

## Changes of Retinal Nerve Fiber Layer Thickness in Patients with Type 2 Diabetes

MOHAMED A. ELSHARQAWI, M.Sc.; AMANY A. EL-SHAZLY, M.D.; SAMAH M. FAWZY, M.D. and DOAA M. ASHOUR, M.D.

The Department of Ophthalmology, Faculty of Medicine, Ain Shams University

### Abstract

**Background:** Deterioration of quality of vision starts early in diabetes, before clinical retinopathy becomes evident. This probably indicates the early signs of neuronal dysfunction. Retinal nerve fiber layer (RNFL) is an important structural neuron in the retinal layer which is often affected in the early pathological stages of diabetic retinopathy.

**Aim of Study:** To assess the changes in retinal nerve fiber layer (RNFL) thickness associated with type 2 diabetes mellitus (DM) with or without diabetic retinopathy (DR) and to correlate these changes with the stage of retinopathy.

**Patients and Methods:** This observational study included 39 patients with type 2 DM in three groups as per the stage of DR. Group 1 included eyes without DR. Group 2 included eyes with mild non-proliferative diabetic retinopathy (NPDR). Group 3 included eyes with moderate NPDR. Severe NPDR, PDR, and/or macular edema were excluded. All participants underwent full ophthalmological assessment followed by imaging via Cirrus HD-OCT 5000 optical coherence tomography (Carl Zeiss Meditec, Jena, Germany). For each participant, macular and optic disc scans were obtained. Blood samples were drawn for serum creatinine and glycosylated haemoglobin (Hb A1c).

**Results:** Thirty-nine eyes of type 2 diabetic patients were enrolled in this study; twelve eyes in group 1, 14 in group 2, and 13 in group 3. We found highly significant differences ( $p < 0.001$ ) between the groups regarding the duration of diabetes and levels of HbA1c. Decremental thinning in the measurements of the average, superior, inferior, and temporal RNFL thickness were observed with the progression of DR. In groups 2 and 3 negative moderate correlations ( $r = 0.55$  and  $0.6$  respectively) were found between the duration of DM and the average thickness of the RNFL.

**Conclusion:** With the advancement in DR, we observed a reduction of RNFL thickness. More research is needed to study the effect of RNFL reduction on the progress of retinopathy.

**Key Words:** Retinal nerve – Nerve fiber thickness – Diabetes.

### Introduction

**DIABETIC** retinopathy (DR) is an incapacitating complication of diabetes mellitus (DM). It is a major cause of visual disability specially in the working age group [1-4]. Also, the presence of DR may indicate the concurrence of other complications in diabetic patients, such as renal, central nervous system, and cardiovascular diseases [5,6]. Thus, early identification and management of DR are major priorities for health services [7]. Accordingly, screening programs have been adopted for years [8].

Several mechanisms have been incriminated in the evolution and progress of diabetic microvascular complications [9]. Also, neurodegenerative changes have been identified even prior to the microvascular changes [10,11]. The exact pathogenesis of such changes is still being worked out however, these changes were proposed to result from the metabolic changes provoked by hyperglycemia and the production of free radicals leading to apoptosis of retinal ganglion cells [13,14]. Such changes are expected to result in a reduction in the thickness of retinal nerve fiber layer (RNFL).

Currently, optical coherence tomography (OCT) machines can quantitatively assess the peripapillary RNFL [14]. Using OCT, recent studies on diabetic eyes have shown reduction and defects in the RNFL [15,16].

The presence of RNFL defects or thinning in diabetics in absence of glaucoma might have a role in the prediction of DR [17]. Also, RNFL thinning, or defects associated with DM might necessitate changes in the glaucoma management approaches adopted in diabetics [16]. Few studies have identified RNFL changes associated with DM however, the relation between these changes and the micro-

**Correspondence to:** Dr. Mohamed A. Elsharqawi,  
[E-Mail: Elsharqawi10@gmail.com](mailto:Elsharqawi10@gmail.com)

vascular manifestation of DR is not well understood.

#### *Aim of the study:*

The primary outcome of the current study was to assess RNFL changes associated with type 2 DM with or without DR. The secondary outcome was to correlate these changes if any with the stage of DR, diabetic control, and serum creatinine.

### **Patients and Methods**

This is a cross-sectional observational study. The study protocol was approved by the local ethical committee. The study abided by research ethics stated by the declaration of Helsinki. Subjects with type 2 DM visiting the outpatient clinic for regular follow-up between January and June 2022 were invited to participate. After a proper explanation of the study's purpose and procedures, all patients signed written informed consent.

For all participants, a careful history has been taken including the duration of diabetes, any other systemic diseases, previous ophthalmic surgery, or laser. Exclusion criteria included any history of systemic diseases rather than type 2 DM or controlled hypertension, glaucoma or glaucoma suspects, media opacity, previous ophthalmic surgery, error of refraction more than  $\pm 6$  diopters, axial length more than 26mm, severe non-proliferative diabetic retinopathy (NPDR), proliferative diabetic retinopathy (PDR), and any other retinal or optic nerve diseases.

Ophthalmological assessment was done for each participant including best corrected visual acuity (BCVA) using the Snellen chart (converted to log MAR for statistical analysis), anterior segment examination, intraocular pressure measurement using air puff to no meter (TOPCON, Tokyo, Japan), Then dilated fundus examination using the 78 diopters lens with the slit lamp biomicroscopy. Patients were assigned into 3 groups according to the stage of DR as per the international diabetic retinopathy disease severity scale [16]. Group 1 included diabetics without clinical signs of DR. Group 2 included diabetics with mild NPDR. Group 3 included diabetics with moderate NPDR. Patients with severe NPDR, PDR, and/or diabetic macular edema were all excluded.

Also, the axial length was measured using Sonomed 300A+ Pac Scan plus A Scan (SONOMED, NY, USA), and finally OCT was done using the Cirrus HD-OCT5000 (Carl Zeiss Meditec, Jena, Germany). For each participant, a macular cube scan (512 x 128) was obtained. Central macular

thickness (thickness in the central 1mm) was obtained from the macular map. Optic disc scan (200 x 200) was obtained. From which, average and vertical cup/disc (C/D) ratio in addition to the average RNFL thickness and RNFL in the four quadrants were obtained and analyzed.

Scans were reviewed for signal strength, automated segmentation, and centration. Scans with a signal strength below 6, decentered, algorithm failure, or motion artifacts were excluded. Only one eye from each participant (randomly selected if both eyes abided by the inclusion criteria) was included.

Blood samples were withdrawn for serum creatinine and glycosylated hemoglobin (Hb A1c). Serum creatinine was tested using the R1 creatinine standard, R2 picric reagent & R3 alkaline reagent (Diamond Diagnostics). HbA1c was tested using HbA1c R1, R2 & R3 reagents (Agappe Diagnostics).

#### *Statistical analysis:*

We employed the 15<sup>th</sup> version of the statistical package for Social Sciences (SPSSv. 15.0; SPSS Inc, Chicago, IL) for analysis. Kolmogorov-Smirnov test was used for data normality assumption. The one-way analysis of variance (ANOVA) was applied for the parametric data sets. In case of overall significance, further analysis was performed using post-hoc-Tukey's honestly significant difference. The Pearson correlation coefficient ( $r$ ) was calculated for correlation between RNFL thickness measurements and other variables. Results with  $p$ -value  $< 0.05$  were considered significant.

### **Results**

Thirty-nine eyes of 39 diabetic patients were enrolled in this research. Twelve eyes without DR were included in Group 1. Fourteen eyes with mild NPDR were included in Group 2 and thirteen eyes with moderate NPDR were included in Group 3.

#### *Demographic data and basic characteristics:*

We have not found significant difference in gender distribution between the groups ( $p=0.581$ ). Group 1 included seven females (58%), group 2 included six females (43%), and group 3 included five females (38%).

Also, no statistically significant differences were found regarding age, BCVE, SE, axial length, IOP, and serum creatinine between the study groups as shown in Table (1). Contrastingly, we found highly significant differences regarding the duration of DM as well as HbA1c level.

*Optic nerve morphological parameters and RNFL thickness:*

We have not found statistically significant differences regarding the average and the vertical C/D ratio (Table 2).

Table (3) shows the results regarding the measurements of the RNFL thickness (average and four quadrants) in the studied groups.

Decremental thinning in the average, superior, inferior, and temporal RNFL thickness measurements were observed. With highly significant differences when comparing the average RNFL between all the studied groups as well as between groups 1 and 3 when comparing the superior RNFL measurements. Also, significant differences were found between groups 1 and 3 when comparing the inferior and temporal RNFL measurements.

Measurements of the nasal quadrant have not shown any significant differences.

*Correlations between RNFL measurements and duration of DM, HbA1c, and serum creatinine in groups 2 and 3:*

We explored the possible correlations between the different RNFL measurements in patients with mild and moderate NPDR and the duration of DM, glycemic control presented as HbA1c, and serum creatinine as an indicator of renal functions. Results are presented in Table (4).

In Fig. (1) we demonstrated the significant negative moderate correlations between the duration of DM ( $r=0.55$  in group 2 and  $r=0.6$  in group 3) with the average measurement of the RNFL thickness.

Table (1): Age, log MAR BCVA, SE, IOP, duration of diabetes mellitus (DM), glycosylated hemoglobin, and serum creatinine among the three studied groups.

Variable	Group 1	Group 2	Group 3	F	p	1vs2	1vs3	2vs3
	Mean ± SD							
Age (years)	53.08±2.27	54.79±2.29	54.23±2.28	1.84	0.173	0.154	0.416	0.811
BCVA (log MAR)	0.00±0.00	0.00±0.00	0.00±0.00	–	–	–	–	–
(Range of Snellen acuities ratios)	(6/6-6/6)	(6/6-6/6)	(6/6-6/6)					
SE (diopters)	0.29±0.72	-0.14±1.00	0.12±1.12	0.66	0.520	0.495	0.889	0.778
IOP (mmHg)	14.92±2.15	15.71±2.30	16.38±1.56	1.63	0.211	0.583	.172	0.682
AL	23.12±0.49	23.49±0.85	23.07±1.07	0.99	0.383	0.514	0.988	0.428
Duration of DM (years)	4.50±1.44	7.29±1.14	7.92±1.38	23.57	<0.001	<0.001	<0.001	0.445
HBA1c%	6.38±0.36	7.14±0.35	7.79±0.59	30.99	<0.001	<0.001	<0.001	0.001
Serum creatinine (mg/dL)	0.93±0.11	0.98±0.11	1.04±0.07	1.50	0.238	0.266	0.324	0.991

SD : Standard deviation. Log MAR : Logarithm of the minimum angle of resolution.  
 F : Freidman correlation coefficient. IOP : Intra ocular pressure.  
 P : Probability value. mmHg : Millimeter of mercury.  
 SE : Spherical equivalent. AL : Axiallength.  
 BCVA : Best corrected visual acuity. HBA1c% : Glycosylated hemoglobin.

Table (2): Average and vertical cup/disc (C/D) ratio in the studied groups.

Variable	Group 1	Group 2	Group 3	F	p	1vs2	1vs3	2vs3
	Mean ± SD							
Average C/D ratio	0.42±0.17	0.40±0.17	0.49±0.09	1.49	0.238	0.891	0.464	0.237
Vertical C/D ratio	0.37±0.16	0.37±0.17	0.47±0.07	2.25	0.120	0.999	0.181	0.166

Table (3): Optical coherence tomographic measurements of retinal nerve fiber layer (RNFL) thickness in the studied groups.

Variable	Group 1	Group 2	Group 3	F	p	1vs2	1vs3	2vs3
	Mean ± SD							
Average RFNL	100.17±4.04	93.07±2.89	86.77±5.43	31.36	<0.001	<0.001	<0.001	0.002
Superior RFNL	129.08±11.22	114.93±6.39	105.92±12.76	15.76	<0.001	0.004	<0.001	0.083
Inferior RFNL	130.08±10.09	122.71±12.63	112.77±13.26	6.43	0.004	0.282	0.002	0.107
Temporal RFNL	70.83±8.37	69.43±8.27	60.69±9.09	5.26	0.010	0.909	0.013	0.036
Nasal RFNL	71.50±9.17	65.29±5.68	67.38±8.35	2.10	0.137	0.119	0.380	0.773

RNFL: Retinal nerve fiber layer

Table (4): Correlations between the retinal nerve fiber layer measurements and duration of diabetes, glycosylated hemoglobin, and serum creatinine.

Variables	Average RNFL thickness		Superior RNFL thickness		Inferior RNFL thickness		Temporal RNFL thickness		Nasal RNFL thickness	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
<i>Group 2:</i>										
Duration of DM (years)	-0.55	0.042	-0.30	0.297	-0.40	0.156	-0.05	0.865	-0.18	0.538
HbA1c %	-0.15	0.609	-0.15	0.609	-0.04	0.892	-0.14	0.633	-0.36	0.206
Serum creatinine (mg/ dL)	-0.31	0.281	0.06	0.839	-0.48	0.082	-0.51	0.062	-0.41	0.145
<i>Group 3:</i>										
Duration of DM (years)	-0.60	0.030	-0.24	0.430	-0.44	0.132	-0.23	0.450	-0.26	0.391
HbA1c %	-0.48	0.097	-0.25	0.410	-0.15	0.625	-0.15	0.625	-0.45	0.123
Serum creatinine (mg/dL)	-0.42	0.153	-0.001	0.997	-0.51	0.075	-0.31	0.303	-0.01	0.974

RNFL: Retinal nerve fiberlayer. DM: Diabetes mellitus. HbA1c: Glycosylated hemoglobin.

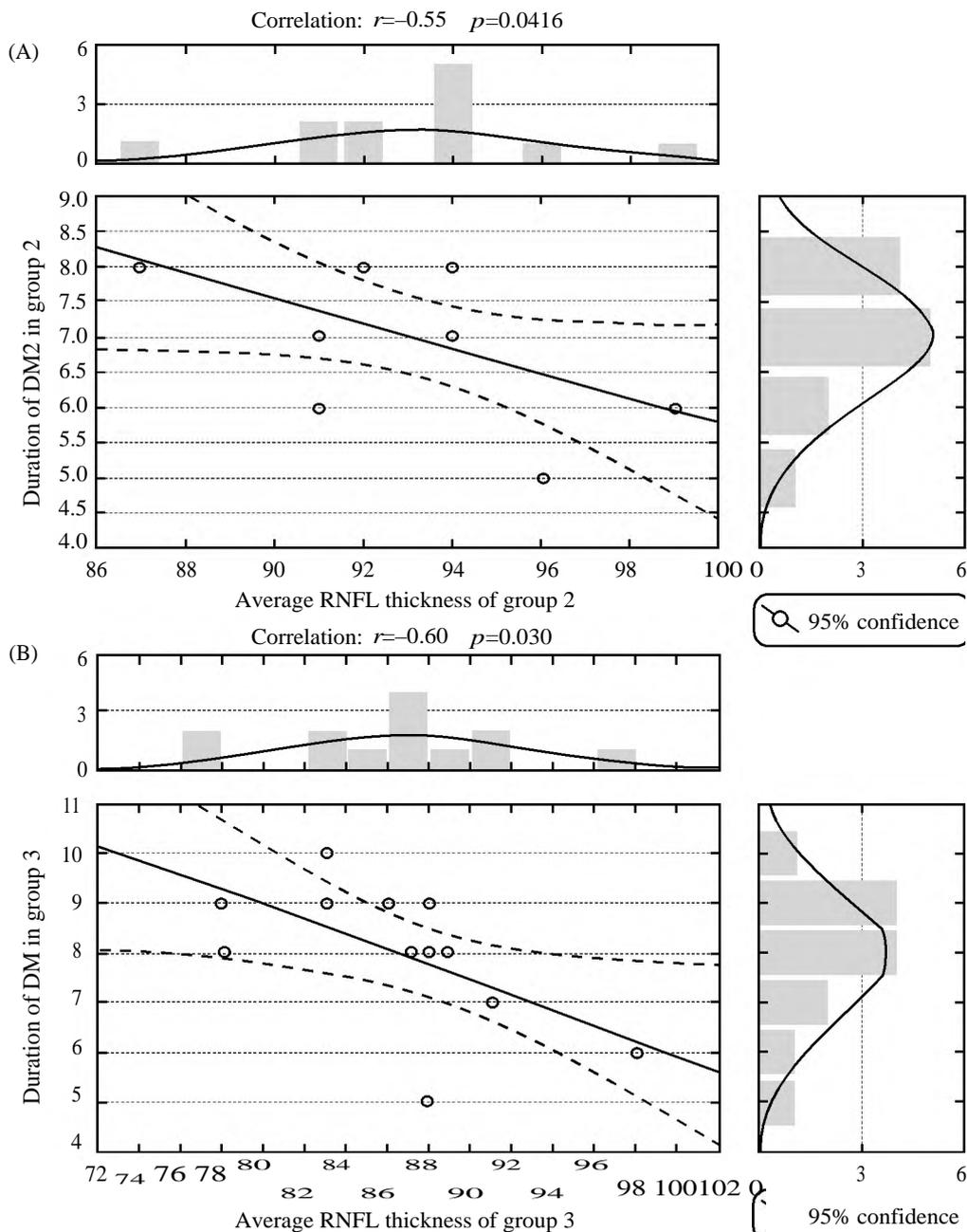


Fig. (1): Correlation between the duration of diabetes mellitus (DM) and the average retinal nerve fiber layer thickness in group 2 (A) and group 3 (B).

## Discussion

Functional and electrophysiological tests on diabetic patients with and without retinopathy have shown significant retinal dysfunction even in the absence of evident DR changes [19,20]. Later, with the advancement in imaging devices structural and microvascular retinal changes have been observed [21,22].

These observations raised the possibility of the participation of different processes in the functional and structural changes observed in eyes of patients with DM. We evaluated the thickness of the peripapillary RNFL in type 2 diabetics either with mild and moderate retinopathy or without retinopathy. A significant reduction was observed with the advancement of the DR stage.

In our study, diabetic patients were assigned into three groups. We excluded diabetics with advanced stages of DR (severe NPDR and PDR) and/or macular edema. Differences regarding the basic characteristics of the patients include age, gender, BCVA, SE, IOP, and axial length between the groups were not significant. However, significant differences have been observed regarding the duration of DM. Duration of DM increased significantly among patients with DR than in patients without clinically evident diabetic changes. This was expected as the relation between the duration of DM and the development of DR has been well established [23].

Differences in the average and superior RNFL measurements were significant, being higher in group 1 (without DR) and less in group 3 (moderate NPDR). These results agreed with Takahashi et al. results using scanning laser polarimetry [24] and Dhasmana et al. [25] results using SD-OCT. Gong et al. also reported decrease of the average RNFL thickness with the progression of DR using swept-source OCT. The RNFL measurements in the nasal quadrants have not shown any significant differences between the different groups. This result agreed with Lim et al. [26] results who reported insignificant differences between normal controls, diabetics without DR, and diabetics with NPDR regarding the thickness of the RNFL in the nasal quadrant.

In our study, moderate negative correlations were found between the duration of diabetes and the average measurements of RNFL thickness in patients with mild and moderate NPDR. In a longitudinal study by Lim et al., reduction of the RNFL was related to the duration of DM. They

reported faster reduction in the presence of DR compared to patients without retinopathy.

Also, decreased RNFL thickness was neither correlated with glycemic control represented as HbA1c nor with serum creatinine level. Similarly, Lim et al., havenot found a significant association between RNFL changes and HbA1c level [26].

Similarly, Dhasmana et al., [25] have not reported significant correlation between the thickness of the RNFL and HbA1c however, they have reported a weak negative correlation between serum creatinine and RNFL measurements. In their study the mean value for serum creatinine for diabetic patients was 1.4mg/dl which is higher than the level of serum creatinine among our patients ( $0.98 \pm 0.11$  and  $1.04 \pm 0.07$  in patients with mild and moderate NPDR).

Our study limitations are the little sample size, absence of assessment of the ganglion cell complex, and lack of follow-up.

The results of this study agreed with the results of the previous studies regarding the reduction of RNFL in diabetic patients with further reduction associated with DR. We are in need of further research to explore the association between the RNFL reduction and occurrence and/or progress of retinal diabetic changes. Also, decreased RNFL thickness among diabetics may warrant the vulnerability to glaucomatous damage. Multicenter international studies are needed to assess if new diagnostic and follow-up approaches are needed for glaucoma assessment in diabetic patients.

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## التغيرات فى سمك طبقة ألياف الشبكية العصبية فى مرضى السكرى من النوع الثانى، مع أو بدون اعتلال الشبكية السكرى

الهدف من البحث : الغرض من هذه الدراسة هو التحقيق فى التغيرات فى سمك طبقة الألياف العصبية للشبكية فى المرضى الذين يعانون من مرض السكرى من النوع الثانى، مع أو بدون اعتلال الشبكية السكرى.

طريقة البحث : تم تقسيم ٣٩ مريضاً من مرضى السكرى من النوع الثانى إلى ثلاث مجموعات. المجموعة الأولى بدون اعتلال الشبكية السكرى القابل للاكتشاف سريرياً، المجموعة الثانية تعاني من اعتلال الشبكية الخفيف والمجموعة الثالثة تعاني من اعتلال الشبكية المعتدل.

خضع جميع المرضى لفحص شامل للعيون، وتحليل للبول من أجل البيلة الألبومينية الدقيقة، ومستوى الهيموجلوبين السكرى، وتحليل مستوى الكرياتينين فى الدم.

النتائج :

- ١- تصبح متوسط سماكات طبقة الألياف العصبية للشبكية والجزاء العليا والأدنى أرق بشكل ملحوظ مع تطور اعتلال الشبكية السكرى.
- ٢- ارتبطت مدة مرض السكر ارتباطاً سلبياً بمتوسط سماكة طبقة الألياف العصبية للشبكية.
- ٣- ارتفع مستوى الهيموجلوبين السكرى بشكل ملحوظ مع تطور اعتلال الشبكية السكرى بين المجموعات.
- ٤- ارتفاع كبير فى نسبة إفراز الألبومين البولى مع تطور اعتلال الشبكية السكرى.
- ٥- ارتباط سلبى كبير بين متوسط نسبة كأس وقرص رأس العصب البصرى وسماكة الجزء الأدنى من طبقة الألياف العصبية للشبكية.
- ٦- تم العثور على ارتباط إيجابى بين المتوسط القياسى لمجال الإبصار ومتوسط سماكات طبقة الألياف العصبية للشبكية والجزء الأدنى.