Retinal Nerve Fiber Layer Thickness Affection in Diabetic Retinopathy

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ABSTRACT

Background: diabetic retinopathy is a chronic progressive, potentially sight-threatening disease of the retinal microvasculature associated with the prolonged hyperglycaemia and other conditions linked to diabetes mellitus such as hypertension. **Objective:** Evaluation of the retinal nerve fiber layer thickness in patients with type II diabetes mellitus. **Patients and Methods:** Cross sectional design;, there is greater confidence the study has internal validity due to the systematic subject selection and equity of groups being compared. This is a prospective comparative study will be carried on forty eyes in patients with Type II Diabetes Mellitus. All investigations were carried out at Al-Azhar University hospitals. Written and informed consent were taken before the investigations.

Results: The male to female ratio in our study showing in diabetic group without retinopathy 12 to 8, while in the NPDR group showing 10 to 10 with a total male to female 22 to 18 in the study. In our study, the difference between NFL thickness measurements between four quadrants (upper, lower, nasal and temporal) between Diabetic group with no diabetic retinopathy and NPDR group is significant.. **Conclusion:** All quadrant retinal nerve fiber layer thinning is associated with peripheral neuropathy in patients with Type 2 diabetes.

Keywords: Ganglion cell layer, nerve fiber layer, intraretinal microvascular abnormalities**.**

INTRODUCTION

The diabetic retinopathy is a chronic progressive, potentially sight-threatening disease of the retinal microvasculature associated with the prolonged hyperglycaemia and other conditions linked to diabetes mellitus such as hypertension **(1).**

Diabetic retinopathy falls into two main classes: non proliferative and proliferative. The word "proliferative" refers to whether or not there is neovascularization (abnormal blood vessel growth) in the retina. Early disease without neovascularization is called nonproliferative diabetic retinopathy (NPDR). As the disease progresses, it may evolve into proliferative diabetic retinopathy (PDR), which is defined by the presence of neovascularization and has a greater potential for serious visual consequences **(2).**

In NPDR, Hyperglycemia results in damage to retinal capillaries. This weakens the capillary walls and results in small outpouchings of the vessel lumens, known as microaneurysms. Microaneurysms eventually rupture to form hemorrhages deep within the retina, confined by the internal limiting membrane (ILM). Because of their dot-like appearance, they are called "dot-and-blot" hemorrhages. The weakened vessels also become leaky, causing fluid to seep into the retina. Fluid deposition under the macula, or macular edema, interferes with the macula's normal function and is a common cause of vision loss in those with DR. Resolution of fluid lakes can leave behind sediment, similar to a receding river after a flood. This sediment is composed of lipid byproducts and appears as waxy, yellow deposits called hard exudates. As NPDR progresses, the affected vessels eventually become obstructed. This obstruction may cause infarction of the nerve fiber layer, resulting in fluffy, white patches called cotton wool spots (CWS) **(1).** In PDR, as mentioned earlier, the retina has a high metabolic requirement, so with continued ischemia, retinal cells respond by releasing angiogenic signals such as vascular endothelial growth factor (VEGF). Angiogenic factors, like VEGF, stimulate growth of new retinal blood vessels to bypass the damaged vessels. This is referred to as neovascularization. In PDR, the fibrovascular proliferation extends beyond the ILM. This may sound like a good idea, but the new vessels are leaky, fragile, and often misdirected. They may even grow off the retina and into the vitreous. As the vitreous shrinks with age, it pulls on these fragile vessels and can cause them to tear, resulting in a vitreous hemorrhage and sudden vision loss. These vessels may also scar down, forming strong anchors between the retina and vitreous causing traction on the retina. If enough force is created, a tractional retinal detachment may occur. This is another mechanism by which DR can cause sudden vision loss. If the retina is not re-attached soon, especially if the macula is involved, vision may be permanently compromised **(2).**

This layer contains about 1.2 million ganglion cells as well as a number of other cell types, including "displaced" amacrine cells, astrocytes, enthothelial cells, and pericytes. The thickness of the ganglion cell layer is greatest in the perifoveal macula consisting of between eight and ten rows of nuclei (60–80 μm), decreases to a single row outside the macula (10–20 μm), and is absent from the foveola itself **(3).**

Ganglionic axons travel towards the optic nerve head within the nerve fiber layer. Thin and difficult to discernin the far periphery, the nerve fiber layer becomes hicker towards the disc as a result of the convergence of all retinal ganglion axon fibers on the optic disc. 44 G.D. Hildebrand and A.R. Fielder The axons are accompanied by astrocytes in the nerve fiber layer and are separated into small bundles by the cellular processes of Müller cells and the internal limiting membrane. The exact cross-sectional ordering of the axonal fibers of the peripheral and central ganglion cells in the retina remains controversial **(4).**

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Temporal to the disc lies the macula, which has the highest density of ganglion cells. Axons from the macula project straight to the disc, forming the papillomacular "bundle." The remaining axons of the temporal retina reach the optic disc only by arcing around the papillomacular bundle. As a result, all temporal ganglion cell axons originating from outside the macula are compressed into the superotemporal and inferotemporal sectors of the optic nerve, above and below the temporal entry of the papillomacular bundle fibers. The superior and inferior nerve fibers are therefore much thicker (almost 200 mm) compared to the papillomacular bundle (65 mm) and easier to see on clinical examination, especially in red-free light. Nasally, axons enter the nasal half of the optic disc more or less straight. In addition, ganglion axon fibers do not cross the horizontal meridian (the horizontal raphe) [(5)]. Optical coherence tomography (OCT) is an established medical imaging technique that uses light to capture micrometer-resolution, three-dimensional images from within optical scattering media. OCT is based on low-coherence interferometry, typically employing near-infrared light. The use of relatively long wavelength light allows it to penetrate into the scattering medium. Confocal microscopy, another optical technique, typically penetrates less deeply into the sample but with higher resolution [(6)].

OCT is heavily used by ophthalmologists and optometrists to obtain high-resolution images of the eye's anterior segment and retina. Owing to its cross-sectional capabilities, OCT provides a straightforward method of assessing axonal integrity in multiple sclerosis and glaucoma.

OCT is also well suited to assess macular degeneration and is considered the new standard for the assessment of diabetic macular edema. More recently, ophthalmic OCT devices have been engineered to perform angiography, and have been used to assess retinal microvasculature pathology in diseases such as glaucoma and diabetic retinopathy. NFL thickness could be an important parameter to study the pathogenesis of macular vision loss in Diabetic retinopathy[(6)].

Macular edema may be noted on fundus exam, but is much more easily appreciated using OCT. OCT is a laser imaging technique which produces an image showing the individual layers of the retina and the shape of the retinal surface.

Fluid accumulation between layers can be appreciated as black patches on the scan and irregular retinal surfaces may reflect the etiology behind a patient's visual abnormalities. It should be noted, however, that in the ETDRS (the study which is used to support the treatment for CSME), OCT was not used. CSME was diagnosed only by clinical appearance [(6)].

AIM OF THE WORK

Is to evaluate the retinal nerve fiber layer thickness in patients with type II diabetes mellitus.

PATIENTS AND METHODS

Study design: Cross sectional design; as we aimed to identify and evaluate NFL in type II diabetic patients, there is greater confidence the study has internal validity due to the systematic subject selection and equity of groups being compared.

Subjects: This is a prospective comparative study was carried on forty eyes in patients with Type II Diabetes Mellitus. All investigations were carried out at Al-Azhar University hospitals. Written and informed consent was taken before the investigation. **The study was approved by the Ethics Board of Al-Azhar University.**

Inclusion criteria: Refractive error <±3.00 diopters. Age group from 20 to 65 years old. Diabetic patients type II. Medically free subjects "control group".

Exclusion criteria: Refractive error (spheric Equivalent) >±3.00 diopters. Visual acuity below 0.1 logarithm of the minimum angle of resolution, had significant media opacity. A history of glaucoma, uveitis, retinal disease, vitreous hemorrhage. History of intraocular surgery, Laser and IV injection in the last 6 months.

Study groups:

The cases were divided into 2 groups: ***Group A*** [Diabetic patients with no diabetic retinopathy group]: 20 eyes. ***Group B*** [NPDR group]: 20eyes.

Methods:

Full ophthalmic examination including: Visual acuity (VA) and best corrected VA (BCVA) using the Snellen chart. Automated refraction using TOPCON auto-refractometer.

Measurement of macular NFL: NFL were measured by ZEISS Cirrus4000 Spectral Domain OCT after dilatation using tropicamide 1% eye drops. All OCT measurements were performed following pupillary dilation using tropicamide 1% eye drops instilled 20-30 minutes prior to examination. The subject's head was fixed by a lower jaw sustainer and a forehead sustainer with the eye focusing on a target for few seconds without blinking during the OCT scanning. Internal fixation was chosen because of better reproducibility than external fixation. In all patients, the fast macular thickness map scan protocol was used and monitored with an infrared-sensitive videocamera. Scan quality was assessed during scanning to maximize the quality of the scans and minimize problems such as decentration and focus. Poor scans were repeated until the best scans were obtained. The central fixation was confirmed in each patient by observing the location of the foveal depression at the centre of each macular scan. Therefore, off-foveola fixation was highly unlikely with the present data.

Statistical Analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when their distribution found parametric while qualitative data were presented as number and percentages. The comparison between two independent groups with quantitative data and parametric

distribution was done by using Independent t-test. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: P > 0.05: Non significant (NS). P < 0.05: Significant (S). P < 0.01: Highly significant (HS).

RESULTS

Sex: The male to female ratio in our study showing in diabetic group without retinopathy 12 to 8, while in the NPDR group showing 10 to 10 with a total male to female 22 to 18 in the study.

Table (1): Gender of studied groups.

Group	Sex	Frequency	Percent
NO DR	Male	12	60 %
	Female	8	40%
	Total	20	
NPDR	Male	10	50%
	Female	10	50%
	Total	20	

Age: In our study the mean age for the diabetic patients without retinopathy group it was 45.5±8.17, while for the NPDR group it was 58.7±10.88.

Table (2): The mean ± SD and range of age in our study.

Group	N	Minimum	Maximum	Mean	SD
NO DR	20	34	59	46.5	8.17
NPDR	20	40	74	58.7	10.88

Average RNFL thickness: In our study the mean average RNFL thickness for the diabetic patients without retinopathy group it was 98.95±5.5, while for the NPDR group it was 82.3±9.08.

Table (3): The mean ± SD and range of average RNFL thickness in our study.

Group	N	Minimum	Maximum	Mean	SD
NO DR	20	90 µ	108 µ	98.95	5.5
NPDR	20	59 µ	96 µ	82.3	9.08

RNFL symmetry: In our study the mean average RNFL symmetry for the diabetic patients without retinopathy group it was 89±3.73, while for the NPDR group it was 73±3.73.

Table (4): The mean ± SD and range of average RNFL symmetry in our study.

Group	N	Minimum	Maximum	Mean	SD
NO DR	20	80%	95%	89	3.73
NPDR	20	61%	85%	73	7.69

RNFL quadrants:

In our study the difference in the thickness in the Inferior quadrant and we found that the mean of NFL thickness in the Inferior quadrant for the non diabetic retinopathy group 128.1 ± 13.75 in comparison to 105.75 ± 15.39 in the NPDR group, the difference in the thickness in the Superior quadrant and we found that the mean of NFL thickness in the Superior quadrant for the non diabetic retinopathy group 122.4± 10.19 in comparison to 100± 18.76 in the NPDR group, the difference in the thickness in the Nasal quadrant and we found that the mean of NFL thickness in the Nasal quadrant for the non diabetic retinopathy group 74.2 ± 6.46 in comparison to 58.7±15.38 in the NPDR group, the difference in the thickness in the Temporal quadrant and we found that the mean of NFL thickness in the Temporal quadrant for the non diabetic retinopathy group 70.65± 9.62 in comparison to 64 ±8.53 in the NPDR group, the results of this study revealed that the NFL is significantly decrease in the NPDR group.

Table (5): The mean ± SD and range of RNFL quadrants

Group	Quadran	N	Minimum	Maximum	Mean	SD
NO DR	Inferior	20	105	150	128.1	13.75
	Superior	20	104	139	122.4	10.19
	Nasal	20	63	86	74.2	6.46
	Temporal	20	58	90	70.65	9.62
NPDR	Inferior	20	68	135	105.75	15.39
	Superior	20	59	130	100	18.76
	Nasal	20	11	78	58.7	15.38
	Temporal	20	46	79	64	8.53

Comparison between groups:

There is significant decrease of average RNFL thickness and RNFL symmetry in NPDR group than diabetic without DR group, P value < 0.05. There is significant decrease of inferior, superior, nasal quadrants of RNFL in in NPDR group than diabetic without DR group. There is non-significant difference of temporal quadrant of RNFL between groups, P > 0.05.

Table (6): Comparison between groups by unpaired t test.

	Unpaired T Test	
	T	P value
Average RNFL Thickness	7.01	< 0.001
RNFL symmetry	8.37	< 0.002
Inferior Quadrant of RNFL	4.84	< 0.003
Superior Quadrant of RNFL	4.69	< 0.004
Nasal Quadrant of RNFL	4.15	< 0.005
Temporal Quadrant of RNFL	2.31	0.26

DISCUSSION

Diabetic retinopathy (DR) is one of the most frequent causes of blindness in the working age population. As the prevalence of diabetes mellitus (DM) increases globally and patients live longer, the development of DR as a microvascular complication of DM also rises. DR classified non-proliferative (NPDR)

and proliferative (PDR). The optical coherence tomography (OCT) has been used for evaluation the thickness of retinal nerve fiber layer (RNFL) and ganglion cell complex (GCC) in some diseases. The purpose of this study was to compare the RNFL between diabetic patients **(6).**

Macular edema may be noted on fundus exam, but is much more easily appreciated using optical coherence tomography (OCT). OCT is a laser imaging technique which produces an image showing the individual layers of the retina and the shape of the retinal surface. Fluid accumulation between layers can be appreciated as black patches on the scan and irregular retinal surfaces may reflect the etiology behind a patient's visual abnormalities. It should be noted, however, that in the ETDRS (the study which is used to support the treatment for CSME), OCT was not used. CSME was diagnosed only by clinical appearance **(6)**.

In our study, the difference between NFL thickness measurements between four quadrants (upper, lower, nasal and temporal) between Diabetic group with no diabetic retinopathy and NPDR group is significant. The previous studies showed agreement for status of RNFL in patients with type 2 DM.

A study found that The RNFL thickness were thinner in diabetic patients with NPDR than diabetic patient without DR **Demir *et al.* (7)** in contrast to our study showing statistically significant decrease in RNFL thickness in NPDR than patient without DR**.**

A study found that at early stage of DR, the macula and RNFL thickness were altered **Oshitari *et al.* *(8)*** showing thinning of RNFL in NPDR while our study showing statistically decrease NFL thickness in NPDR.

A study showed that only inferior RNFL thinning associated with peripheral neuropathy but age, duration of disease and retinopathy levels did not significantly influence RNFL thickness **Shahidi *et al.* (9)** while our study shows stastistically significant decrease in NFL thickness in all quadrants in NPDR.

There were some limitations to our study: The approximately 20 eyes per each group is a relatively small number. Because we measured the NFL thickness using the automatic method the results might contain slight errors and this was the best clinical method currently available with the current OCT equipment, estimating the disctance between the ILM and IPL/INL. We did not consider the axial length, it has been reported that the eyes with longer axial length have a thinner retinal layers.

CONCLUSION

All quadrant retinal nerve fiber layer thinning is associated with peripheral neuropathy in patients with Type 2 diabetes.

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