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Role of Inflammation in the Pathogenesis of Diabetic Peripheral Neuropathy

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Abstract

BACKGROUND: Diabetic peripheral neuropathy (DPN) means the presence of symptoms and/or signs of peripheral nerve damage that occur to people with diabetes, excluding all other causes of neuropathy. Chronic hyperglycaemia leads to increased secretion of tumour necrotic factor-alpha (TNF-α), with the development of micro and macroangiopathy, damage to nerve fibres and local demyelination.

AIM: To determine the role of inflammation in the peripheral nerve damage process concerning people suffering from type II diabetes mellitus.

MATERIAL AND METHODS: The study included a total of 80 subjects, men and women, divided into two groups: an examined group (n = 50) consisting of subjects with DPN at the age from 30 to 80 years and a control group (n = 30) of healthy subjects aged from 18 to 45. In the investigated group, a neurological examination was performed using the Diabetic Neuropathy Symptoms (DNS) Score and Electroneurography. All the subjects had the blood plasma concentration of TNF-α by ELISA technique.

RESULTS: The average value of TNF-α in the test group was 8.24 ± 2.899 pg/ml, while the control group was 4.36 ± 2.622 pg/ml ($p < 0.0001$). The average value of TNF-α was correlated with the achieved DNS score in the investigated group ($p = 0.005$). Concerning the linear association of the concentration of TNF-α with the peripheral nerve velocity in the investigated group, no statistical significance was detected.

CONCLUSION: Inflammation can play a role in the pathogenesis of diabetic autonomic neuropathy and cranial neuritis.

Introduction

Diabetic peripheral neuropathy (DPN) is the most common microvascular complication in type I and type II diabetes mellitus, with an average in 30% of cases is with clinical manifestation as a painful neuropathy [1]. DPN means the presence of symptoms and/or signs of peripheral nerve damage that occur to people with diabetes, excluding all other causes of neuropathy. In the first years of diabetes, neuropathies develop about 5-10% of the patients, and after 20 years the duration of diabetes is thought to be about 60-70% of the patients develop some of the forms of diabetic peripheral neuropathy [2]. People who suffer from diabetic sensory neuropathy have a 25% greater risk of developing an ulcer on the feet and amputation of the limbs. The three-year survival of people with diabetic neuropathy is about 20% lower compared to people suffering from diabetes mellitus but have no peripheral neuropathy [3].

TNF-α (also known as cachectin) is a pro-inflammatory cytokine that plays a major role in the emergence of diabetic neuropathy; unlike other microvascular complications such as retinopathy or nephropathy (where the dominant role is played by IL-6 and CRP). In animal models, the role of TNF-α in the peripheral and central sensitisation mechanisms has been proven, and hence the occurrence of neuropathic pain [4]. Human TNF-α is synthesised as a 26 kDa type II transmembrane protein consisting of 35 amino acids in the cytoplasmic domain, 21 amino acids in the transmembrane segment and 177 amino

acids in the extracellular domain. In the extracellular domain, the human TNF-α possesses amino acid sequences that are 97% identical to those of rhesus monkeys; 71-92% identical to the amino acids detected in TNF-α in cows, dogs, horses, mice, rats. It creates various types of cells: immune, epithelial, endothelial, and tumor cells.

Chronic hyperglycaemia leads to the stimulation of macrophages, such as cells that dominantly secrete TNF-α and increased secretion of this cytokine, by developing micro and macroangiopathy. TNF-α increases the expression of endothelial cell adhesion molecules and thus accelerates the process of atherosclerosis [5]. The increased production of TNF-α secondary to hyperglycemia is a factor of exacerbation of insulin resistance in ill-controlled diabetes. The influence of TNF-α on Schwann cells should also be neglected, which explains local demyelination in the pathological process of peripheral neuropathy.

The aim of the study was to determine the role of inflammation in the process of peripheral nerve damage concerning people suffering from type II diabetes mellitus.

Material and Methods

A total of 80 examinees, men and women, were divided into two groups: a study group (n = 50) consisting of subjects with the symptomatology of DPN, aged 30 to 80 years, and a control group (n = 30) composed of healthy subjects, aged 18 to 45 years. Criteria for inclusion in the research were: respondents with diagnosed type II diabetes mellitus, lasting one to 40 years; on regular therapy with oral antidiabetics and / or insulin, with symptoms and signs of DPN. Criteria for exclusion from the study were: pre-diagnosed diabetic retinopathy and nephropathy; ischemic/haemorrhagic stroke or acute myocardial infarction over the past 12 months; acute and/or chronic skin infection, respiratory and gastrointestinal infection, known malignancy and autoimmune diseases, where higher plasma concentrations of TNF-α and diabetic foot or gangrene can be expected.

In the respondents from the investigated group (n = 50) clinical and neurological examination was first performed to determine the clinical type of DPN. To assess the severity of the clinical picture in our study, we used the Diabetic Neuropathy Symptom score-DNS, which assessed the following symptoms: pain, stiffness, tingling and the presence of ataxia. An objective electrophysiological assessment of the degree of damage to the peripheral nerves was made using electroneurography, which included the following peripheral nerves: n. medianus, n. ulnaris, n. peroneus profundus and n. suralis.

Subsequently, a sample of blood from the vein was taken for all subjects to determine the concentration of a TNF-α. Blood samples were first centrifuged to separate the blood plasma from the cellular elements, and then the blood plasma samples (about 1-2 ml) were frozen at -70°C. The concentration of the TNF-α was determined by the enzyme-linked immunosorbent assay (ELISA) technique. Anti-TNF-α antibodies that are absorbed in the wells bind to TNF-α which is present in the sample or standards. Subsequently, the addition of secondary biotinized anti-TNF-α antibodies that bind to TNF-α which is bound to the primary antibody. After incubation, unbound biotinised anti-TNF-α antibodies are removed by rinsing. The next step involves the addition of horseradish peroxidase (HRP) that is conjugated with streptavidin and which binds to biotin-conjugated anti-TNF-α antibodies.

After incubation, the unbound streptavidin HRP is removed by rinsing and a substrate solution for peroxidase, tetramethylbenzidine (TMB), which forms a colored product and whose coloring is proportional to the concentration of TNF-α in the sample or standard solution, is added. The reaction is stopped with 1 M phosphoric acid and the absorbance is measured at 450 nm. A standard curve of 7 standard solutions of TNF-α is formed.

For the analysis of the material were used the following statistical methods: frequency, percentage, contingency table, χ^2-Chi square test, Fisher Exact Test (FET), p index of statistical significance, mean value, standard deviation, Student t-test, Fisher F-test and Pearson correlation coefficient r.

Results

Based on the clinical presentation of DPN, the subjects (n = 50) were divided into 4 categories: 11 (22%) with a clinical picture of sensory neuropathy, 29 (58%) were with senso-motor neuropathy; 4 (8%) with cranial mononeuritis and 6 (12%) with dominant autonomic symptomatology (autonomic neuropathy). Regarding the achieved number of points on the DNS scale, the respondents were divided into four groups: with 1 point were 13 respondents, of which 6 (12%) were men, and 7 (14%) were women. With 2 points, there were 17 respondents, 7 (14%) men, 10 (20%) women. With 3 points on the DNS score were 10 respondents and 6 (12%) were men, and 4 (8%) were women. Ten respondents had 4 points on the DNS score with equal representation of men and women, respectively 5 (10%).

The average age of respondents in the study group was 65.5 years, while healthy subjects were 33.6 years old. The control group was composed of younger respondents because of the lower likelihood

of other chronic illnesses, which would give higher values of the investigated pro-inflammatory marker TNF-α. Based on the statistical analysis, it is noted that there is a statistically significant difference between the average age of subjects in the investigated and control group at the level $p < 0.0001$.

The average TNF-α blood plasma sample of the test group (n = 50) was 8.24 ± 2.899 pg/ml, while the control group (n = 30) was 4.36 ± 2.622 pg/ml ($p < 0.0001$). The average plasma TNF-α sample in the subjects from the test group (n = 50) was 8.24 pg/ml, 8.05 pg/ml for men and 8.40 pg/ml for women. The conducted statistical analyses showed that there was no statistically significant difference between the average values of TNF-α in men and women ($p = 0.671$). The average value of TNF-a does not depend on gender.

The average plasma concentration of TNF-α considering subjects with diabetic senso-motor peripheral neuropathy (n = 29) was 8.08 pg/ml, in subjects with diabetic sensory peripheral neuropathy (n = 11) was 6.81 pg/ml. The average TNF-α concentration in subjects with diabetic autonomic neuropathy (n = 6) was 11.2 pg/ml, while those with cranial mononeuritis (n = 4) was 8.85 pg/ml.

The average value of TNF-α was correlated with the achieved DNS score in the examined group ($p = 0.005$). The results are shown in Table 1.

Table 1: The average value of TNF-α dependence on DNS score in the examined group (n = 50) and statistical significance

DNS score	TNF-α (pg/ml) Average value ± S.D.	n	F (p)
1	6.138 ± 1.975	13	4.84
2	8.352 ± 3.058	17	
3	8.83 ± 3.27	10	P = 0.005
4	10.18 ± 1.503	10	

F-Fisher test; S.D. standard deviation; p-index of statistical significance.

Regarding the DNS score connection, the score with the DPN type was found a statistically significant difference at the level $p = 0.0244$. Respondents with autonomic neuropathy and cranial neuritis had higher scores on the DNS scale. This is shown in Table 2.

Table 2: Distribution by clinical type of diabetic peripheral neuropathy depending on the DNS score in the examined group (n = 50) and statistical significance

DPN type	DNS score				FET p
	1	2	3	4	
Sensomotor	6 (12 %)	11 (22 %)	8 (16 %)	4 (8 %)	P = 0.0244
Sensory	5 (10 %)	5 (10 %)	1 (2 %)	0 (0 %)	
Autonomous	0 (0 %)	1 (2 %)	1 (2%)	4 (8 %)	
Cranial mononeuritis	2 (4 %)	0 (0 %)	0 (0 %)	2 (4 %)	

FET-Fisher Exact Test; p-index of statistical significance.

Regarding the linear association of the concentration of TNF-α with the peripheral nerve velocity in the examined group, no statistical significance was found of the Pearson correlation coefficient r in any of the motor and sensory nerves conduction velocity.

Table 3: Correlation between TNF-α and the rates of nerve testing in the examined group (n = 50) and statistical significance

	Pearson correlation coefficient r	Relation	t (p)
n. medianus	-0.103	TNF-α = 9.699 – 0.030	-0.721 p = 0.474
n. ulnaris	-0.1	TNF-α = 10.125 – 3.841	-0.699 p = 0.488
n. peroneus	-0.178	TNF-α= 10.103 – 4.887	-1.257 p = 0.215
n. medianus	0.009	TNF-α = 8.182 + 1.419	6.559 p = 0.948
n. ulnaris	-0.093	TNF-α = 8.734 – 0.0129	0.653 p = 0.516
n. suralis	0.058	TNF-α = 8.044 + 9.932	0.405 p = 0.687

t-Student test; p-index of statistical significance.

Discussion

Diabetic peripheral neuropathy is the most common microvascular complication in diabetes mellitus. There are many different mechanisms involved in peripheral nerve damage in diabetic peripheral neuropathy, of which the key role in oxidative stress, inflammation and mitochondrial dysfunction [6]. Diabetes causes functional deficiency of nitric monoxide, activation of alternative metabolic pathways, accumulation of end-products of glycation, oxidative stress and inflammation, by activating inflammatory molecules. People with diabetes mellitus have an increased expression of pro-inflammatory cytokines such as C-reactive protein, TNF-α and IL-6. Chronic hyperglycemia leads to infiltration of cytokines into the vascular tissue and thus reduces the ability to repair.

Obesity increases the risk of developing neuropathy precisely because the fat tissue has increased expression of TNF-α, which in turn is involved in the mechanisms of insulin resistance. There is also a positive correlation between the increased plasma concentration of TNF-α and the number of macrophages with the progression of diabetic peripheral neuropathy [7].

In 2009, Herder et al. investigated the association of inflammation with diabetic peripheral neuropathy (by analysing 10 inflammatory markers of a total of 227 subjects who had type 2 diabetes mellitus). In this study, a high degree of association was found between CRP and IL-6 concentrations in people suffering from diabetic peripheral neuropathy [8].

In another study, an inverse association was found between the level of TNF-α and the nerves conduction velocities of n. suralis, n. medianus, and n. ulnaris, by analyzing the inflammatory marker in people who did not suffer from diabetic peripheral neuropathy and those who had undergone less than or more than 8 years of diagnosing diabetic neuropathy. Respondents who had diabetic neuropathy had higher serum TNF-α concentrations compared to others, with an upward trend in the duration of the disease [9].

In our study, subjects with diabetic peripheral neuropathy had higher concentrations of TNF-α in blood plasma related to healthy subjects, or individuals without diabetic neuropathy. This confirms the finding of numerous research studies where the role of TNF-α in the pathogenesis of DPN was examined [10], [11].

For a clinical assessment of the severity of DPN in our research, we used the DNS score as a simplified system for the diagnosis of distal diabetic neuropathy. Regarding the impact of the clinical type of diabetic peripheral neuropathy on the average value of TNF-α, in our study, higher TNF-α concentrations in the blood plasma sample had subjects in whom damage of the autonomic nervous system and cranial nerves were clinically dominant. The highest percentage of subjects diagnosed with diabetic autonomic neuropathy and cranial mononeuritis had a maximum number of points on the DNS score. The correlation between the levels of TNF-α with the severity of DPN has been proven in numerous research studies [7], [12], [13].

When it comes to the association of TNF-α concentration in blood plasma with the motor and sensory conduction velocities the examined nerves, no correlation was found in any of our motor and sensory implementation rates. That is, the increased plasma concentration of TNF-α as pro-inflammatory cytokine does not affect the neuropathic characteristics of the peripheral nerves. Regarding the link of the DNS score with the motor and sensory velocities of conducting the examined nerves, no positive correlation between these two parameters was found in our research study, that is, the severity of the clinical picture in subjects with diabetic peripheral neuropathy does not depend on the conduction velocities of the examined peripheral nerves.

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