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Assessment of Retinal Nerve Fiber Layer Thickness Using Optical Coherence Tomography in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Background: Diabetic retinopathy (DR) is the most common ocular complication of diabetes mellitus (DM) and considered one of the leading causes of blindness in developed countries. Diabetic retinopathy is predominantly a microangiopathy in which high glucose levels can make small blood vessels particularly, vulnerable to damage.

Objective: The aim of this work was to assess the thickness of peripapillary retinal nerve fiber layer (RNFL) measured by Swept Source optical coherence tomography (SS-OCT) in patients with Type 2 Diabetes Mellitus (DM).

Patients and Methods: The study was an observational cross-sectional study. The study was conducted on 40 eyes of diabetic patients from the outpatient clinic in ophthalmology department of Al-Azhar University Hospitals. All patients were subjected to a complete ophthalmic examination including OCT.

Results: The current study showed a negative correlation between parameters related to DM (duration of DM, and state of glycemic control measured by HbA1C) and all the parameters related to RNFL, and RGCL thickness but this correlation was statistically insignificant, and there was statistically significant decrease in superior RNFL thickness in patients with mild DR than patients with no DR, however, this difference was statistically insignificant in all parameters related to RGCL thickness in the two groups.

Conclusion: Optical coherence tomography (OCT) provides non-invasive, quantitative and objective measurement of RNFL thickness, optic nerve head, and RGCL thickness with high resolution and accuracy. This could be the method of choice for monitoring the neurodegenerative changes in DR.

Keywords: Retinal Nerve Fiber Layer Thickness, Optical Coherence Tomography, Diabetes Mellitus.

INTRODUCTION

Diabetic retinopathy (DR) is the most common ocular complication of diabetes mellitus (DM) and considered one of the leading causes of blindness in developed countries [(1)]. Diabetic retinopathy is predominantly a microangiopathy in which high glucose levels can make small blood vessels particularly, vulnerable to damage. Direct hyperglycemic effect on retinal cells is also likely to play a role [(2)].

Normal vision depends on many factors, one of them is the normal function of the retinal neurons to produce a good quality of vision. Deterioration of quality of vision starts early in diabetes, before clinical retinopathy becomes an evident, this probably indicates the early signs of neuronal dysfunction [(3)]. Retinal nerve fiber layer (RNFL) is one of the important structural neuron in the retinal layers which is often shown to be affected in the early pathological stages of diabetic retinopathy. RNFL thinning or defects have been reported by several studies in people with diabetes[(4, 5)].

Retinal nerve fiber layer loss also has been reported in diabetic patients without diabetic retinopathy but their blood glucose was poorly controlled[(1)]. In this regard, defect in RNFL could be considered another ocular association of diabetes other than diabetic retinopathy[(6)].

Assessment of RNFL loss is crucial because RNFL loss is irreversible and may contribute to diabetic optic nerve dysfunction [(7, 8)].

Optical coherence tomography (OCT) enables interpretation of a variety of eye diseases [(9)]. It also provides an objective and quantitative measurement of RNFL thickness and optic nerve head with high resolution and accuracy [(10)].

Different authors have reported clinical decrease in total central retinal or single cellular layer thickness in diabetic eyes with or without signs of DR compared to control groups (subjects without DM).[(15, 16)]Others have shown reduction in the inner retinal thickness in the macular area in diabetic patients with mild DR, this may represent initial loss in ganglion cells in the pericentral areas followed by thinning of retinal nerve fiber layer in the peripheral macula [(5)].

Vujosevic and Midena has concluded that automatic layering of retinal structures by SD-OCT may be a useful tool in diagnosing and monitoring early intraretinal changes in diabetic retinopathy[(11)]. Subsequently, detection of early RNFL thinning may help ophthalmologists to provide effective treatment of diabetic retinopathy and early prevention, thus reducing vision loss[(3)].

The aim of this work was to assess the thickness of peripapillary retinal nerve fiber layer (RNFL) measured by Swept Source optical coherence tomography (SS-OCT) in patients with Type 2Diabetes Mellitus (DM).

SUBJECTS AND METHODS

This observational cross-sectional study included a total of 40 eyes of diabetic patients attending at the

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outpatient clinic, Department of Ophthalmology, Al-Azhar University Hospitals. Written informed consent from all the subjects were obtained.

Ethical approval:

Approval of the ethics committee of the Faculty of Medicine, Al-Azhar University was obtained. The study followed the principles of the Declaration of Helsinki [(12)].

Inclusion criteria:

Type 2 diabetic patients over 10 years with prior diagnostic criteria based on the American Diabetes Association and/or World Health Organization criteria. Regular use of insulin and/or oral hypoglycemic agents for the treatment of diabetes can facilitate the selection of required subjects [(13)].

Exclusion criteria:

1. Type 1 Diabetes Mellitus.
2. Type 2 diabetic patients with disease history less than 10 years.
3. Criteria related to visual acuity:

a. Best corrected visual acuity less than 6/12 Snellen (UK in meter) or 0.5 using decimal system, 20/40 Snellen (US in feet).
b. Myopia > 5 Diopters.

4. Presence of media opacities affecting quality of OCT images (signal strength below 60) as: corneal opacities, cataract, vitreous hemorrhage.
5. Presence of any type of glaucoma, history of using or current usage of intraocular pressure lowering agents, history of glaucoma surgeries or filtration procedures or history of previous attacks of elevated intra ocular pressure.
6. Intra ocular pressure > 21 mmHg.

All patients were subjected to the following:

Full History taking (with particular emphasis on):

- **a.** Type and duration of diabetes mellitus.
- **b.** State of glycemic control (HbA_{1c}).
- **c.** Presence or absence of any systemic disease e.g. hypertension, renal impairment.
- **d.** History of previous eye surgery.

2. Complete ophthalmic examination including:

- **a.** Visual acuity (uncorrected and best corrected visual acuity) tested using Snellen's chart (metric). Snellen VA was converted to LogMAR for statistical analysis.
- **b.** Anterior segment examination.
- **c.** Intra ocular pressure (IOP) measurement using the Goldman applanation tonometry.
- **d.** Dilated Fundus examination by stereoscopic slit-lamp biomicroscopy using 90-diopter noncontact lens.

Table (I) : Different notation of visual acuity values as LogMAR values and Snellen fractions[(14)].

LogMAR	Snellen Fractions
1.52	1/60
1.4	2/60
1.3	3/60
1.22	4/60
1.1	5/60
1.0	6/60
0.92	6/48
0.8	6/38
0.7	6/30
0.6	6/24
0.5	6/19
0.4	6/15
0.3	6/12
0.22	6/9.5
0.1	6/7.5
0.0	6/6.0
-0.1	6/4.8
-0.2	6/3.8
-0.3	6/3.0
-0.4	6/2.4

3. Ophthalmic investigation: OCT scan was done using DRI - Triton (plus) Swept Source OCT (SS-OCT):

The Topcon DRI OCT Triton (plus) is a swept source OCT with a non-mydriatic color fundus camera and a monochrome camera for fluorescein angiography and fundus auto fluorescence utilizing the exclusive Spaide auto fluorescence filters. Utilizing a 1,050 nm wavelength light source, and a scanning speed of 100,000 A Scans/sec, it provides uniform scanning sensitivity allowing superior visualization of the vitreous and choroid in the same scan. Invisible OCT scanning light along with high scanning speeds reduce the effect of patient eye movement and allow for more data to be collected [(15)]. A 12 mm x 9 mm wide field scan along with 7 layer automated layer segmentation (including choroid) provides measurement and topographical maps of the optic nerve and macula in one scan.

The easy-to-use, intuitive IMAGEnet®6 software enables dynamic viewing of the OCT data, providing 3D, 2D and fundus images simultaneously. Pin-Point™ Registration properly indicates the location of the OCT image within the fundus image. In addition, the compare and follow up scan functions allow users to view serial exams as well as scan the exact same location of the retina. EnView software, based on en face technology, with layer flattening application allows for visualization of the various layers of the retina. Enhanced Vitreous Visualization (EVV) application allows the user to easily see the structures of the vitreous[(16)].

Figure (1): DRI-Triton (plus), Swept source OCT (ver.10.07)

Statistical analysis

Data management and analysis will be performed using statistical package SPSS version 25 for windows (SPSS Inc., Chicago, IL). The numerical data will be statistically presented in terms of range, mean, standard deviation, median and interquartile range (IQR).Cat egorical data will be summarized as percentages. Comparisons between numerical variables will be done by Student's unpaired t-test or Mann-Whitney U test for parametric data. Comparing categorical variables will be done by Chi-square test or Fisher exact test for small sample size. Correlations between various variables were done using Pearson moment correlation equation for linear relation, value was considered significant when P-values less than 0.05.

RESULTS

Patients' characteristics:

Demographic Data:

This study was carried on 40 eyes (including 21 right eyes and 19 left eyes) of 21 diabetic patients suffering from type 2 DM. The cases were chosen according to the criteria mentioned before. 12 patients were females (57.2%) and 9 were males (42.8%). Their ages ranged from 48 to 67 years and the mean age of the study population was (57.19 ± 5.618)

Table (2): Distribution of the studied cases according to Age (years) (n=21)

N	21
Mean ± SD	57.19 ±5.618
Median	56.00
Range	19
Min - Max	48 - 67

Table (3): Distribution of the studied cases according to their sex (n=21)

	Frequency	Percent
Male	9	42.8
Female	12	57.2
Total	21	100.0

Table (4): Distribution of the studied eyes according to Laterality (n=40)

	Frequency	Percent
OD	21	52.5
OS	19	47.5
Total	40	100.0

Correlation between RNFL thickness (µm) and Age: Pearson's r test was done, and it showed a statistically significant negative correlation between age and some of the parameters related to RNFL thickness [Temporal RNFL thickness (r = -0.504, p = 0.020), and Total RNFL thickness (r = -0.480, p = 0.028]. These RNFL thickness parameters decreased in thickness with increased age.

Table (5): Correlation between RNFL thickness (µm) and Age (n = 21)

		RNFL Thickness (um)				
		Superior	Inferior	Nasal	Temporal	Total
Age	R	-.384	-.410	-.149	-.504*	-.480*
	P value	.086	.065	.520	.020*	.028*

r: Pearson coefficient. * : p value ≤ 0.05

Correlation between RNFL thickness (µm) and parameters related to DM:

a) Diabetes Control

Student's unpaired t-test was done to compare between patients controlled by oral hypoglycemic drugs and those controlled by insulin according to different parameters of RNFL thickness, and showed statistically insignificant difference in all parameters related to RNFL thickness (superior, inferior, nasal, temporal, and total) between the two groups.

Table (6): Correlation between RNFL thickness and method of DM control (n = 21)

RNFL Thickness (µm)	oral		insulin		
	N	Mean ± SD	N	Mean ± SD	P value
Superior	15	117.40 ± 8.365	6	123.33 ± 12.738	0.328
Inferior	15	130.40 ± 17.278	6	125.50 ± 11.005	0.451
Nasal	15	84.13 ± 10.006	6	82.50 ± 14.707	0.810
Temporal	15	66.87 ± 7.615	6	70.17 ± 2.229	0.145
Total	15	99.73 ± 8.481	6	100.67 ± 7.474	0.809

b) DM Duration (years)

Pearson's r test was done and there was statistically insignificant negative correlation between the duration of DM and all the parameters related to RNFL thickness.

Table (7): Correlation between RNFL thickness and duration of DM (n = 21)

		RNFL Thickness (um)				
		Superior	Inferior	Nasal	Temporal	Total
DM Duration(years)	R	-.415	-.298	-.174	-.24	-.401
	P value	.061	.190	.451	.276	.071

r: Pearson coefficient

c) Glycemic control, HbA1C (%)

Pearson's r test was done and there was statistically insignificant negative correlation between the state of glycemic control measured by HbA1C and all the parameters related to RNFL thickness.

Table (8): Correlation between RNFL thickness and HbA1C (n = 21)

		RNFL Thickness (um)				
		Superior	Inferior	Nasal	Temporal	Total
HbA1C (%)	R	-.350	-.171	-.175	.072	-.239
	P value	.120	.458	.447	.756	.297

r: Pearson coefficient

Correlation between RNFL thickness (µm) and Clinical Data :

a) **Sphere/equivalent:**

Pearson's r test was done and there was statistically significant positive correlation between sphere equivalent and superior RNFL thickness (r = 0.443, p = 0.004), and there was statistically insignificant positive correlation between sphere equivalent and other parameters related to RNFL thickness (inferior, nasal, temporal, and total).

Table (9): Correlation between RNFL thickness and sphere equivalent (n = 40)

		RNFL Thickness (um)				
		Superior	Inferior	Nasal	Temporal	Total
Sphere/Equivalent	R	.443*	.215	.016	.017	.255
	P value	.004*	.183	.923	.917	.113

r: Pearson coefficient, * : p value ≤ 0.05

b) **BCVA (Log MAR):**

Pearson's r test was done and there was statistically significant negative correlation between BCVA in (Log MAR) and nasal RNFL thickness (r = - 0.407, p = 0.009), and there was statistically insignificant negative correlation between BCVA in (Log MAR) and other parameters related to RNFL thickness (superior, inferior, temporal, and total).

Table (10): Correlation between RNFL thickness and BCVA (LogMAR) (n = 40)

		RNFL Thickness (um)				
		Superior	Inferior	Nasal	Temporal	Total
BCVA (Log MAR)	R	-.013	-.224	-.407	-.057	-.266
	P value	.937	.164	.009*	.728	.097

r: Pearson coefficient, * : p value ≤ 0.05

c) **IOP :**

Pearson's r test was done and there was statistically insignificant negative correlation between IOP and all the parameters related to RNFL thickness.

Table (11): Correlation between RNFL thickness and IOP (n = 40)

		RNFL Thickness (um)				
		Superior	Inferior	Nasal	Temporal	Total
IOP	R	.144	-.064	-.096	-.241	-.104
	P value	.374	.694	.554	.133	.525

r: Pearson coefficient

d) **Fundus Examination:**

Student's unpaired t-test was done to compare between patients with no DR and those with mild NPDR according to different parameters of RNFL thickness, and showed statistically significant decrease in superior RNFL thickness (p value = 0.006) in patients with mild NPDR than those with no DR, and there was statistically insignificant decrease in other parameters related to RNFL thickness (inferior, nasal, temporal, and total) in patients with mild NPDR than those with no DR.

Table (12): Comparison between patients with no DR and Mild NPDR according RNFL thickness (n = 40)

RNFL Thickness(µm)	Free (no DR)		Mild NPDR		
	N	Mean ± SD	N	Mean ± SD	P value
Superior	31	123.03 ± 9.053	9	111.56 ± 9.342	.006*
Inferior	31	129.06 ± 17.368	9	124.22 ± 19.511	.515
Nasal	31	82.81 ± 11.262	9	82.00 ± 12.738	.867
Temporal	31	67.23 ± 5.920	9	71.78 ± 17.768	.470
Total	31	100.68 ± 8.631	9	97.44 ± 8.748	.346

*: p value $\leq$ 0.05

Further statistical analysis was done using different statistical tests to detect presence of statistically significant correlation between GCL thickness and: Duration of DM, state of glycemic control by HbA1C, BCVA (LogMAR), and fundus examination.

I. Correlation between GCL thickness (µm) and Duration of DM:

Pearson's r test was done and there was statistically insignificant negative correlation between the duration of DM and all the parameters related to GCL thickness.

Table (13): Correlation between GCL thickness and duration of DM (n = 21)

		GCL superior (um)	GCL inferior (um)	GCL total (um)
DM Duration (years)	R	-.118	-.174	-.158
	P value	.610	.450	.493

r: Pearson coefficient
Insignificant negative correlation

II. Correlation between GCL thickness (µm) and HbA1C:

Pearson's r test was done and there was statistically insignificant negative correlation between the state of glycemic control measured by HbA1C and all the parameters related to GCL thickness.

Table (14): Correlation between GCL thickness and HbA1C (%) (n = 21)

		GCL superior (um)	GCL inferior (um)	GCL total (um)
HbA1C (%)	R	-.019	-.116	-.075
	P value	.935	.617	.746

r : Pearson coefficient
Insignificant negative correlation

III. Correlation between GCL thickness (µm) and BCVA:

Pearson's r test was done and there was statistically insignificant very weak negative correlation between BCVA in (LogMAR) and all the parameters related to GCL thickness.

Table (15): Correlation between GCL thickness and BCVA (LogMAR) (n = 21)

		GCL superior (um)	GCL inferior (um)	GCL total (um)
BCVA(LogMAR)	R	-.038	.008	-.019
	P value	.818	.959	.908

r: Pearson coefficient
Insignificant very weak negative correlation

IV. Correlation between GCL thickness (µm) and Fundus examination:

Student's unpaired t-test was done to compare between patients with no DR and those with mild NPDR according to different parameters of GCL thickness, and showed statistically insignificant decrease in all parameters related to GCL thickness (superior, inferior, and total) in patients with mild NPDR than those with no DR.

Table (16): Comparison between patients with no DR and Mild NPDR according GCL thickness (n = 40)

GCL thickness (um)	Free (no DR)		Mild NPDR		
	N	Mean ± SD	N	Mean ± SD	P value
Superior	31	66.42 ± 7.645	9	63.56 ± 2.833	.281
Inferior	31	66.26 ± 7.992	9	61.89 ± 4.014	.124
Total	31	66.16 ± 7.703	9	62.44 ± 2.920	.167

DISCUSSION

Lee *et al.* [17] reported that peripapillary RNFL thickness measured by the Cirrus HD-OCT was associated with age. The correlations were prominent in superior and inferior areas, whereas those in nasal and temporal areas were less remarkable. While **Alasil *et al.*** [18] reported that the thickest RNFL measurements were found in the inferior quadrant, followed by the superior, nasal, and temporal quadrants (ISNT rule applied to the RNFL). Thinner RNFL measurements were associated with older age and increasing myopia.

The current study showed statistically significant negative correlation between age and some of the parameters related to RNFL thickness [Temporal RNFL thickness (r = -0.504, p = 0.020), and Total RNFL thickness (r = -0.480, p = 0.028]. These RNFL thickness parameters decreased in thickness with increased age.

Chihara *et al.* [4] reported the risk factors for RNFL defect as a higher level of diabetic retinopathy, systemic hypertension, and advanced age, but visual acuity and HbA1c level at the time of examination were reported to be not correlated with these defects.

Ozdec *et al.* [1] made the first clinical study demonstrating the effects of diabetic glucose regulation level on RNFL by using scanning laser polarimetry (NFA - GDx). RNFL thickness was seen to decrease with development of diabetic retinopathy and with impairment of metabolic regulation. They have demonstrated that all of the studied NFAGDx variables except symmetry were statistically significantly lower in diabetic patients with poor metabolic control as compared with the healthy control group and the group of good metabolic control. The values were even lower in the group with diabetic retinopathy independent of metabolic control.

In the current study there was negative correlation between parameters related to DM (duration of DM, and state of glycemic control measured by HbA1C) and all the parameters related to RNFL thickness (superior, inferior, nasal, temporal, and total) but this correlation was statistically insignificant. In addition, there was statistically significant decrease in superior RNFL thickness (p value = 0.006) in patients with mild NPDR than those with no DR, and there was statistically insignificant decrease in other parameters related to RNFL thickness (inferior, nasal, temporal, and total) in patients with mild NPDR than those with no DR. In our study there was statistically insignificant difference in all parameters related to RNFL thickness (superior, inferior, nasal, temporal, and total) between patient controlled by oral hypoglycemic drugs, and patient controlled by insulin.

Another study done by **Chen *et al.*** [19] found that the RNFL around the optic disc was thinner in the patients with DM than in the controls only at the 9 o'clock position, which may prove that RNFL degeneration occurs in the onset stage of diabetes, however, it is difficult to determine which area is the first to be affected. **Kim *et al.*** [20], analyzed the relationship between sphere equivalent/axial length and RNFL parameters measured with TOPCON 3D-OCT, and found individuals with highly myopia had thinner RNFLs, except for the temporal quadrant. In the current study there was statistically significant positive correlation between sphere equivalent and superior RNFL thickness (r = 0.443, p = 0.004), and there was statistically insignificant positive correlation between sphere equivalent and other parameters related to RNFL thickness (inferior, nasal, temporal, and total).

Moreover, the current study showed statistically significant negative correlation between BCVA in (Log MAR) and nasal RNFL thickness (r = - 0.407, p = 0.009), and there was statistically insignificant negative correlation between BCVA in (Log MAR) and other parameters related to RNFL thickness (superior, inferior, temporal, and total).

Chin *et al.* [19] **and Carpineto *et al.*** [21] found statistically insignificant correlation between RNFL thickness BCVA, while, there was statistically significant negative correlation between the average, inferior and superior RNFL thickness and the (Log MAR) BCVA in another study done by **Srivastav *et al.*** [22] The difference in the current study may be due to the exclusion criteria of the study as we excluded patients with BCVA less than 6/12 in Snellen's fraction (0.3 using LogMAR) and patients with myopia more than - 5.0 diopters.

Regarding RGCL, there was negative correlation between parameters related to DM (duration of DM, and state of glycemic control measured by HbA1C) and all the parameters related to GCL thickness (superior, inferior, and total), but this correlation was statistically insignificant. In addition, this study showed statistically insignificant decrease in all parameters related to GCL thickness in patients with mild NPDR than those with no DR. This came in agreement with the study done by **Carpineto *et al.*** [21] **and van Dijkt *et al.*** [23] they stated that there was no correlation between the duration of DM and neither the GC-IPL thickness nor the RNFL thickness because the disease process was unclear in patients with type 2 diabetes, and glucose metabolism could be altered years before diabetes diagnosis. Therefore, the possible correlation between the thinning of GC-IPL, RNFL and the duration of disease couldn't be precisely demonstrated.

While, **Chin *et al.*** [19] stated that both ganglion cells loss and axonal loss were correlated with HbA_{c1} and duration of DM. **Carpineto *et al.*** [21] also reported the presence of a correlation between the GC-IPL and RNFL average thickness values and HbA_{1c} level.

There was also statistically insignificant very weak negative correlation between BCVA in (LogMAR) and all the parameters related to GCL thickness. This may be because our patients has early stages of diabetic retinopathy and the ganglion cell layer was not affected that much to influence the visual acuity, this also was agreed by **Chin *et al.*** [19] **and Carpineto *et al.*** [21].

The findings in the current study showed that mean temporal and total RNFL thickness parameters are inversely correlated with age, and nasal RNFL thickness has a negative correlation with LogMAR

BCVA, while, superior RNFL is directly correlated with spherical equivalent.

The current study also showed a negative correlation between parameters related to DM (duration of DM, and state of glycemic control measured by HbA1C) and all the parameters related to RNFL, and RGCL thickness but this correlation was statistically insignificant, and there was statistically significant decrease in superior RNFL thickness in patients with mild DR than patients with no DR, however, this difference was statistically insignificant in all parameters related to RGCL thickness in the two groups. These results comes with the speculation that neurodegeneration occurs during the early stages of DR[19]. However, the differences were not significantly meaningful at all of the tested points; thus, we may need to increase our sample size. Continuous follow-up of these subjects is also needed for further study.

CONCLUSIONS

- Optical coherence tomography (OCT) provides non-invasive, quantitative and objective measurement of RNFL thickness, optic nerve head, and RGCL thickness with high resolution and accuracy. This could be the method of choice for monitoring the neurodegenerative changes in Diabetic Retinopathy.

RECOMMENDATIONS

- Further studies with large sample size are recommended, and it must include more sever forms of DR like sever NPDR and PDR to get more statistically significant results.
- OCT can be used to assess early neurodegenerative changes by providing a non-invasive, quantitative measurement of RNFL and RGCL which appears during the early stages of Diabetic Retinopathy.

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