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A COMPARATIVE STUDY OF THE ROLE OF RETINAL NERVE FIBER LAYER THICKNESS MEASURED BY OPTICAL COHERENCE TOMOGRAPHY VERSUS NERVE CONDUCTION VELOCITY FOR EARLY DIAGNOSIS OF PERIPHERAL NEUROPATHY IN TYPE TWO DIABETES MELLITUS

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ABSTRACT

Diabetic neuropathy may carry the risk of more complications and even mortality in patients with diabetes mellitus. Current techniques for early clinical assessment of diabetic peripheral neuropathy (DPN) are either relatively insensitive or invasive. There is a need for non-invasive, safe evaluation methods. Recently many studies evaluate the retina as an important site for the diagnosis and monitoring of (diabetic peripheral neuropathy) DPN. This study aim is to evaluate retinal nerve fiber layer (RNFL) thickness assessed by optical coherence tomography (OCT) in early detection of DPN compared with currently used golden standard methods. Thirty-eight diabetic patients were assessed clinically for neuropathy using neuropathy Mitchigan score and nerve conduction study of sural, popliteal and ulnar nerves. The subjects were divided into diabetic patients with neuropathy (patient group) and diabetic patients without neuropathy (control group); the patient group was further subdivided into mild, moderate and severe groups according to Toronto clinical neuropathy score system (TCSS). The RNFL thickness was measured in both groups. In our study twenty patients have DPN, in which RNFL thinning was demonstrated in both peripapillary and macular areas compared with the controls, and was more prominent in the inferior half. Moreover; the inferior macular RNFL thinning was statistically significant in patients with mild DPN compared with the control; whereas the nerve conduction study (NCS) failed to differentiate between mild diabetic neuropathy patient and control group. The RNFL thickness in type 2 diabetes mellitus significantly reduced with the severity of DPN and it can be used for early detection of DPN before the appearance of the NCS abnormalities.

INTRODUCTION

Diabetes affects nearly 285–347 million people worldwide and expected to reach 552 million in 2030 [1]. Neuropathy remains one of the commonest complications of diabetes, affecting up to 50% of all diabetic patients [2]. The risk factors for development of diabetic peripheral neuropathy (DPN) are not well understood, but it was found that its prevalence increases with increasing the duration and degree of hyperglycemia [3].

The development of DPN is an insidious and progressive process, and its severity is poorly linked with the development of symptoms [4] At present, underdiagnosis of the DPN prevent its early identification and interfering with its early management and prevention of neuropathy-related sequelae [5]. The nerve conduction study (NCS) are fundamentally the most widely accepted objective test for the diagnosis of late stage DPN and its sequelae [6-9], but it lack the sensitivity to detect early neuropathic changes [10], which highlights the urgent need for a valid clinical test as predictive markers for the early onset of neuropathy [11].

The gold-standard method for evaluating small, unmyelinated, and thinly myelinated Aδ- and C-type nerve fibers morphological changes can be accomplished by the examination of intraepidermal nerve fibers in skin biopsy but this procedure is invasive and expensive [12].

Recent studies have indicated that the retina may also be an important site for the diagnosis and monitoring of neuropathy [13]. As an alternative to skin biopsy, the small nerve fibers in the retina can be directly visualized reliably and noninvasively by a technique of optical coherence tomography (OCT).

OCT was first introduced by Huang and colleagues in 1991 [14]. It is a novel technology for cross-sectional and three-dimensional imaging in biological systems with ultra high resolution approaching that of histology [15], it can provide images at cellular level [16], OCT measures the intensity of back-reflected near-infrared light to measure the thickness of different biological tissues [17]. It is a rapid noncontact noninvasive method that allows in vivo imaging of the retina, optic nerve head and retinal nerve fiber layer (RNFL) thickness that can be qualitatively and quantitatively evaluated [18].

OCT is a promising tool for the genesis of biomedical images, which enables tissue pathology to be imaged in situ and in real time [19]. It can provide quantitative information on retinal pathology and monitor disease progression in vivo that can't be obtained by any other method [20].

PATIENTS AND METHODS

The protocol and consent procedures were approved by the scientific committee at Riyadh National Hospital and adhered to the tenets of the Declaration of Helsinki of 1975, as revised in 1983. All participants have provided written informed consent. Thirty eight participants with type II diabetes were examined with Michigan neuropathy screening instrument (MNSI) between 2012 and 2013 for detection of DPN; 18 of them show no clinical DPN from the current analysis While 20 patients were diagnosed with DPN. Patients with DPN were assessed by TCSS for detection of neuropathy severity. The RNFL thickness was examined by the OCT technology in all participants.

A data collecting sheet was filled by the internal medicine doctor to record demographic data and relevant medical history and then was screened by a neurologist for DPN using MNSI; MNSI consists of a two-step program: The first part: assessed a neuropathic symptom by a history questionnaire which includes 15 "yes or no" questions on foot sensation including pain, numbness and temperature sensitivity. The second part: is a brief physical examination involving an inspection of the feet and evaluation of ankle reflexes, vibration sensation and fine touch. Neuropathy is defined operationally as seven or more positive responses on the MNSI questionnaire or a score >2.0 on the MNSI examination, thresholds defined by prior validation studies. [21]. The screening methods for ankle reflex, fine touch sensation and vibration perception were done using 10-g SWM, 128-Hz tuning fork and reflex hummer respectively and followed the practical guideline from Michigan Diabetes Research and Training Center [22].

After clinical diagnosis of DPN the neurologist assessed the neuropathy severity by TCSS: Sensory testing was performed on the first toe. Symptoms scores were as follow: present = 1; absent = 0. Reflex scores: absent = 2; reduced = 1, normal = 0. Sensory test score: abnormal = 1; normal = 0. Total scores range from normal = 0 to maximum of 19. A score ≤5 considered no neuropathy, 6–8 mild, 9–11 moderate, and >11 correlated to severe neuropathy.

NCS were conducted in the electrophysiology unit at Riyadh National hospital using the counterpoint instrument (Medtronic) according to the standards of the American Association for Neuromuscular Disorders [23]. Recordings were performed with temperature control (32–34 C), careful distance measurements, and recording of artifact-free responses. Amplitude and latency values were calculated automatically from the operating machine. The nerve parameters recorded were: sural sensory nerve action potential amplitude and conduction velocity, peroneal compound muscle action potential amplitude and ulnar nerve action potential amplitude and conduction velocity. Testing was performed bilaterally with mean values used in statistical analyses. In individuals with only unilateral measurements (for example, in participants with a limb amputation) the single unilateral value was taken.

A comprehensive evaluation was performed to exclude other etiologies for neuropathy such as familial, nutritional, and uremic polyneuropathy. This included a general medical exam as well as biochemical testing included serum creatinine, glycated hemoglobin A1c, serum lipids, complete blood count, vitamin B12 and folate levels, urinary albumin excretion, and thyroid hormone levels. All patients underwent a complete ophthalmologic examination, including assessment of refractive error, slit-lamp biomicroscopy, fundus examination, and intraocular pressure measurement, then examined with The OCT system (Topcon 3D-OCT 2000; Topcon Corporation, Tokyo, Japan) which employs a broad-band light source, delivering an output power of less than 0.65 mW at the central wavelength of 840 nm with a bandwidth of 50 nm. During examination the patient was seated with the chin

rested and the machine properly aligned, the contralateral eye was covered and the OCT lens was adjusted for the patient's refractive error. Polarization was optimized to maximize the reflective signal. A single operator collects all measurements and adjusts the aiming circle to match the macula. All OCT' study cases were obtained using the Macular Thickness Map (MTM) protocol. Each raster scan was separately analysed by using the retinal thickness algorithm to generate total retinal thickness values in micrometres. This process is automatically generated by the OCT software.

After automated segmentation of the retinal layers, the ophthalmologist measure two retinal areas; peripapillary area a donut-shaped ring centered on the disc and the whole macular area (6mm x 6mm). The RNFL thickness (average, superior and inferior half's values) of each layer areas was calculated automatically to be analyzed.

The frequency and percentage were calculated for qualitative variables, the mean values ± standard deviation (SD), and range were used for quantitative variables. Student t-test was used to compare between two groups. Statistical computations were done using the software SPSS version 16 (Chicago, IL, USA). Statistical significance was predefined as $P \leq 0.05$.

RESULTS

Thirty-eight diabetic patients were screened using MNSI, of whom twenty (52.6%) had DPN, the rest of diabetic patients was used as control group, 55% of the patients were female and their ages ranged from 36 to 68 years, with mean age of 54.20±8.61 years, while the ages of control ranged from 38 to 64 years, with mean age of 52.28±7.46 years. Clinical characteristics of the patient and control group are summarized in (Table 1).

Age, BMI, HbA1c within the last 3 months, lipid profile, diastolic blood pressure, serum creatinine and liver enzymes (ALT & AST) were comparable among patients and controls; the results failed to demonstrate any statistically significant difference between the two groups, while longer duration of diabetes was statistically significant in patients compared to controls (Table 1).

Peripapillary and macular OCT imaging were performed on The eyes of the studied group Examples of RNFL assessment in one patient eye and in one control eye are shown in Figure 1A and 1B respectively. The macular and peripapillary RNFL thickness in patients was reduced significantly compared to age-matched controls ($P < 0.05$) (Table 1).

By comparing the means of NCS of sural, popliteal and ulnar nerves in patients and controls according to the polyneuropathy severity, it didn't show a statistically significant difference between patients with mild polyneuropathy and control group, however; in patients with moderate polyneuropathy there was reduction of NCV and action potential amplitude compared to controls This reduction was only statistically significant in sural nerve study and in lateral popliteal action potential amplitude. The NCV and action potential amplitude were reduced significantly in all examined nerves in patient with severe diabetic polyneuropathy compared to controls (Table 2).

Significant Macular RNFL thinning was noticed in patients compared to controls in different neuropathy severity group ($P< 0.05$); while the peripapillary RNFL thinning become significant only in moderate and severe DPN (Table 2).

As showed in table 3 the macular thinning was greater in lower half compared to the upper half, this reduction in macular RNFL was statistically significant when compared with the upper half and also when compared with the control group ($P< 0.05$).

The peripapillary RNFL thinning was greater in lower half compared to the upper half, this reduction was statistically significant when compared with the upper half.

This thinning failed to reach a statistically significant value when compared to control group in patients who developed mild DPN and only became significant in patient with severe DPN, also it became significant in the inferior half in patients with moderate DPN (Table 3).

Table 1. Clinical characteristics and laboratory results

	+ve Neuropathy	-ve Neuropathy	P
Clinical Characteristics			
Number	20	18	
Age (ys)	54.20±8.61	52.28±7.46	0.47
Male Sex (%)	9 (45%)	8 (44.4%)	0.15
Duration of diabetes (ys)	13.80±6.03	6.17±2.53	0.000
Physical Examination			
Height	167.70±8.93	167.83±9.15	0.96
Weight	97.28±11.56	94.86±13.06	0.55
Waist Circumference	107.00±7.46	105.17±7.55	0.46
BMI (kg/m^2)	34.62±3.39	33.86±5.15	0.13

SBP (mmHg)	127.25±15.17	128.89±15.39	0.74
DBP (mmHg)	78.75±11.34	81.67±8.40	0.38
Laboratory Investigations			
HbA1C (%)	10.24±2.24	9.91±2.093	0.64
Total cholesterol (mmol/L)	4.54±.84	4.39±.82	0.61
LDL cholesterol (mmol/L)	2.48±.68	2.49±.73	0.96
HDL cholesterol (mmol/L)	1.15±.24	1.19±0.20	0.59
Triglycerides (mmol/L)	2.00±1.08	1.94±1.09	0.89
S-creatinine (mmol/L)	92.10±15.83	85.24±25.38	0.42
S-Albumin (g/L)	38.20±6.79	39.72±8.49	0.19
Alanine transaminase (ALT)	47.65±16.11	40.83±18.64	0.23
Aspartate transaminase (AST)	33.75±13.82	30.45±15.94	0.50
Individual NCS Parameters			
Sural Amp (mV)	2.52±1.87	5.492.33±	0.000
Sural CV (m/s)	26.90±16.66	43.67±16.68	0.000
Popliteal Amp (mV)	5.35±1.42	6.37±0.90	0.013
Popliteal CV (m/s)	40.53±5.29	44.85±2.00	0.002
Ulnar Amp (mV)	7.40±3.08	10.15±2.98	0.008
Ulnar CV (m/s)	46.99±10.49	54.44±14.35	0.074
Retinal nerve fiber layer thickness			
Macular RNFL thickness, µm	29.80±4.53	41.94±2.04	0.000
Peripapillary RNFL thickness, µm	25.45±4.72	31.31±2.40	0.000

Data are means ± SD, median (range), or n (%) unless otherwise indicated. NS= non significant statistically.

Table 2. The means of the electrophysiological parameters and RNFL compared to the mean of the controls according to neuropathy severity

	Mild		Moderate		Severe	
	Mean (SD)	P	Mean (SD)	P	Mean (SD)	P
Individual NCS Parameters						
Sural Amp (mV)	3.55±2.17	0.110	2.8±2.18	0.021	1.76±1.28	P< 0.05
Sural CV (m/s)	33.45±18.76	0.250	26.22±20.38	0.047	23.70±13.61	P< 0.05
Popliteal Amp (mV	6.56±1.30	0.709	6.00±1.12	0.428	4.24±0.73	P< 0.05
Popliteal CV (m/s)	43.04±2.63	0.108	42.7183	0.030	37.68±6.65	P< 0.05
Ulnar Amp (mV)	9.96±3.70	0.909	8.70±1.84	0.279	5.11±1.51	P< 0.05
Ulnar CV (m/s)	53.55±15.04	0.904	50.28±6.94	0.506	41.15±6.68	P< 0.05
Retinal nerve fiber layer thickness						
Macular RNFL thickness, µm	36.00±1.58	0.000	30.67±1.63	0.000	25.78±1.72	P< 0.05
Peripapillary RNFL thickness, µm	29.70±2.28	0.195	28.83±2.66	0.044	20.83±1.39	P< 0.05

Data are mean ± SD.P value measured in comparison to control group.

Table 3. Retinal nerve fiber layer thickness generated by OCT compared to control group in different neuropathy severity

Neuropathy Severity	Mild		Moderate		Severe	
	Mean (SD)	P	Mean (SD)	P	Mean (SD)	P
Macular RNFL thickness, µm						
Superior	39.40±.95	P< 0.001	32.50±2.59	P< 0.001	29.44±2.07	P< 0.001
Inferior	32.60±2.41	P< 0.001	28.83±1.17	P< 0.001	22.56±2.19	P< 0.001
Average	36.00±1.58	P< 0.001	30.67±1.63	P< 0.001	25.78±1.72	P< 0.001

Peripapillary RNFL thickness, µm						
Superior	31.202.68±	0.249	30.00±2.53	0.04	22.11±1.54	P< 0.001
Inferior	28.20±2.05	0.195	27.67±2.80	0.085	19.56±1.51	P< 0.001
Average	29.70±2.28	0.195	28.83±2.66	0.044	20.83±1.39	P< 0.001

Data are mean ± SD.

Figure 1A. OCT in an eye of diabetic patient without diabetic neuropathy, showing normal RNFL thickness, the box below showed the measurement of the superior, inferior and average thickness measured in µm.

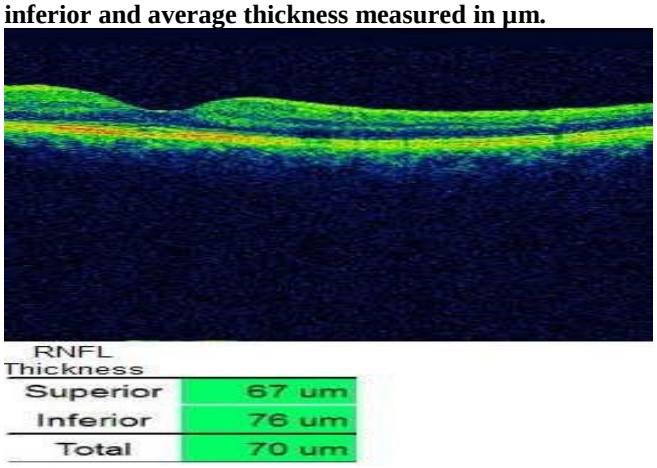
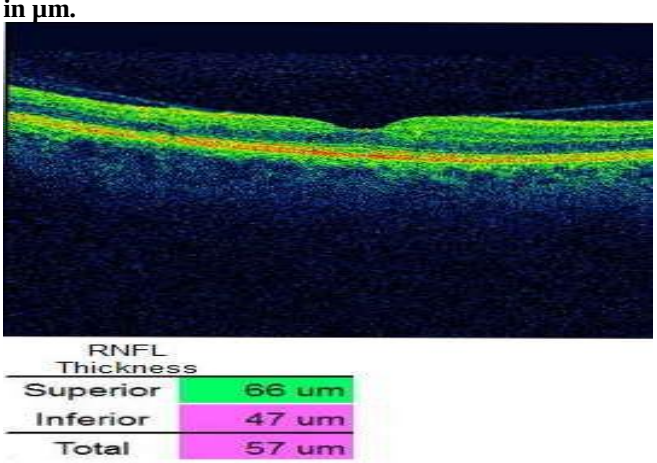


Figure 1: OCT in an eye of DPN patient, showing reduced RNFL, the box below showed the measurement of the superior, inferior and average thickness measured in µm.



DISCUSSION

In our study the risk of developing DPN increased with increasing the duration of diabetes confirming the previously reported strong contributions of duration of diabetes to the risk of neuropathy [24]. The RNFL thinning significantly increase with increasing duration of diabetes; these result was consisted with the study by Asefzadeh and colleagues (2008) who found that the macular and foveal thicknesses were significantly thinner with longer duration of diabetes [25].

The results of our study suggest that the RNFL is more susceptible to initial damage and can reflect the patient susceptibility to develop DPN; also the RNFL thickness correlated to the diabetic severity; this was in agreement with the study conducted by Shahidi and colleagues (2012), who found that RNFL thinning was associated with higher neuropathy disability scores [26], also with the study of Cabrera and Somfai (2010), who concluded that there was a significant reduction of RNFL thickness in the pericentral and peripheral regions among DPN patient [27]. The macular RNFL thinning was statistically significant among all DPN patients, while the peripapillary RNFL thinning was statistically significant only in moderate, and severe DPN, for that it was concluded that the macular RNFL thickness is more sensitive in early detection of neuropathy compared to peripapillary RNFL thickness, This was observed also in a study of Vujosevic and Midena, (2013), they revealed that decreased RNFL thickness in diabetics without DR or with initial DR suggest early alterations in the inner retina [28], also Oshitari and colleagues (2009) concluded that at the early stage of diabetic retinopathy (DR), the macular RNFL thicknesses was altered [29].

This study showed significant thinning of the inferior fiber of macular RNFL among all DPN patients; while in peripapillary RNFL this thinning only became statistically significant in moderate and sever DPN patients , This was in agreement with study of Shahidi and colleagues (2012) who has found that Inferior quadrant RNFL thinning was associated with DPN in Type 2 diabetic patients 26, However, in a study performed by Kanamori and colleagues (2004) it was concluded that superior macular RNFL is the most affected part in diabetic patients [30].

To date, few investigators have examined the relationships between different measures of NCS in DPN [31-35]. In the current study, the TCSS and NCS was further validated with the RNFL thickness. Our study showed that sural nerve amplitude and conduction velocity was statistically significant only in moderate diabetic neuropathy patient, while failed to reach significant differences in mild DPN patients, also ulnar and lateral popliteal nerves amplitude and conduction velocity measurements only became significant in severe DPN patients; this is in consistent with the study conducted by Zahed and colleagues (2008), who revealed that Sural nerve NCS has the highest abnormalities in early DNP patients [36]. While NCS failed to reach significant differences between mild DPN, macular RNFL thickness was significantly reduced in mild DPN, This was in agreement with the study of Shahidi and colleagues (2012)

who found that RNFL thinning is associated with DPN in patients with Type 2 diabetes 26, also Krajka-Lauer and colleagues (2007) who concluded that diabetic patients with painful DPN not only peripheral nerves but also optic nerve were damaged [37], This was in the contrary to study of Abdollahi and colleagues (2009) who found no correlation between DR and DPN, However, this can be explained by lack of assessment of DPN severity in their study [38]. Further research should be done on larger scale to specify an optimal set of diagnostic categories and the role of OCT in early detection of DPN.

CONCLUSION

This study revealed that the macular RNFL thickness measured can be used as a safe, noninvasive and rapid tool in investigating diabetic patients not only for early detection of DR but also for prediction of DPN.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

STATEMENT OF HUMAN AND ANIMAL RIGHTS

All procedures performed in human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

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