RESEARCH

Early detection of peripheral neuropathy in patients with diabetes mellitus type 2

Ahmed W. Fadel[1], Amin E. Nawar[2], Loai M. Elahwal[3], Azza A. Ghali[1] and Osama A. Ragab[1]*

Abstract

Background Early diagnosis of diabetic polyneuropathy (DPN) can significantly improve the prognosis and help prevent severe complications. The aim of this work was to study clinical, radiological, laboratory and neurophysiological findings for early detection of peripheral neuropathy in T2DM.

Methods A total of 60 diabetic patients were classified according to Toronto Clinical Neuropathy Score (TCNS) into: Group 1: 20 diabetic patients with no evident neuropathy. Group 2: 20 diabetic patients with mild neuropathy. Group 3: 20 diabetic patients with moderate and severe neuropathy. All patients underwent a neurological examination, nerve conduction studies and optical coherence tomography (OCT) to assess retinal nerve fiber layer (RNFL) thickness. Additionally, ELISA technique to measure serum interleukin-6 (IL-6).

Results The analysis of gender and age distributions among the groups revealed no significant differences. There were statistically significant differences regarding disease duration, HBA1c, body mass index Systolic and diastolic blood pressure. Group 3 had such significant impairment that resulted in an inability to record the measurements of sural nerves. The study's statistical analysis results for OCT variables, and post hoc comparisons revealed significant differences between all three groups. The results demonstrated significant variations in Serum IL6 levels among the groups, with Group 3 having the highest IL6 levels. In groups 1, 2, and 3 the area under the curve for IL-6 and RNFL showed a good differentiation ability between groups.

Conclusion We conclude that the total thickness RNFL and serum IL-6 levels are a potential biomarker in prediction the severity of DPN.

Keywords Diabetes mellitus type 2, Diabetic polyneuropathy, Retinal nerve fiber layer, Interleukin-6

Introduction

Diabetes mellitus (DM) is a prevalent metabolic disorder characterized by chronic high blood glucose levels. DM is associated with various complications, such as heart disease, stroke, peripheral neuropathy, renal disease, and blindness [1]. Type 2 diabetes mellitus (T2DM) is a chronic health condition that has become a major concern worldwide. In an Egyptian study, the age-adjusted prevalence of diabetes was found to be 16.8% [2].

Diabetic neuropathy is a broad term used to describe the presence of symptoms and signs indicating dysfunction of peripheral nerves in individuals with diabetes, once other potential causes have been excluded [3]. In Egypt, approximately 29.3% of diabetic patients develop peripheral neuropathy [4]. In T2DM, oxidative stress, vascular issues, and metabolic disturbances play significant roles in driving diabetic polyneuropathy [5].

Early diagnosis of diabetic polyneuropathy (DPN) can significantly improve the prognosis and help prevent severe complications. It is recommended that all patients

*Correspondence:
Osama A. Ragab
osama.ragab@med.tanta.edu.eg
[1] Neurology Department, Tanta University, Tanta, Egypt
[2] Ophthalmology Department, Tanta University, Tanta, Egypt
[3] Internal Medicine Department, Tanta University, Tanta, Egypt

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with diabetes undergo neuropathy screening at least once a year, starting from the diagnosis of T2DM [6].

T2DM is now known to have an inflammatory component that significantly contributes to the development of several complications, including DPN. Studies have shown increased IL-6 expression in sural nerve biopsies of diabetic neuropathic patients compared to controls, as well as in diabetic patients with foot ulcers [7].

The retina, like the peripheral nerves, contains a dense population of sensory neurons, making it susceptible to potential injury related to DPN. DM leads to loss of photoreceptors and ganglion cells (GCs), as well as thinning of the retinal nerve fiber layer (RNFL). Patients with DPN also exhibit significant thinning of the macular retinal nerve fiber layer (mRNFL) [8]. The study of retinal changes in diabetes is essential as it provides insights into potential early indicators of complications. The aim of this work was to study clinical, radiological, laboratory and neurophysiological findings for early detection of peripheral neuropathy in T2DM.

Methods

This study was conducted on patients with diagnosed withT2DM according to the American Diabetic Association criteria [9], who attended the Departments of Neuropsychiatry and Internal Medicine.

A total of 60 diabetic patients were classified according to Toronto Clinical Neuropathy Score (TCNS) [10] into: Group 1: 20 diabetic patients with no evident neuropathy "clinically or by nerve conduction study" (TCNS ranged from 0 to 3). Group 2: 20 diabetic patients with mild neuropathy (TCNS ranged from 6 to 8. Group 3: 20 diabetic patients with moderate and severe neuropathy (TCNS ranged from 9 to 18).

The authors excluded patients suffering from other medical conditions that may be associated with neuropathy. These conditions include nutritional deficiencies, malignancies, hereditary neuropathy, hepatic or renal disease, thyroid disorders, and toxic exposure.

All patients underwent a thorough detailed medical and neurological examination. The severity of neuropathy was measured and ranked using the TCNS.

Neurophysiological examination, all patients underwent two main assessments. Firstly, Nerve Conduction studies were performed using a Nicolet EDX®EMG/NCS, Middelton, USA machine. In the upper limbs, the median and ulnar nerves were examined, recording compound motor action potentials (CMAPs) amplitude in millivolt (mV). Motor conduction velocities (MCVs) meter per second (m/s) between wrist and elbow were measured, and sensory nerve action potentials (SNAPs) amplitude in microvolt (µV) were assessed at the wrist, calculating sensory conduction velocities (SCVs) expressed as meter

per second (m/s) using the antidromic method. In the lower limbs, CMAPs and MCV of the peroneal and tibial nerves were recorded, and sural nerve conduction studies were conducted using the antidromic method.

Optical coherence tomography (OCT) was utilized to precisely measure the thickness of the retinal nerve fiber layer (RNFL). Circular profile 3.4 mm centered on the optic disc that was manually adjusted to the optic disc margins was taken to assess the RNFL thickness. The thickness of the mean peripapillary RNFL area and of the four quadrants were evaluated using Topcon 3D camera™ Capelle aan den Ijssel, Netherlands.

Laboratory studies were carried out, including routine investigations such as complete blood count, liver and renal function tests, and HbA1c levels. Additionally, a specific laboratory investigation employed the ELISA (CatchPoint® SimpleStep ELISA® Kit, San Jose, CA, USA) technique to measure Serum Interleukin-6 (IL-6).

The collected data were subjected to organization, tabulation, and statistical analysis using SPSS version 24, a software developed by IBM in Illinois, Chicago, USA. Descriptive statistics were utilized to present the data in terms of numbers and percentages. To assess the differences between two mean values, an independent sample t-test (t) was performed, while differences among the three subject groups were examined using a one-way analysis of variance (ANOVA) test (F). Post hoc Tukey tests were conducted to compare each group with the others. Additionally, logistic regression analysis was conducted to evaluate the influence of one or more independent variables on a dependent variable. Furthermore, Receiver operating characteristic (ROC) curve analysis was performed to assess the effectiveness of serum IL6 and RNFL total thickness as a diagnostic classification method for determining neuropathy severity. Throughout the statistical analyses, a p-value of less than 0.05 was considered significant.

Results

The current study involved 60 patients with T2DM who were divided into three groups. In Group 1, there were 9 males (45.0%) and 11 females (55.0%). Group 2 consisted of 8 males (40.0%) and 12 females (60.0%). In Group 3, there were 12 males (60.0%) and 8 females (40.0%). The analysis of gender distributions among the groups revealed no significant differences, as the p-value obtained was 0.42.

The results showed no statistically significant difference regarding mean age in years among studied groups, and showed statistically significant differences regarding disease duration, HBA1c, body mass index (BMI), systolic and diastolic blood pressure, and total cholesterol levels

where higher values of each were found in group 3 followed by group 2 patients as illustrated in Table 1.

The results showed statistically significant difference in mean CMAP amplitude and CV of both median and ulnar motor nerves among studied groups. Post hoc comparison showed statistically significant difference between each group and the other, with highly statistically significant difference in median motor CMAP amplitude and both ulnar motor CMAP amplitude and CV. Regarding the lower limbs there were highly statistically significant differences in CMAP amplitude and CV of the peroneal and tibial nerves among studied groups. Post hoc comparison showed high statistically significant difference in mean peroneal motor CMAP amplitude and CV between each group and the other, while it showed highly statistically significant difference in mean tibial motor CMAP amplitude and CV, between group 1 and group 3. Detailed results are shown in Table 2.

The study results demonstrated highly statistically significant differences in median sensory SNAP amplitude and CV, as well as ulnar sensory SNAP amplitude and CV, among the studied groups. Post hoc comparisons revealed highly statistically significant differences between each group and the others in ulnar sensory

SNAP amplitude and CV. Additionally, there was a highly statistically significant difference in median sensory SNAP amplitude and CV between patients in group 1 and group 3. The full data are presented in Table 3.

For Sural PL, the mean and standard deviation were reported for Group 1 (4.61 ± 0.64 ms), and for Group 2 (4.96 ± 0.56 ms). The *t*-test was performed between Group 1 and Group 2, showing a *p*-value of 0.070, indicating no statistically significant difference between the two groups. For Sural SNAP amplitude, the mean and standard deviation were reported for Group 1 (6.29 ± 0.85 µV), and for Group 2 (3.66 ± 0.75 µV). The *t*-test between Group 1 and Group 2 revealed a highly statistically significant difference, and a *p*-value of 0.001. Similarly, for Sural CV, the mean and standard deviation were reported for Group 1 (33.29 ± 2.82 m/s), and for Group 2 (27.70 ± 3.37 m/s). The *t*-test between Group 1 and Group 2 also showed a highly statistically significant difference, with a *p*-value of 0.001. Please note that there is no data available for Group 3 in these parameters, as there were no recordable signals indicating severe nerve affection. This absence of data suggests that Group 3 had such significant nerve impairment that it resulted in an inability to record the measurements for Sural PL, Sural

Table 1 Age and clinical characteristics of the studied groups

Variable	Group	Mean ± SD	F	p		
Age	Group 1	55.00 ± 6.22	0.249	0.780	P1	0.613
	Group 2	54.10 ± 4.71			P2	0.866
	Group 3	55.30 ± 5.75			P3	0.500
Disease duration (years)	Group 1	3.30 ± 1.30	28.729	0.001*	P1	0.012*
	Group 2	6.60 ± 1.39			P2	0.001*
	Group 3	12.75 ± 6.66			P3	0.001*
HBA1c	Group 1	6.07 ± 0.13	158.127	0.001*	P1	0.001*
	Group 2	7.70 ± 0.49			P2	0.001*
	Group 3	9.11 ± 0.79			P3	0.001*
BMI	Group 1	22.37 ± 1.82	42.797	0.001*	P1	0.001*
	Group 2	24.97 ± 2.41			P2	0.001*
	Group 3	29.01 ± 2.57			P3	0.001*
Diastolic blood pressure	Group 1	77.00 ± 7.33	10.095	0.001*	P1	0.037*
	Group 2	82.00 ± 7.68			P2	0.001*
	Group 3	87.50 ± 7.16			P3	0.022*
Systolic blood pressure	Group 1	125.00 ± 11.12	5.278	0.008*	P1	0.021*
	Group 2	127.50 ± 15.85			P2	0.004*
	Group 3	140.25 ± 19.63			P3	0.014*
Total cholesterol (mg/dl)	Group 1	186.42 ± 12.83	16.250	0.001*	P1	0.021*
	Group 2	196.41 ± 6.39			P2	0.001*
	Group 3	210.27 ± 18.00			P3	0.001*

BMI body mass index, *P1* post hoc significance between group 1 and 2, *P2* post hoc significance between group 1 and 3, *P3* post hoc significance between group 2 and 3

* means statistically significant

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Table 2 Motor nerve conduction studies of the upper and lower limbs in the studied groups

Nerve	Group	Mean ± SD	F	p		
Median motor DL (ms)	Group 1	4.61 ± 1.82	0.228	0.797	P1	0.715
	Group 2	4.77 ± 1.19			P2	0.502
	Group 3	4.90 ± 1.03			P3	0.759
Median motor CMAP amplitude (mV)	Group 1	7.02 ± 1.47	84.248	0.001*	P1	0.001*
	Group 2	4.30 ± 1.31			P2	0.001*
	Group 3	2.21 ± 0.52			P3	0.001*
Median motor CV (m/s)	Group 1	46.85 ± 4.87	16.338	0.001*	P1	0.008*
	Group 2	41.56 ± 2.51			P2	0.001*
	Group 3	39.18 ± 5.16			P3	0.024*
Ulnar motor DL (ms)	Group 1	4.07 ± 0.55	0.647	0.527	P1	0.696
	Group 2	4.14 ± 0.46			P2	0.267
	Group 3	4.27 ± 0.64			P3	0.469
Ulnar motor CMAP amplitude (mV)	Group 1	8.74 ± 1.86	167.985	0.001*	P1	0.001*
	Group 2	4.24 ± 0.61			P2	0.001*
	Group 3	2.13 ± 0.49			P3	0.001*
Ulnar motor CV (m/s)	Group 1	47.90 ± 10.45	26.772	0.001*	P1	0.001*
	Group 2	39.69 ± 2.99			P2	0.001*
	Group 3	33.05 ± 2.42			P3	0.001*
Peroneal motor DL (ms)	Group 1	6.41 ± 1.15	2.858	0.166	P1	0.201
	Group 2	6.81 ± 0.69			P2	0.059
	Group 3	7.14 ± 1.03			P3	0.279
Peroneal motor CMAP amplitude (mV)	Group 1	2.33 ± 0.77	57.371	0.001*	P1	0.001*
	Group 2	1.41 ± 0.15			P2	0.001*
	Group 3	0.70 ± 0.28			P3	0.001*
Peroneal motor CV (m/s)	Group 1	24.66 ± 3.09	102.651	0.001*	P1	0.001*
	Group 2	31.11 ± 1.46			P2	0.001*
	Group 3	35.30 ± 2.27			P3	0.001*
Tibial motor DL (ms)	Group 1	6.54 ± 1.31	1.142	0.326	P1	0.334
	Group 2	6.84 ± 0.57			P2	0.142
	Group 3	7.00 ± 0.87			P3	0.610
Tibial motor CMAP amplitude (mV)	Group 1	2.46 ± 0.49	18.046	0.001*	P1	0.008*
	Group 2	1.88 ± 0.32			P2	0.001*
	Group 3	1.23 ± 0.36			P3	0.007*
Tibial motor CV (m/s)	Group 1	33.59 ± 3.28	16.655	0.001*	P1	0.009*
	Group 2	30.11 ± 2.91			P2	0.001*
	Group 3	27.72 ± 3.50			P3	0.023*

DL distal latency, *CMAP* compound motor action potential, *CV* conduction velocity, (ms) millisecond, (mv) millivolt, (m/s) meter per second, *P1* post hoc significance between group 1 and 2, *P2* post hoc significance between group 1 and 3, *P3* post hoc significance between group 2 and 3

* means statistically significant

SNAP amplitude, and Sural CV. Therefore, the statistical analysis and comparison for these parameters were only performed between Group 1 and Group 2.

The study's statistical analysis results for OCT variables (total thickness, superior RNFL, inferior RNFL, temporal RNFL, and nasal RNFL) in three groups are summarized in Table 4. The one-way analysis of variance (ANOVA) for total thickness showed a highly statistically significant difference among the groups, and

post hoc comparisons revealed significant differences between all three groups. Similarly, for inferior RNFL and temporal RNFL, post hoc comparisons also indicated highly statistically significant differences between all three groups. However, the ANOVA test for nasal RNFL and superior RNFL did not show any statistically significant differences among the groups, and the post hoc comparisons confirmed no significant differences between any of the groups.

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Table 3 Sensory nerve conduction studies of the upper and lower limbs in studied groups

Nerve	Group	Mean ± SD	F	p		
Median sensory PL (ms)	Group 1	4.06 ± 1.07	0.293	0.747	P1	0.679
	Group 2	4.19 ± 0.78			P2	0.448
	Group 3	4.29 ± 1.00			P3	0.729
Median sensory SNAP amplitude (µV)	Group 1	12.97 ± 2.41	24.558	0.001*	P1	0.006*
	Group 2	10.61 ± 0.87			P2	0.001*
	Group 3	9.13 ± 1.61			P3	0.010*
Median sensory CV (m/s)	Group 1	31.25 ± 3.52	12.502	0.001*	P1	0.043*
	Group 2	29.31 ± 3.02			P2	0.001*
	Group 3	26.45 ± 2.55			P3	0.007*
Ulnar sensory PL (ms)	Group 1	4.10 ± 0.50	1.281	0.286	P1	0.272
	Group 2	4.30 ± 0.55			P2	0.126
	Group 3	4.38 ± 0.64			P3	0.658
Ulnar sensory SNAP amplitude (µV)	Group 1	17.55 ± 2.13	315.160	0.001*	P1	0.001*
	Group 2	9.46 ± 1.34			P2	0.001*
	Group 3	5.41 ± 0.97			P3	0.001*
Ulnar sensory CV (m/s)	Group 1	46.26 ± 5.01	123.668	0.001*	P1	0.001*
	Group 2	38.78 ± 3.27			P2	0.001*
	Group 3	22.25 ± 6.12			P3	0.001*

PL peak latency, *SNAP* sensory nerve action potential, *CV* conduction velocity, (ms) millisecond, (µV) microvolt, (m/s) meter per second, *P1* post hoc significance between group 1 and 2, *P2* post hoc significance between group 1 and 3, *P3* post hoc significance between group 2 and 3

* means statistically significant

Table 4 Optical coherence topography (OCT) in studied groups

Variable	Group	Mean ± S. D	F	p		
Total thickness (µm)	Group 1	110.70 ± 3.04	271.978	0.001*	P1	0.001*
	Group 2	105.28 ± 5.68			P2	0.001*
	Group 3	79.33 ± 4.53			P3	0.001*
Superior RNFL (µm)	Group 1	114.32 ± 5.42	0.842	0.436	P1	0.645
	Group 2	113.35 ± 5.81			P2	0.416
	Group 3	116.04 ± 8.32			P3	0.205
Inferior RNFL (µm)	Group 1	109.54 ± 2.11	2675.600	0.001*	P1	0.001*
	Group 2	99.45 ± 1.90			P2	0.001*
	Group 3	67.87 ± 1.59			P3	0.001*
Temporal RNFL (µm)	Group 1	115.48 ± 3.43	2441.391	0.001*	P1	0.001*
	Group 2	98.15 ± 2.45			P2	0.001*
	Group 3	49.70 ± 3.29			P3	0.001*
Nasal RNFL (µm)	Group 1	103.24 ± 2.72	0.133	0.876	P1	0.838
	Group 2	103.07 ± 3.02			P2	0.611
	Group 3	102.82 ± 2.07			P3	0.760

RNFL retinal nerve fiber layer, (µm) micrometer, *P1* post hoc significance between group 1 and 2, *P2* post hoc significance between group 1 and 3, *P3* post hoc significance between group 2 and 3

* means statistically significant

The study analyzed serum IL6 levels in three groups. Post hoc comparisons confirmed highly statistically significant differences between all three groups. The results demonstrated significant variations in serum IL6 levels among the groups, with Group 3 having the highest IL6 levels among the studied groups as explained in Table 5.

Regarding RNFL total thickness measured using OCT, the study found highly statistically significant strong,

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Table 5 Serum interleukin-6 (IL6) in studied groups

	Group	Mean ± SD	F	p		
Serum IL6 (pg/mL)	Group 1	28.54 ± 5.61	64.917	0.001*	P1	0.001*
	Group 2	41.96 ± 6.82			P2	0.001*
	Group 3	51.93 ± 7.02			P3	0.001*

* means statistically significant

Table 6 Correlation between each of RNFL total thickness in OCT and serum IL6 and different clinical and electrophysiological parameters

Variable	RNFL total thickness in OCT		Serum IL6	
	R	p	r	p
Serum IL6	− 0.701	0.001*		
Disease duration	− 0.665	0.001*	0.542	0.001*
HBA1c	− 0.827	0.001*	0.759	0.001*
TCNS	− 0.831	0.001*	0.782	0.001*
Sural PL	− 0.106	0.234	0.136	0.315
Sural SNAP	0.711	0.001*	− 0.763	0.001*
Sural CV	0.572	0.001*	− 0.740	0.001*

RNFL retinal nerve fiber layer, *OCT* optical coherence topography, *TCNS* Toronto Clinical Neuropathy Scale, *PL* peak latency, *SNAP* sensory nerve action potential, *CV* conduction velocity

* means statistically significant

positive correlations between RNFL thickness and both sural SNAP and sural CV. Additionally, there were highly statistically significant strong negative correlations between RNFL thickness and each of serum IL6 levels, disease duration, HBA1c levels, and TCNS. As for serum IL6 levels, the study revealed highly statistically significant strong, positive correlations between IL6 levels and disease duration, HBA1c levels, and TCNS scores. On the other hand, there were highly statistically significant strong negative correlations between IL6 levels and both sural SNAP and sural CV. Numerical data are presented in Table 6.

The regression analysis for RNFL (retinal nerve fiber layer) total thickness in OCT with various predictors revealed significant associations. HBA1c levels and TCNS (Toronto Clinical Neuropathy Score) showed highly statistically significant negative relationships with RNFL total thickness, indicating that higher HBA1c levels and more severe neuropathy (higher TCNS scores) were associated with thinner RNFL. Conversely, sural SNAP amplitude and sural CV exhibited statistically significant positive relationships with RNFL total thickness, suggesting that higher sural SNAP amplitude and sural CV values were associated with thicker RNFL. However, there were no statistically significant relationships

observed between RNFL total thickness and disease duration or sural PL (peak latency).

The regression analysis for serum IL6 levels with various predictors showed significant associations. HBA1c levels and TCNS exhibited highly statistically significant positive relationships with serum IL6 levels, indicating that higher HBA1c levels and more severe neuropathy (higher TCNS scores) were associated with increased IL6 levels. On the other hand, there were no statistically significant relationships observed between serum IL6 levels and disease duration, sural PL, or sural SNAP amplitude. However, sural CV showed a statistically significant negative relationship with serum IL6 levels, suggesting that higher sural CV values were associated with lower IL6 levels.

In groups 1 and 2, the area under the curve (AUC) for IL-6 was 0.896, indicating a good discriminatory ability of this biomarker. The optimal cutoff value for IL-6 was 34 pg/ml. At this cutoff, the sensitivity was 88%, and the specificity was 84%. In groups 2 and 3, the AUC for IL-6 was 0.896, indicating a good discriminatory ability of this biomarker. The optimal cutoff value for IL-6 was 45 pg/ml. At this cutoff, the sensitivity was 88%, and the specificity was 80% (Fig. 1).

In groups 1 and 2, the AUC for total thickness measured using OCT was 0.812, indicating a good discriminatory ability of this parameter. The optimal cutoff value for total thickness was 108 μm. At this cutoff, the sensitivity was 84%, and the specificity was 80%. In groups 2 and 3, the AUC for total thickness measured using OCT was 0.918, indicating an excellent discriminatory ability of this parameter. The optimal cutoff value for Total thickness was 97 μm. At this cutoff, the sensitivity was 92%, meaning that the test correctly identified 92% of the individuals with the condition. The specificity was 84%, indicating that the test correctly identified 84% of the individuals without the condition (Fig. 2).

Discussion

Diabetic polyneuropathy (DPN) can be diagnosed when there are symptoms and/or signs of peripheral nerve damage in diabetics after exclusion of other neuropathy causes, while abnormalities in nerve conduction studies especially of lower limb nerves offer more proof. Many
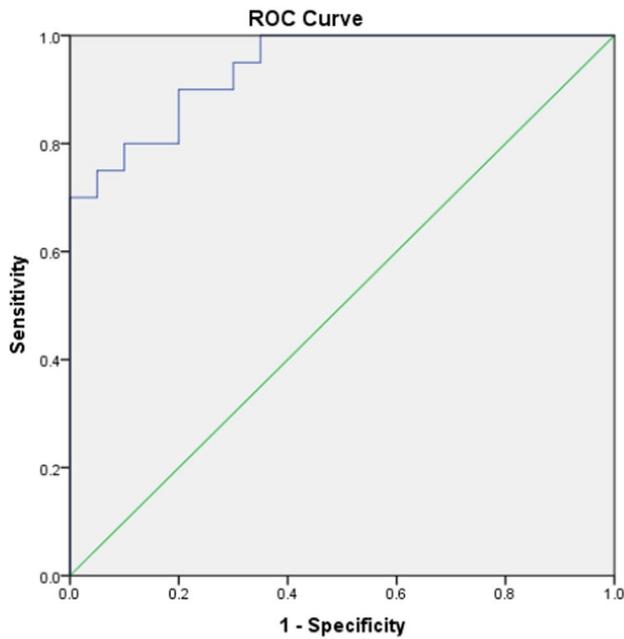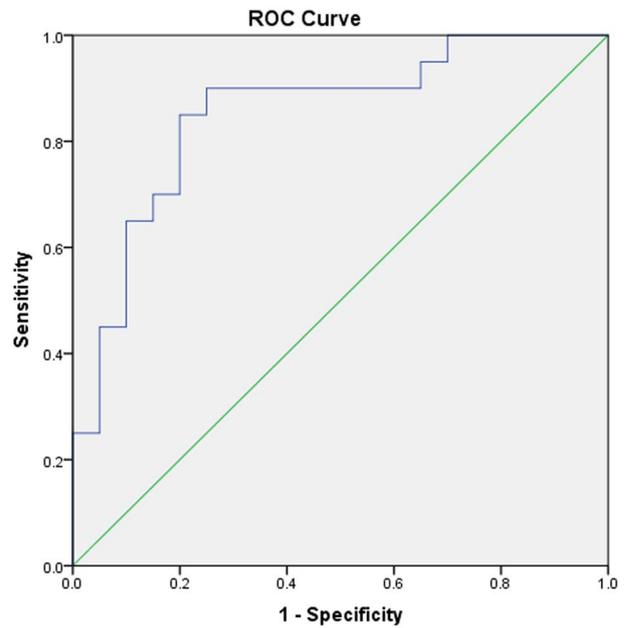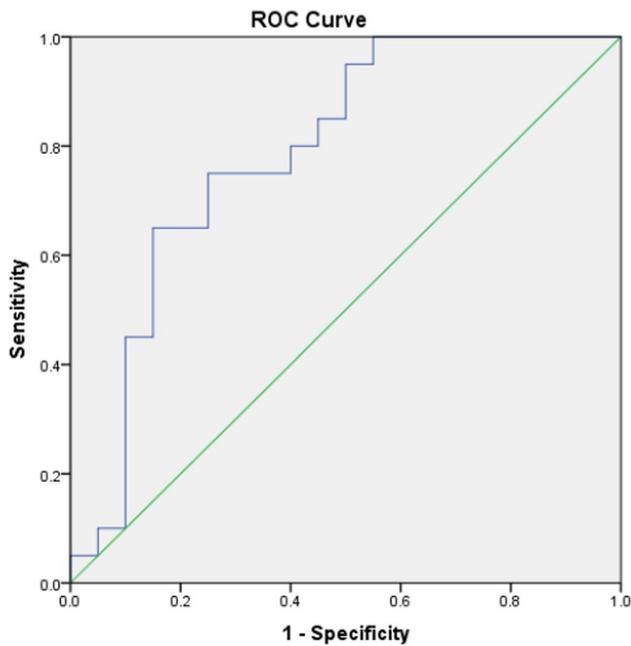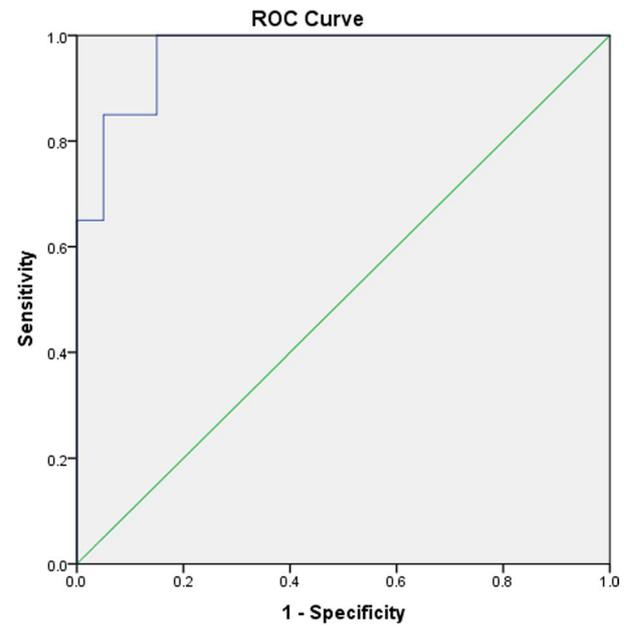
Fig. 1 **a** ROC curve for IL-6 in patient groups 1 and 2. **b** ROC curve for IL-6 in patient groups 2 and 3

Fig. 2 **a** ROC curve for RNFL total thickness in OCT in patient groups 1 and 2. **b** ROC curve for RNFL total thickness in OCT in patient groups 2 and 3

scales have been developed for rapid and integrated evaluation of symptoms and signs of DPN. One of these scales that defines, and grades neuropathy is the TCNS, which is a valid and reliable, widely used clinical test in research and practice [10]. In the present study, TCNS was used in grading the neuropathy as: no neuropathy (group 1), mild and moderate neuropathy (group 2) and severe neuropathy (group 3).

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The pathogenesis of symptoms in different patients with DPN can be very complex. Several risk factors are correlated with the development and severity of peripheral neuropathy in patients with type 2 diabetes mellitus (DM). In this study, some of these factors were evaluated: duration of diagnosis of diabetes mellitus, glycated hemoglobin (HbA1c), body mass index, systolic and diastolic blood pressure and total cholesterol level.

In the present study the duration of DM diagnosis varied significantly among the studied groups, where group 1 with no neuropathy had least duration in years since diagnosis of DM, while severe neuropathy had longest duration of DM. Duration was reported to be a well-established factor in development of neuropathy in diabetic patients, independent of patient's age [11]. The exact mechanisms by which diabetes duration contributes to the development and severity of DPN are not fully understood. However, it is believed that it is not the duration itself that contributes but may be chronic hyperglycemia and/or metabolic derangement, which could lead to nerves damage over time [12].

Glycated hemoglobin (HbA1c) measures an individual's average blood glucose levels over the past two to three months. It is considered a useful tool in monitoring the long-term glycemic control of individuals with diabetes. Maintaining optimal HbA1c levels is essential to prevent or delay the onset of diabetic complications such as neuropathy [13]. In the current study, HbA1c was significantly related to the development and severity of DPN. BMI, systolic and diastolic blood pressure levels, and total cholesterol levels were significantly related to development and severity of DPN. This agreed with other studies which stated that obesity and low physical activity have been related to DPN in T2DM [14]. Dyslipidemia including elevated total cholesterol level was reported to have a strong impact on DPN, and this could be due to its role in oxidative stress on dorsal root ganglion sensory neurons and endothelial dysfunction [15].

In the present study, there was a statistically significant difference among studied groups regarding the conduction velocity and amplitude of the studied motor and sensory nerves, where the groups with more severe neuropathy had shorter conduction velocities and lesser SNAP amplitude and CMAP amplitude. Sensory nerves were affected more than motor nerves, and sural nerve was most of the sensory nerves affected; its response was even absent in more severe neuropathy in group 3, and peroneal nerve was mostly affected in motor nerves, followed by the tibial nerve and then ulnar and median nerves. These findings are similar to previous published studies, even sural nerve abnormality was considered a necessity in diagnosis of DPN [16], although Kakrani et al. [17] concluded that sural and tibial nerves are the most affected in DPN. Typically, sensory nerves are affected earlier than motor ones, and this is suggested to be due to the smaller diameter of the motor nerves and the longer distance that sensory nerves travel in the body, and thus being more exposed to injury [18].

Recently, the increases in levels of several pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α) and IL-6 is thought to be a main contributor to the neuroinflammatory process that induces neuropathic pain, and could be related to the severity of DPN [19].

The study results showed that higher levels of serum IL-6 were found in patient groups with more severe DPN. These findings in general were in concordance with previous studies, one which was conducted in Germany, revealed significantly higher levels of IL-6 in patients with DPN versus patients without DPN [20]. These findings weren't the same as another one conducted in Emirati population, where patients with DNP had lower mean serum IL-6 values in relation to patients without DNP with no significant difference between the two patient groups [21]. Racial variations of serum IL-6 have been reported in previous studies with observed lower levels in Asian population [22].

Interestingly, serum IL-6 levels were significantly correlated directly with the disease duration and HbA1c levels and inversely with sural nerve amplitude and conduction velocity. Higher levels for IL-6 were associated with increased likelihood of sural nerve axonal injury. These findings in general were similar to a previous study that also reported a significant and an inverse relation between SNAP amplitude and CMAP amplitude of the peripheral nerves [23]. These findings could preliminarily support the role of IL-6 in peripheral axonal nerve injury in diabetic patients.

IL-6 is a pleiotropic cytokine generated after tissue injury or infection. that stimulates innate and adaptive immune responses. When pattern recognition receptors activate leukocytes and stromal cells, they release IL-6, which then attracts B and T cells, and this could have an impact on neurons and glial cells. IL-6 can be also released under advantageous situations, such as exercise, where it promotes anti-inflammatory and metabolic effects in physical adaptation to strenuous exercise [24]. This could somewhat explain the contradictory results regarding the effect of serum IL-6 in patients with DPN, where higher levels could be protective in some patients with good physical activity profiles.

Serum IL-6 role is still not fully understood in DPN. Magrinelli et, al. had explored role in of serum IL-6 in both large and small nerve fiber affection in DPN, and they found that increased serum levels of IL-6 were associated with sensory and motor axonal damage in large nerve fibers, but they didn't find a qualified

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relation between these levels, pain characteristics, clinical, and electrodiagnostic assessment of small nerve fibers function· [23] Interestingly in a recent study, serum IL-6 levels were significantly related to presence of neuropathic pain in patients with DPN, so it could be a biomarker for the neuropathic pain in these patients, and a potential target for future therapeutic studies [25].

Research on diabetes-related ocular problems has been extensive. Some investigations identified structural and functional neural alterations preceding vascular changes, as some authors observed decreased retinal nerve fiber layer (RNFL) thickness before clinically apparent vasculopathy in diabetic patients compared to healthy controls. Interestingly, the degree of affection of RNFL measured by optical coherence tomography (OCT) was related to the presence and/or the degree of neuropathy in diabetic patients [26].

Our results showed that each of the total, inferior and temporal thickness of RNFL were significantly lower with the progression of neuropathy. Also, total thickness of RNFL was significantly correlated directly with sural nerve SNAP and conduction velocity. This could reveal that retinal thickness may be correlated to development and progression of peripheral neuropathy.

It has been reported that DPN and diabetic retinopathy (DR) are both related to the glycemic state of diabetic patients and disease duration especially in poor metabolic control [27]. However, Srinivasan et al., [28] didn't find any significant relationship between RNFL thickness and HbA1c levels, and this could clarify the fact that the actual relation between HbA1c and the occurrence and progression of complication in diabetic patients is a nonlinear one. In fact, rapid reduction of HbA1c could lead to worsening in the state of either DPN and DR [29].

Previous studies had also reported the significant relation between the degenerative changes that occur in the peripheral nerves in patients with DPN and the structural changes that occur in the RNFL. Kim et al. have found that RNFL was significantly thinner in patients with DPN in relation to ones without. They also have compared total thickness of RNFL in relation to total retina layer and have found also a significant decrease in DPN [30]. Also, Srinivasen et al. have found that the inferior layers thickness could be used to differentiate more severe degrees of DPN [31].

These findings raise the question to the possible sequential mechanisms that could affect the retina and the peripheral nerves in diabetes; including vascular and neural cell apoptosis, that may be induced by the oxidative stress and glutamate excitotoxicity. In fact, it has been found that particularly the inferior peripapillary RNFL was more prone to this vascular insult and thus pathological thinning than other layers, as it requires more oxygen and blood supply, being the thickest of all quadrants [32].

The non-invasive attribute of OCT has encouraged authors to implement its use further in patients with DPN. Jia et al. revealed that both the inferior and temporal peripapillary RNFL thickness were more prone to injury in diabetic patients, and could be used as an early biomarker for DR [33]. Also, Srinivasan et al. have found that decreased total RNFL along with high BMI could predict a 4-year incidence of DPN and thus OCT may serve as a potential biomarker for early detection of DPN [31].

Finally, the ROC analysis was performed to assess the effectiveness of serum IL6 and RNFL total thickness as a diagnostic classification method for determining neuropathy severity. In both groups 1 and 2, the biomarker IL-6 exhibited a high discriminatory ability with an AUC of 0.896. In group 2 and 3, the AUC remained at 0.896, indicating the biomarker's consistent performance. Regarding total thickness of RNFL, in both groups 1 and 2, the parameter of Total thickness, measured using OCT, displayed a good discriminatory ability with an AUC of 0.812. However, in groups 2 and 3, the discriminatory ability of total thickness significantly improved, with an AUC of 0.918, classified as excellent.

Conclusion

This study concludes that the total thickness of the RNFL measured by OCT, along with the inferior and temporal peripapillary thickness, can serve as predictive markers for the occurrence and severity of neuropathy in T2DM. Additionally, serum IL-6 levels were found to be indicative of neuropathy presence and severity in these patients, suggesting its potential as a sensitive and specific marker for DPN in T2DM.

Abbreviations

AUC	Area under the curve
CMAPs	Compound motor action potentials
CV	Conduction velocity
DPN	Diabetic polyneuropathy
GCs	Ganglion cells
HbA1c	Glycated hemoglobin
IL-6	Interleukin-6
MCVs	Motor conduction velocities
mRNFL	Macular retinal nerve fiber layer
OCT	Optical coherence tomography
PL	Peak latency
RNFL	Retinal nerve fiber
SCVs	Sensory conduction velocities
SNAPs	Sensory nerve action potentials
T2DM	Type 2 diabetes mellitus
TCNS	Toronto Clinical Neuropathy Score

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Availability of data and materials
All raw data will be available on the editor request.

Declarations

Ethics approval and consent for participate
The study protocol was approved by the ethical committee in Tanta University, Egypt, under the code number (34413/1/21). Participation was voluntary and all contributors received detailed information about the aims of this research work and an informed written consent was obtained prior to the commencement of the study.

Consent for publication
Not applicable.

Competing interests
The authors have no conflict of interest to disclose.

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