Tumor necrosis factor-α is a novel biomarker for peripheral neuropathy in type II diabetes mellitus: a clinical and electrophysiological study

Mohja A. El-Badawy[a], Dina A.B. Farrag[a], Samia M.R. Abd El-Rehem[a], Amira R. El-Mahdi[b], Alyaa A. El-Sherbeny[c], Emad A.M. Abdel Hady[d], Hoda A. Abdel-Sattar[e], Doaa M. Abdelaziz[e]

Departments of [a]Physical Medicine, Rheumatology and Rehabilitation, [b]Internal Medicine, Allergy and Immunology, [c]Internal Medicine, Endocrinology, [d]Internal Medicine, Hematology, [e]Clinical Pathology, Faculty of Medicine, Ain Shams University Hospital, Ain Shams University, Cairo, Egypt

Correspondence to Mohja A. El-Badawy, MD, Assistant Professor of Physical Medicine, Rheumatology & Rehabilitation, Faculty of Medicine, Ain Shams University, 211 Abdel-Hamid Keshk Street, Hadaeq EL-Quba, 11646 Cairo, Egypt; Tel: +20 100 504 8716/ 20 111 143 4418; e-mail: mohjaaelbadawy@gmail.com

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Background
Tumor necrosis factor-α (TNF-α) is an adipocytokine locally produced by Schwann cells and has a role in nerve regeneration and regulation of apoptosis. The role of TNF-α in the development of diabetic peripheral neuropathy (DPN) is controversial.
Objective
The objective of this study was to evaluate TNF-α serum level in a group of type II diabetes mellitus (DM) patients with and without neuropathy in comparison with healthy age-matched control group.
Design
This is a cross-sectional case–control study.
Settings
The study was conducted in outpatient clinics of the diabetes and physical medicine, rheumatology, and rehabilitation departments.
Patients
Ninety patients diagnosed with type II diabetes were included in the study.
Main outcome measures
All patients were assessed for clinical neuropathy using neuropathy symptom score and neuropathy disability score. All patients underwent nerve conduction studies of both upper and lower limbs. They were divided into two groups: group I with confirmed DPN (n=60) and group II with DM but no peripheral neuropathy (n=30). Serum TNF-α level was measured in all previous DM patients (90 patients) in addition to 48 healthy age-matched controls.
Results
A statistically significant difference was detected between serum TNF-α level in controls and diabetic patients. Similarly, a significant difference was detected between its level in non-DPN patients and confirmed DPN patients, being higher in the latter. A positive significant correlation has been detected between TNF-α level and patients' age, as well as blood cholesterol level. A positive significant correlation has been found between TNF-α level and both neuropathy symptom score and neuropathy disability score. A significant negative correlation had been detected between TNF-α level and motor amplitudes of both tibial nerves.
Conclusion
Serum TNF-α level might be a potential biomarker for peripheral neuropathy in type II DM.

Keywords:
peripheral neuropathy, tumor necrosis factor-α, type II diabetes mellitus

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Introduction

Diabetic peripheral neuropathy (DPN) is a common complication of diabetes mellitus (DM) that can have a serious impact on the quality of life [1].

A number of neuropoietic cytokines that are produced locally by residual and infiltrating macrophages, lymphocytes, mast cells, Schwann cells, and sensory neurons exhibit pleiotrophic effects on glia cells and neurons, which is vital for the homeostasis of the peripheral, central, and autonomic nervous system. These neuropoietic cytokines include interleukin-1, interleukin-6, transforming growth factor β-1, leukemia inhibitory factors, ciliary neurotrophic factors, and tumor necrosis factor-α (TNF-α) [2].

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TNF-α is locally produced by Schwann cells and has a role in peripheral nerve regeneration and regulation of apoptosis [2]. The metabolic changes induced by hyperglycemia lead to disturbance of cytokine control [3].

Under chronic hyperglycemia, endogenous TNF-α production is accelerated in microvascular and neural tissues, leading to increased microvascular permeability, hypercoagulability, and nerve damage, thus promoting the development of characteristic lesions of diabetic microangiopathy and polyneuropathy [4].

The effect of TNF-α on neurons seems to be mediated, directly and/or indirectly, by the phosphorylation of extracellular-regulated kinase and P38 mitogen-activated protein kinase [5], translocation of nuclear factor κβ to the nucleus, and activation of Cox-2-dependent prostanoid release [6].

TNF-α also activates nuclear factor κβ for the initiation of nitric oxide synthase, and nitric oxide production. Inhibition of serum TNF-α and nitric oxide levels with insulin attenuates DN pain, as TNF-α plays an important role in the development of DPN [7].

TNF-α increases the permeability of the endothelium through the release of nitric oxide [8] and increases thrombogenesis through plasminogen activator inhibitor-1 overexpression [9].

The present study is designed to explore the serum level of TNF-α in type II diabetic patients as a risk factor for the development of DPN.

Patients and methods

The present study is a cross-sectional conducted in the Ain Shams University Hospital. It includes ninety patients with type II DM according to the American Diabetes Association criteria [10] whose ages ranged from 35 to 60 years. Patients were recruited at random from the diabetes and physical medicine, rheumatology, and rehabilitation outpatient clinics. The study was approved by the local ethics committee and an informed consent was obtained from all participants. The 90 DM patients are subdivided into 60 patients with peripheral neuropathy (PN) (group I) and 30 patients with no PN (group II).

PN due to causes other than diabetes was also excluded, such as alcohol abuse, liver or renal disease, metabolic or nutritional disorders, rheumatologic or endocrine diseases, inflammatory diseases, or monoclonal gammopathies. Patients with trauma, limb swelling, or skin lesion that could interfere with nerve conduction were excluded.

Patient data and anthropometric measurement

Age, diabetes duration, weight, height, and blood pressure were recorded. BMI was calculated using the Quetelet formula (weight in kilograms divided by the square of height in meters).

Patients underwent full history taking and clinical examination. Patient symptoms due to PN was assessed by the neuropathy symptom score (NSS) [11]. Patients were asked about their experience of pain or discomfort in the legs: if the patient described burning, numbness, or tingling, a score of 2 was assigned; fatigue, cramping, or aching scored 1. The presence of symptoms in the feet was assigned a score of 2, the calves 1, and elsewhere a score of 0. Nocturnal exacerbation of symptoms scored 2 versus 1 for both day and night and 0 for daytime alone. A score of 1 was added if the symptoms had ever awakened the patient from sleep. The patients were asked if any maneuver could reduce the symptoms: walking was assigned a score of 2, standing as 1, and sitting or lying down was 0. The maximum symptom score was 9 [12].

Neuropathy disability score (NDS) was used for grading DM-PN severity, which is a composite score (0–10). NDS assesses four items: the presence or absence of pain sensation (pin prick), vibration sensation with a tuning fork, the temperature sensation on the dorsum of the foot with cold and warm rods, and Achilles tendon reflexes (present, re-enforcements, absent). On the basis of the outcome, Young *et al.* [12], have proposed a neuropathy severity classification system. According to this system, the classification is as follows: no PN (0–2), mild PN (3–5), moderate PN (6–8), and severe PN (9–10).

Laboratory investigations

Six milliliters of venous blood was collected under complete aseptic precautions from each subject. The collected blood was divided among an EDTA tube for glycated hemoglobin and a plain test tube for serum separation. After clotting, samples were centrifuged at 1000*g* for 15 min and sera were separated. Hemolyzed samples were discarded, and repeated freezing and thawing was avoided.

Serum glucose, total serum cholesterol, and triglycerides were determined by enzymatic colorimetric assay (Synhron CX-9; Beckman Instruments Inc., Fullerton, California, USA). High-density lipoprotein cholesterol

was determined enzymatically in the supernatant after dextran–magnesium-induced precipitation of other lipoproteins. Low-density lipoprotein cholesterol was calculated using the Friedewald formula.

HbA1c levels were determined by an ion-exchange HPLC method (Bio-Rad D10 HbA1c; BioRad Laboratories, Hercules, California, USA).

Quantification of serum TNF-α in serum samples was performed using sandwich ELISA (Assay pro LLC, St Charles, Missouri, USA) according to the manufacturer's protocol. The detection limit was 0.01 ng/ml (Assay pro LLC). Serum TNF-α was measured in all patients and 48 age-matched and sex-matched healthy controls.

Electrophysiological studies

Electrophysiological evaluation was performed in all DM patients to confirm PN using nerve conduction studies (by using Toennies Neuroscreen Plus; Toennies, Hoechberg, Germany). In motor studies, we used parameters of the sweep of 5 ms/division and a gain of 4 mV. In sensory studies, the sweep was adjusted at 2 ms and gain at 20 μV. The tests were performed at room temperature.

In the lower limbs, peroneal nerve and tibial nerve motor conduction studies and sural sensory nerve conduction study were performed bilaterally. In the upper limbs, both median and ulnar nerves motor nerve conduction and median sensory nerve conduction studies were performed.

Diagnosis of diabetic peripheral neuropathy

DPN was categorized into two levels, according to clinical findings and nerve conduction results based on the modified Toronto Expert Consensus [13]: non-DPN, in which DM patients had neither clinically evident PN nor abnormal nerve conduction tests, and confirmed DPN, in which DM patients had at least one abnormal nerve parameter of nerve conduction velocity (NCV), amplitude, and latency in two nerves among the median, peroneal, and sural nerves [14] with or without clinical signs and symptoms. An abnormality is more than or equal to 99th or less than or equal to first percentile according to Kimura [15]. The category of only clinically evident DPN could not be found in our patients. Clinically evident DPN was defined for patients who had at least two positive results among sensory symptoms, signs, or reflex abnormalities in accordance with a distal symmetrical polyneuropathy, and with the normal nerve conduction studies.

Statistical analysis

Continuous variables are expressed as mean and SD. Categorical variables are expressed as frequencies and percents. Student's *t*-test and Mann–Whitney test were used to assess the statistical significance of the difference between quantitative variables. Spearman's correlation was used to assess the correlation between TNF and other parameters. A significance level of *P* value less than 0.05 was used in all tests. All statistical procedures were carried out using SPSS, version 20 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Our DM patients were divided into two groups based on the presence or absence of PN. Group I consists of 60 DM patients with PN and group II consists of 30 DM patients with no PN in addition to 48 healthy age-matched and sex-matched controls. The demographic and clinical characteristics of both groups of diabetic patients are shown in Table 1. No statistically significant differences were found between both groups at baseline for anthropometric parameters and medications. However, statistically significant differences were found between groups in disease duration and both scores of neuropathy disability symptoms and of clinical PN. Diabetic patients in both groups showed nonsignificant differences in fasting plasma glucose, postprandial glucose, HbA1c, and serum lipids (Table 2).

Motor nerve conduction velocities and amplitudes and distal latencies in group I diabetic patients with PN were compared with group II diabetic patients without PN in Table 3.

Motor nerve amplitudes of median, ulnar, peroneal, and tibial nerves were found to be significantly decreased ($P<0.05$) in group I, whereas motor nerve conduction velocities of the studied nerves, sensory conduction velocities, and sensory amplitudes of median and sural nerve differences were not statistically significant. Both tibial motor distal latencies were the only ones to show a statistically significant difference between both groups.

We found a mixed axonal and demyelinating neuropathy in 65% and a demyelinating neuropathy in 35% of DPN patients (group I).

We found a highly significant increase in serum TNF-α in our diabetic patients when compared with the control group ($P=0.000$) (Table 4).

Group I patients with PN showed a significant increase in serum TNF-α when compared with group II patients without PN (P=0.038) (Table 5).

Serum TNF-α showed a significant positive correlation with age (P=0.016), serum cholesterol level (P=0.04), score of neuropathy disability symptoms (P=0.018), and score of clinical PN (P=0.039), whereas no significant correlations were found with BMI, duration of diabetes, and all studied biochemical tests in DPN patients (Table 6).

Serum TNF-α showed a nonsignificant negative correlation with conduction velocities of all studied motor and sensory nerves in DPN patients (Table 7).

Serum TNF-α showed a nonsignificant negative correlation with amplitudes of all studied motor and

Table 1 Baseline characteristics of two groups of patients (group I: confirmed diabetic peripheral neuropathy, group II: no diabetic peripheral neuropathy)

	Groups	*N*	Mean±SD	*P*-value	Significance
Age	Group I	60	48.05±11.62	0.25	NS
	Group II	30	42.40±13.55		
Sex (female) [*n* (%)]	Group I	60	39 (65)	0.416	NS
	Group II	30	24 (80)		
DM duration (years)	Group I	60	3.46±1.92	0.012	S
	Group II	30	1.73±0.98		
Weight	Group I	60	79.85±16.53	0.273	NS
	Group II	30	87.40±19.26		
Height	Group I	60	165.65±10.97	0.763	NS
	Group II	30	164.50±6.43		
BMI	Group I	60	28.93±6.47	0.170	NS
	Group II	30	32.50±6.75		
SBP	Group I	60	131±18.89	0.235	NS
	Group II	30	122.80±13.93		
DBP	Group I	60	83.75±13.27	0.409	NS
	Group II	30	80.00±6.67		
Score of neuropathy disability symptoms	Group I	60	6.10±1.65	0.000	HS
	Group II	30	1.10±0.88		
Neuropathy symptom score	Group I	60	6.00±1.75	0.000	HS
	Group II	30	1.20±1.14		
Medication					
Insulin [*n* (%)]	Group I	60	27 (45)	0.803	NS
	Group II	30	12(40)		
Oral hypoglycemic [*n* (%)]	Group I	60	33 (55)	0.803	NS
	Group II	30	18 (60)		

DBP, diastolic blood pressure; DPN, diabetic peripheral neuropathy; HS, highly significant; S, significant; SBP, systolic blood pressure.

Table 2 Biochemical characteristics of type 2 diabetic patients with (group I) and without peripheral neuropathy (group II)

	Neuropathy	*N*	Mean±SD	*P*-value	Significance
Fasting plasma glucose (mg/dl)	Group I	60	176.90±67.41	0.592	NS
	Group II	30	163.30±58.90		
Postprandial glucose (mg/dl)	Group I	60	272.90±96.80	0.687	NS
	Group II	30	258.40±81.19		
HbA1c (%)	Group I	60	8.72±1.65	0.803	NS
	Group II	30	8.86±1.05		
Serum cholesterol (mg/dl)	Group I	60	212.40±45.89	0.226	NS
	Group II	30	235.00±49.64		
Serum triglycerides (mg/dl)	Group I	60	140.50±38.58	0.335	NS
	Group II	30	154.50±33.00		
LDL cholesterol (mg/dl)	Group I	60	117.20±28.95	0.991	NS
	Group II	30	117.32±21.81		
HDL cholesterol (mg/dl)	Group I	60	45.50±11.49	0.374	NS
	Group II	30	49.90±14.58		

HDL, high density lipoprotein; LDL, low density lipoprotein.

sensory nerves except for the motor amplitude of both tibial nerves where a significant negative correlation was found in DPN patients (Table 8).

Discussion

DPN is one of the most common chronic complications of DM and is the leading cause of disability among patients with type 2 DM.

Mechanisms underlying the development of DPN have not been fully elucidated. Previous studies have shown the multifactorial nature of DPN that it can be attributed to genetic factors, hyperglycemia, abnormal fat and protein metabolism, and vascular abnormalities [16–18].

Multiple immune factors were evidenced in the occurrence of DPN [19,20]. TNF-α is an important immune cytokine involved in the development of many inflammatory, infectious, and autoimmune diseases, and functions to inhibit tumor cells, increase phagocytic activity of neutrophils, and stimulate the production of other cytokines [21].

The immune response, in which self-antigen components get exposed, is the main factor leading to demyelination in case of DPN. T cells activated by these antigens produce different cytokines, including TNF-α, which induces a positive feedback loop cycle to further increase its own immune response that mediates the inflammatory reaction [21].

Table 3 Comparison of electrophysiological studies between two groups of patients (groups I and II)

	Groups	*N*	Right			Left		
			Mean±SD	*P*	Significance	Mean±SD	*P*	Significance
Median motor CV	Group I	60	51.26±7.35	0.299	NS	52.04±9.33	0.981	NS
	Group II	30	54.08±5.82			51.95±10.41		
Median motor DL	Group I	60	4.24±2.01	0.159	NS	3.98±1.73	0.201	NS
	Group II	30	3.30±0.43			3.23±0.64		
Median motor amp.	Group I	60	9.05±3.76	0.003	S	9.82±3.84	0.007	S
	Group II	30	14.39±5.12			14.20±4.05		
Ulnar motor CV	Group I	60	58.52±11.99	0.997	NS	52.69±12.77	0.178	NS
	Group II	30	58.53±8.69			58.96±9.14		
Ulnar motor DL	Group I	60	2.62±0.52	0.116	NS	2.56±0.63	0.286	NS
	Group II	30	2.33±0.27			2.32±0.36		
Ulnar motor amp.	Group I	60	11.87±3.64	0.053	S	11.92±3.39	0.024	S
	Group II	30	14.36±1.89			14.65±1.70		
Median sensory CV	Group I	60	30.41±12.37	0.326	NS	26.15±13.14 ±13.14	0.146	NS
	Group II	30	35.24±11.59			34.09±13.51		
Median sensory DL	Group I	60	4.31±2.12	0.113	NS	4.17±2.19	0.256	NS
	Group II	30	3.06±1.42			3.27±1.46		
Median sensory amp.	Group I	60	20.19±6.51	0.517	NS	22.11±16.37	0.651	NS
	Group II	30	23.21 ±15.86			19.10±7.91		
Peroneal motor CV	Group I	60	43.21±8.78	0.336	NS	40.84±8.83	0.137	NS
	Group II	30	45.41±7.65			45.44±4.61		
Peroneal motor DL	Group I	60	4.42±1.84	0.565	NS	7.27±10.09	0.273	NS
	Group II	30	4.02±1.55			3.66±0.66		
Peroneal motor amp.	Group I	60	2.90±1.80	0.007	S	2.82±2.25	0.001	S
	Group II	30	5.54±3.17			6.74±3.21		
Tibial motor CV	Group I	60	40.90±11.73	0.272	NS	38.68±9.84	0.288	NS
	Group II	30	45.32±5.66			42.51±7.38		
Tibial motor DL	Group I	60	5.21±1.53	0.005	S	5.22±1.51	0.002	S
	Group II	30	3.49±1.32			3.44±0.93		
Tibial motor amp.	Group I	60	7.35±6.61	0.002	S	7.47±5.74	0.003	S
	Group II	30	16.13±6.70			14.95±6.44		
Sural sensory CV	Group I	60	35.60±15.01	0.251	NS	34.85±12.75	0.201	NS
	Group II	30	41.44±5.43			41.62±11.87		
Sural sensory DL	Group I	60	4.94±2.80	0.080	NS	4.89±2.43	0.567	NS
	Group II	30	3.30±0.46			6.22±8.41		
Sural sensory amp.	Group I	60	26.09±14.50	0.116	NS	23.48±17.35	0.307	NS
	Group II	30	15.96±12.20			16.20±7.34		

Amp., amplitude; CV, conduction velocity; DL, distal latency; Lt, left; Rt, right; S, significant.

Table 4 Comparison between diabetes mellitus cases and controls as regards tumor necrosis factor level

	N	Mean±SD	*P*-value	Significance
DM cases	90	0.12±0.07	0.000	HS
Controls	48	0.03±0.02		

DM, diabetes mellitus; HS, highly significant.

Table 5 Comparison of tumor necrosis factor level between both groups of diabetes mellitus patients

	N	Mean±SD	*P*-value	Significance
Group I	60	0.14±0.08	0.038	S
Group II	30	0.09±0.04		

S, significant.

Table 6 Correlations between personal, biochemical parameters, and clinical scores of peripheral neuropathy and serum tumor necrosis factor level among diabetes mellitus cases with peripheral neuropathy (group I)

Personal and biochemical parameters	Correlation coefficient	*P*-value	Significance
Age	0.533	0.016	S
BMI	–0.280	0.232	NS
DM duration	0.234	0.320	NS
FBS	0.232	0.324	NS
2HPP	0.037	0.878	NS
HbA1c (%)	–0.233	0.322	NS
Cholesterol	–0.462	0.040	S
HDL	–0.190	0.423	NS
Triglyceride	–0.266	0.257	NS
LDL	–0.341	0.142	NS
Score of neuropathy disability symptoms	0.523	0.018	S
Score of clinical PN	0.464	0.039	S

DM, diabetes mellitus; FBS, fasting blood sugar; HDL, high density lipoprotein; LDL, low density lipoprotein; PN, peripheral neuropathy; S, significant; 2HPP, 2 hours post prandial.

In the present study, we measured the serum level of TNF-α in type II DM patients with PN and compared such a level with its level in patients with type II DM without neuropathy.

Our study results support the hypothesis that a relationship between serum TNF-α levels and DPN exists. Serum TNF-α was found to be statistically significantly higher in diabetic patients as compared with age-matched and sex-matched controls. Furthermore, serum TNF-α level in DPN patients was found to be much higher than in non-DPN patients (i.e. only DM with no PN).

We found a mixed axonal and demyelinating neuropathy in 65% and a demyelinating neuropathy in 35% of DPN patients. The pattern of neuropathy noted in the present study is consistent with previous neurophysiologic studies undertaken in type 2 diabetic patients [22–25].

Table 7 Correlations between electrophysiological parameters in nerve conduction velocity and tumor necrosis factor-α among diabetes mellitus cases with peripheral neuropathy (group I)

Electrophysiological parameters	Correlation coefficient	*P*-value	Significance
Rt median motor CV	–0.329	0.157	NS
Lt median motor CV	–0.024	0.919	NS
Rt ulnar motor CV	–0.344	0.138	NS
Lt ulnar motor CV	–0.034	0.888	NS
Rt median sensory CV	–0.390	0.121	NS
Rt median sensory CV	–0.274	0.271	NS
Lt median sensory CV	–0.359	0.157	NS
Lt median sensory CV	–0.421	0.092	NS
Rt peroneal motor CV	–0.112	0.637	NS
Lt peroneal motor CV	–0.183	0.454	NS
Rt tibial motor CV	–0.172	0.469	NS
Lt tibial motor CV	–0. 167	0.482	NS
Rt sural sensory CV	–0.192	0.476	NS
Lt sural sensory CV	–0.005	0.988	NS

CV, conduction velocity; Lt, left; Rt, right.

Table 8 Correlations between electrophysiological parameters in amplitude and tumor necrosis factor-α among diabetes mellitus cases with peripheral neuropathy (group I)

Electrophysiological parameters	Correlation coefficient	*P*-value	Significance
Rt median motor amp.	–0.249	0.291	NS
Lt median motor amp.	–0.409	0.073	NS
Rt ulnar motor amp.	–0.434	0.065	NS
Lt ulnar motor amp.	–0.270	0.249	NS
Rt median sensory amp.	–0.030	0.915	NS
Lt median sensory amp.	–0.032	0.907	NS
Rt peroneal motor amp.	–0.385	0.093	NS
Lt peroneal motor amp.	–0.104	0.671	NS
Rt tibial motor amp.	–0.434	0.050	S
Lt tibial motor amp.	–0.473	0.035	S
Rt sural sensory amp.	–0.174	0.570	NS
Lt sural sensory amp.	–0.014	0.963	NS

Amp, amplitude; Lt, left; Rt, right; S, significant.

In the present study, levels of TNF-α in the DPN patient showed a nonsignificant negative correlation with motor NCV in the median, ulnar, common peroneal, and tibial nerves and sensory NCV in median and sural nerves. Serum TNF-α in DPN patients showed a significant negative correlation with motor amplitudes of both tibial nerves, and no significant correlations were found with motor and sensory amplitudes of other studied nerves.

However, levels of TNF-α in DPN patient showed statistically significant positive correlations with score of neuropathy disability symptoms (NDS) and score of clinical PN (NSS).

Several experimental and clinical studies have previously mentioned the association between TNF-α and diabetic

neuropathy [26–29]. Matsuda *et al.* [28] reported a significant negative correlation between the levels of TNF-α and the index of age-corrected sensory NCVs in patients with type 2 DM.

Our results are in accordance with those of Hussain *et al.* [29], where the mean value of serum TNF-α level was found to be significantly increased in DPN patients of both shorter and longer duration when compared with DM patients without neuropathy. However, a point of contradiction is that Hussain *et al.* [29] found that TNF-α level in various groups of neuropathy patients showed a statistically significant negative correlation with motor NCV (in the median and ulnar nerves) and sensory NCV (in sural nerve).

This point of contradiction may be attributed to two points of difference between both studies. First, the study by Hussain and colleagues included diabetic patients with clinically detectable PN based on NSS and NDS, whereas the present study included confirmed DPN based on nerve conduction studies. Second, in nerve conduction studies conducted in our patients, we found a mixed axonal and demyelinating neuropathies in 65% and a demyelinating neuropathy in 35% of DPN patients. We found a statistically significant difference in motor amplitudes of median, ulnar common peroneal, and tibial nerves between diabetic patients with PN and those with no PN, whereas motor NCV of median, ulnar common peroneal, and tibial nerves showed a nonsignificant difference between both groups. On the other hand, PN patients in the study of Hussain and colleagues seem to have only demyelinating neuropathies of studied nerves.

However, the study of Duskal *et al.* [30] was in accordance with our study, in which authors found plasma TNF levels to be significantly higher in type II diabetes patients compared with controls, but no correlations were found between TNF-α levels and conduction velocities in the studied nerves.

High level of TNF-α in serum of type II DPN patients in the present study is supported by the results of Navarro and Mora [31] and Skundrik and Lisak [32] in which the role of neuropoitic cytokines in diabetic PN was analyzed.

Furthermore, previous studies showed that TNF-α contributes to the development of diabetic complications [33,34].

The study of Purwata [1] supports the role of TNF-α in DPN pathogenesis. A positive correlation was found between DPN pain intensity and plasma TNF-α level [1].

In the study of Shi *et al.* [35], the inhibition of TNF-α in the DPN rats resulted in a significant recovery from DPN signs.

During the pathologic process of DPN, TNF-α may cause nerve dysfunction via multiple pathways. Microvascular damage elicited by TNF-α may cause nerve ischemia and increased vascular permeability, thus permitting the exposure of harmful substances to nerve fibers. On the other hand, local TNF-α exerts demyelination by attacking Schwann cells [36].

Furthermore, the proinflammatory cytokine TNF-α has been implicated in mechanical and thermal hyperalgesia in addition to ectopic firing of sensory neurons [1,37,38], which occurs in a dose-dependent manner [37].

Previous studies have proposed that TNF-α stimulates the expression of specific proteins relevant to cellular damage, such as aldose reductase [39], protein kinase C [40], mitogen-activated protein kinase [41], and inducible nitric oxide synthase [8,42], all of which potentially play a role in the pathogenesis of diabetic polyneuropathy [43]. However, the precise mechanisms of how TNF-α is responsible for the pathogenesis of DPN remain to be elucidated.

As with all studies, there are some limitations. First, the study did not include type I diabetic patients and diabetic patients with good glycemic control, meaning that it would not be possible to directly apply the study results to these groups of diabetic patients. Second, this study could not evaluate the 'cause and effect' relationship between elevated serum TNF-α levels and DPN, which is a characteristic of a cross-sectional study. Third, the study population did not include patients with neuropathy due to other etiologies. As such, larger-scale studies that include additional categories of PN patients are warranted in the future.

Conclusion

We conclude that serum TNF-α is associated with clinical scores of PN in type II diabetic patients. Significant negative correlations were found with motor amplitudes of both tibial nerves as an index for PN. Thus, we conclude that TNF-α could be used as a biomarker for DPN.

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Conflicts of interest

There are no conflicts of interest.

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