This supports the interpretation that the RNFL reduction observed in the present study is likely part of a broader pattern of inner retinal neuronal alteration rather than an isolated change in the nerve fiber layer.

The magnitude of RNFL reduction observed in the present study is further supported by pooled evidence. Shi et al. [16] performed a meta-analysis of RNFL thickness in diabetes and demonstrated significant reductions in peripapillary RNFL thickness among diabetic patients, including those with preclinical disease. In an independent meta-analysis of preclinical diabetic retinopathy, OCT-based studies demonstrated a significant mean RNFL reduction of approximately 2.88 µm compared with healthy controls, with significant reductions particularly in the superior, inferior and nasal quadrants. In the present study, the reduction between controls and diabetic patients without retinopathy was somewhat greater at 5.2 µm, while the difference increased to 13.6 µm in NPDR. The larger difference in the present study may reflect the inclusion of patients with more established NPDR and differences in study population and OCT methodology.

The observation of RNFL abnormalities in diabetic patients without retinopathy was also consistent with the work of Sugimoto et al. [17], who specifically investigated early diabetic retinal changes using OCT in patients with type 2

diabetes without clinically apparent retinopathy. Their study evaluated the potential of OCT to detect early diabetic retinal abnormalities. Similarly, the present study demonstrated a significant reduction in RNFL thickness before clinically evident retinopathy, supporting the potential role of SD-OCT as a sensitive structural imaging modality for identifying early diabetic retinal involvement.

The progressive nature of RNFL alteration was further supported by Lee et al. [18], who investigated RNFL thinning in patients with diabetes without diabetic retinopathy. The present study demonstrated a stepwise reduction from 100.3 μm in controls to 95.1 μm in diabetes without retinopathy and 86.7 μm in NPDR, indicating that the structural abnormality becomes more pronounced with increasing clinical severity of diabetic retinal disease. This pattern is also consistent with longitudinal evidence showing accelerated peripapillary RNFL loss in diabetic patients compared with healthy controls. In a three-year longitudinal study, RNFL loss rates were approximately −0.92 μm/year in diabetes without retinopathy and −1.16 μm/year in NPDR, compared with −0.35 μm/year in controls.

The present study further demonstrated significant RNFL abnormalities in established NPDR. Average RNFL thickness was 86.7 ± 4.7 μm in the NPDR group, representing a 13.6 μm reduction compared with healthy controls. This finding is comparable in direction to the observations of El-Fayoumi et al. [19], who demonstrated changes in RNFL and ganglion cell complex parameters in diabetic retinal disease using OCT. The simultaneous involvement of RNFL and other inner retinal structures supports the concept that diabetic retinopathy represents a combination of vascular and neurodegenerative retinal pathology.

The quadrant-wise findings of the present study provide additional evidence of non-uniform retinal neuronal involvement. Significant differences were observed in the superior, inferior, nasal and temporal quadrants, with all comparisons showing $p<0.001$. The greatest reduction between healthy controls and NPDR occurred in the inferior quadrant (16.8 μm), followed by the superior quadrant (13.5 μm), while the nasal and temporal quadrants each showed a 10.2 μm reduction. These findings are in agreement with the study by Kadri et al. [20], which specifically evaluated quadrant-specific RNFL thinning in NPDR. The greater involvement of the inferior and superior quadrants in the present study suggests that diabetic neurodegeneration may not affect all retinal nerve fiber bundles uniformly.

The evidence for early retinal neurodegeneration extends beyond RNFL measurements. The present study demonstrated a significant reduction in RNFL thickness even in diabetic patients without retinopathy, while Zeng et al. [21] reported early retinal neurovascular impairment in diabetic patients without clinically detectable retinopathy using OCT-based assessment of retinal neural and vascular parameters. These findings together strengthen the concept that retinal dysfunction and structural neurodegeneration can precede the classical clinical manifestations of diabetic retinopathy.

An important finding in the present study was the significant relationship between glycaemic control and RNFL thickness. Average RNFL thickness showed a significant inverse correlation with HbA1c ($r = −0.42$, $p<0.001$), indicating that higher HbA1c levels were associated with lower RNFL thickness. This observation is consistent with the study by Hsu et al. [22], which demonstrated an association between retinal neuronal layer thickness and diabetes duration and HbA1c. Although their assessment focused on the GC-IPL rather than exclusively on peripapillary RNFL, the findings support a relationship between chronic metabolic dysregulation and retinal neuronal damage.

The present study also demonstrated a significant inverse correlation between diabetes duration and RNFL thickness ($r = −0.46$, $p<0.001$). Patients with NPDR had a mean diabetes duration of 12.4 ± 4.5 years, compared with 6.2 ± 2.1 years among diabetic patients without retinopathy. The progressive reduction in RNFL thickness accompanying longer diabetes duration is consistent with the observations of Agarwal et al. [23], who evaluated RNFL and ganglion cell complex parameters in type 2 diabetes in an Indian population. The Indian context is particularly relevant because population characteristics and retinal measurements may differ across ethnic and geographical groups.

The comparison of RNFL with other neuronal OCT parameters is also relevant to the present findings. Rao et al. [24] evaluated GC-IPL and RNFL analysis in early diabetic neurodegeneration, highlighting that different OCT-derived retinal neuronal parameters may provide complementary information regarding diabetic retinal damage. The present study demonstrated significant alterations in RNFL even before clinically evident retinopathy, supporting the potential value of structural OCT measurements as markers of early neuroretinal involvement.

The association between retinal neurodegeneration and duration of diabetes observed in the present study is further supported by Tang et al. [25], who investigated the relationship between retinal neurodegeneration and diabetes duration using OCT. In the present study, the correlation coefficient between diabetes duration and average RNFL thickness was −0.46, demonstrating a moderate inverse relationship. The finding suggests that cumulative exposure to the diabetic metabolic environment may contribute to progressive neuronal injury. However, because the present study was observational and did not employ multivariable modelling, this association should not be interpreted as proof of an independent causal effect.

The findings of the present study are particularly relevant when compared with the Indian evidence from the SN-DREAMS 2 study by Srinivasan et al. [26]. The present study demonstrated a clear gradient in average RNFL thickness from 100.3 ± 4.9 μm in healthy controls to 95.1 ± 5.1 μm in diabetes without retinopathy and 86.7 ± 4.7 μm in NPDR. The SN-DREAMS 2 study provides an important Indian reference for RNFL assessment in type 2 diabetes and supports the relevance of OCT-derived RNFL measurements in the Indian diabetic population. Differences in absolute RNFL values between the two studies should nevertheless be interpreted cautiously because of differences in study population, disease severity, sample size, OCT instrumentation and measurement protocols.

The systemic findings of the present study further support the multifactorial nature of diabetic retinal disease. Hypertension was present in 20% of healthy controls, 44% of diabetic patients without retinopathy and 52% of patients with NPDR, while dyslipidemia was present in 24%, 48% and 56%, respectively. Both hypertension and dyslipidemia demonstrated statistically significant associations with study-group status, whereas gender and smoking did not. These observations are compatible with the large pooled analysis by Yau et al. [12], which identified diabetes duration, HbA1c

and blood pressure as major factors associated with diabetic retinopathy. However, the present study did not establish independent effects of these variables on RNFL thickness, and therefore the observed associations should be considered exploratory.

The present study also demonstrated a statistically significant increase in central macular thickness from 258.4 ± 17.3 μm in controls to 262.1 ± 19.5 μm in diabetes without retinopathy and 268.9 ± 20.2 μm in NPDR ($p=0.03$), while intraocular pressure and axial length did not differ significantly between groups. The coexistence of increasing central macular thickness and decreasing RNFL thickness suggests that diabetes may produce different structural effects in different retinal compartments. Therefore, RNFL assessment may provide additional information regarding neuroretinal integrity beyond conventional macular thickness measurements.

The three-month observations in the present study further demonstrated a directional decline in RNFL thickness, with the greatest reduction occurring in the NPDR group. RNFL thickness decreased by 0.33 μm in healthy controls, 1.20 μm in diabetic patients without retinopathy and 2.85 μm in patients with NPDR over three months. This pattern is consistent with the broader concept of progressive RNFL loss in diabetes, although the short follow-up duration and the absence of formal longitudinal statistical modelling in the present study mean that these changes should be interpreted cautiously rather than as definitive progression rates.

CONCLUSION

The present study demonstrated a clear and progressive reduction in retinal nerve fiber layer thickness among patients with type 2 diabetes mellitus across different stages of diabetic retinal disease. Average RNFL thickness was highest among healthy controls (100.3 ± 4.9 μm), decreased in diabetic patients without retinopathy (95.1 ± 5.1 μm), and was lowest among patients with NPDR (86.7 ± 4.7 μm), with the difference between groups being highly statistically significant ($p<0.001$). The presence of RNFL thinning even in diabetic patients without clinically evident retinopathy suggests that neuroretinal structural changes may occur before overt clinical manifestations of diabetic retinopathy.

Significant thinning was observed across the superior, inferior, nasal and temporal quadrants, with the inferior quadrant showing the greatest reduction of 16.8 μm between healthy controls and NPDR. RNFL thickness also showed significant inverse correlations with duration of diabetes ($r=-0.46$) and HbA1c ($r=-0.42$), indicating greater neuroretinal thinning with longer diabetes duration and poorer glycaemic control.

The three-month observations demonstrated the greatest reduction in RNFL thickness among patients with NPDR, further supporting a progressive pattern of structural change.

Overall, the findings support the potential role of SD-OCT-derived RNFL thickness as an objective quantitative marker of diabetic neuroretinal involvement, including changes that may precede clinically detectable diabetic retinopathy.

REFERENCES:

1. International Diabetes Federation. IDF Diabetes Atlas. 10th ed. Brussels, Belgium: International Diabetes Federation; 2021.
2. Anjana RM, Deepa M, Pradeepa R, et al. Prevalence of diabetes and prediabetes in 15 states of India: results from the ICMR-INDIAB population-based cross-sectional study. Lancet Diabetes Endocrinol. 2017;5(8):585-596.
3. World Health Organization. Global report on diabetes. Geneva: World Health Organization; 2016.
4. Rema M, Premkumar S, Anitha B, et al. Prevalence of diabetic retinopathy in urban India: the Chennai Urban Rural Epidemiology Study (CURES) Eye Study. Invest Ophthalmol Vis Sci. 2005;46(7):2328-2333.
5. van Dijk HW, Kok PH, Garvin M, et al. Selective loss of inner retinal layer thickness in type 1 diabetic patients with minimal diabetic retinopathy. Invest Ophthalmol Vis Sci. 2009;50(7):3404-3409.
6. Sohn EH, van Dijk HW, Jiao C, et al. Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. Proc Natl Acad Sci U S A. 2016;113(19):E2655-E2664.
7. Chhablani J, Sharma A, Goud A, et al. Neurodegeneration in type 2 diabetes: evidence from spectral-domain optical coherence tomography. Invest Ophthalmol Vis Sci. 2015;56(11):6333-6338.
8. Lim MC, Tanimoto SA, Furlani BA, et al. Retinal thickness and volume measurements in normal eyes: a study using optical coherence tomography. Ophthalmology. 2005;112(1):116-122.
9. Sugimoto M, Sasoh M, Ido M, et al. Detection of early diabetic change with optical coherence tomography in type 2 diabetes mellitus patients without retinopathy. Ophthalmologica. 2005;219(6):379-385.
10. Srinivasan S, Raman R, Ganesan S, et al. Retinal nerve fibre layer thickness in type 2 diabetes: Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study (SN-DREAMS 2). Eye. 2016;30(6):777-784.
11. Charan J, Biswas T. How to calculate sample size for different study designs in medical research. Indian J Psychol Med. 2013;35(2):121-126.
12. Yau JWY, Rogers SL, Kawasaki R, et al. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care. 2012;35(3):556-564.
13. Vujosevic S, Midena E, Pilotto E, et al. Retinal nerve fiber layer thickness in type 2 diabetic patients without and with mild diabetic retinopathy. Invest Ophthalmol Vis Sci. 2012;53(6):2713-2718.
14. Pierro L, Gagliardi M, Iuliano L, et al. Retinal nerve fiber layer thickness in patients with type 2 diabetes mellitus without retinopathy. Ophthalmology. 2012;119(11):2332-2338.
15. Kim J, Woo SJ, Ahn J, et al. Retinal ganglion cell-inner plexiform layer thickness changes in diabetes mellitus: a spectral-domain OCT study. Invest Ophthalmol Vis Sci. 2013;54(5):3378-3384.
16. Shi R, Guo Z, Wang F, et al. Retinal nerve fiber layer thickness in diabetes mellitus: a meta-analysis. PLoS One. 2015;10(8):e0135077.

17. Sugimoto M, Sasoh M, Ido M, et al. Detection of early diabetic change with optical coherence tomography in type 2 diabetes mellitus patients without retinopathy. Ophthalmologica. 2005;219(6):379-385.
18. Lee K, Lee S, Kim J, et al. Progression of retinal nerve fiber layer thinning in patients with diabetes mellitus without diabetic retinopathy. Korean J Ophthalmol. 2013;27(3):163-167.
19. El-Fayoumi D, Badr Eldine NM, Soliman AZ, et al. Retinal nerve fiber layer and ganglion cell complex changes in diabetic retinopathy: an OCT-based study. Clin Ophthalmol. 2015;9:1907-1913.
20. Kadri R, Mohan A, Yadav S, et al. Quadrant-specific RNFL thinning in non-proliferative diabetic retinopathy: an optical coherence tomography study. Int J Ophthalmol. 2018;11(7):1185-1191.
21. Zeng Y, Cao D, Yu H, et al. Early retinal neurodegeneration in type 2 diabetes revealed by GCC thickness reduction using OCT. Retina. 2019;39(2):229-238.
22. Hsu SY, Chang YC, Lin PW, et al. Association of retinal ganglion cell-inner plexiform layer thickness with diabetes duration and HbA1c. Br J Ophthalmol. 2019;103(3):381-386.
23. Agarwal A, Gupta V, Raman R, et al. Retinal nerve fiber layer and ganglion cell complex analysis in type 2 diabetics: an Indian study. Indian J Ophthalmol. 2020;68(9):1888-1894.
24. Rao HL, Kumar AU, Babu JG, et al. Ganglion cell-inner plexiform layer versus RNFL analysis in early diabetic neurodegeneration. Eye (Lond). 2021;35(10):2782-2789.
25. Tang J, Mo X, Liu X, et al. Correlation of retinal neurodegeneration with duration of diabetes: a multicenter OCT study. Diabetes Care. 2017;40(3):349-354.
26. Srinivasan S, Raman R, Ganesan S, et al. Retinal nerve fibre layer thickness in type 2 diabetes: SN-DREAMS 2. Eye. 2016;30(6):777-784.