ORIGINAL ARTICLE

Clinical use of an analysis of oxidative stress and IL-6 as the promoters of diabetic polyneuropathy

Emina Karahmet[1], Besim Prnjavorac[2,3], Tamer Bego[4], Adaleta Softić[5], Lejla Begić[6], Edin Begić[7,8], Esma Karahmet[4], Lejla Prnjavorac[2], Irfan Prnjavorac[2]

[1] Faculty of Pharmacy, University of Tuzla, Tuzla, [2]General Hospital, Tešanj, [3]Department of Pathophysiology, Faculty of Pharmacy, Sarajevo, [4]Department of Clinical Biochemistry, Faculty of Pharmacy, Sarajevo, [5]Department of Biochemistry, University of Tuzla, Tuzla, [6]Department of Biochemistry, Faculty of Pharmacy, University of Tuzla, Tuzla, [7]Department of Cardiology, General Hospital "Prim.dr. Abdulah Nakaš", Sarajevo, [8]Department of Pharmacology, School of Medicine, Sarajevo School of Science and Technology, Sarajevo; Bosnia and Herzegovina

ABSTRACT

Aim To investigate interleukin 6 (IL-6) values depending on duration of diabetes mellitus (DM) and evaluate possible correlation with diabetic polyneuropathy.

Methods The research study included 90 patients with DM divided into three groups (30 patients each) according to the duration of DM: group A – patients who had DM for less than 10 years, group B - duration of DM was 10 to 20 years, and group C - patients with DM over 20 years. Control group (K) included 30 healthy participants.

Results IL-6 was significantly higher in the healthy control group, 180.318 pg/mL±94.18, than in group A, 47.23pg/ml±34.8, group B, 43.31pg/ml±33.17, and group C, 70.39 pg/ml±59.26 (p=0.0001). All groups had significantly different values of IL-6 between each other (p=0.0001). Level of IL-6 was in correlation with diabetic polyneuropathy in the group A (the youngest participants) (p=0.0001). In other groups there was no significant correlation between IL-6 and diabetic polyneuropathy.

Conclusion The level of IL-6 was in correlation with neuropathy among younger patients. A higher level of IL-6 in the control group than in diabetic groups is a sign of stronger inflammatory response among younger and healthy people than in patients with DM.

Key words: diabetes, interleukins, inflammation, neuropathy

Corresponding author:
Besim Prnjavorac
Department of Pathophysiology,
Faculty of Pharmacy
Zmaja od Bosne 8, 71000 Sarajevo,
Bosnia and Herzegovina
Phone: +387 33 586 182;
Fax: +387 33 666 189;
E-mail: pbesim@bih.net.ba;
Emina Karahmet ORCID ID https://orcid.org/0000-0002-0913-5759

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INTRODUCTION

Diabetic peripheral neuropathy is a common complication of diabetes mellitus (DM), which mainly appears in long duration of the disease. It is present in 30-50% of patients with DM, but statistical results show that it occurs more frequently in DM type 1 (54-59%) than in DM type 2 (45%) (1). In 13-26 % of patients it occurs in the form of painful distal neuropathy (1).

The mechanism of pathophysiology is very complex. There are several accepted explanations: disorder of polyol and myoinositol metabolism, formation of highly reactive oxygen species (ROS), reduction of Na/K ATP-enzyme, ischemic endo-neural microvascular disorder, neurotrophic disorder, disorder of axonal transmission and non-enzymatic glycosylation of proteins (2). Commonly used explanation is oxidative stress. (3) High ROS makes changes to the axonal and dendritic surface and changes its main rule (4). Other effects of oxidative stress are the decrease of glutathione and ascorbate levels, increase of lipid peroxidation and protein nitrosylation. In patients treated with antioxidant therapy a very good therapeutic effect was shown (5). It is important to consider that spinal microglia is highly activated with these neuronal disorders, and it starts to produce CD11b and Iba1 signals, and p38 mitogen protein kinase phosphorylation is induced. It results with high production of inflammatory cytokines IL-6, interelukin-1 (IL-1), tumour necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) (6). These cytokines can influence the spinal synaptic transmission by increasing excitability of dorsal horn neurons and partially decreasing inhibitory synaptic transmission (2). At the same time, glucotoxicity and lipotoxicity in pancreas induce releasing of free fatty acids (especially palmitic acid, where the process of lipid peroxidation starts), that results with new oxidative stress and damaging of insulin producing β cells of pancreas (2). Inflammatory cytokines have the main role in this process too because of their inflammatory promoting activity. The same cytokines are included in this process, IL-1β, IL-6, TNF-α, TGF-β but chemokine, IL-8 and NF-κβ (nuclear factor) (2). IL-6 is double ruled cytokine, pro-inflammatory and anti-inflammatory myokine (8). It is produced by macrophages and T-cells, as an immune answer to stimulation (tissue damaging for example) (8), previously explained process in the spine and pancreas (2). Tunica media of many blood vessels produces IL-6 too, and it plays pro-inflammatory role. Its anti-inflammatory role is manifested by inhibition of TNF-α and IL-1, and by activation of IL-1ra and IL-10 (5). IL-6 is an important inflammatory promoter and reactant of the acute phase of the inflammatory reaction (9). A very important fact is that in stress, human body starts an intense production of the inflammatory cytokines, especially IL-6 (10). Considering all these facts, it is obvious that inflammatory cytokines can be a good screening method for consideration of diabetic neuropathy (11).

The aim of this study was to investigate values of IL-6 and check any dependence of IL-6 on DM duration and its use as a diagnostic marker for diabetic polyneuropathy, as well to test active inflammatory processes related to diabetic polyneuropathy.

PATIENTS AND METHODS

Patients and study design

This retrospective-prospective study included 90 DM patients (type 1 and type 2) age 18-80, who were divided into three groups (30 patients in each group) and a control group (K, 30 healthy participants). All study subjects were processed at the Department of Internal Medicine, General Hospital Tešanj, over the period of 18 months (during 2018 and 2019).

The groups were formed according to the duration of DM: group A included patients with the duration of DM less than 10 years, group B with the duration of DM 10-20 years and group C with more than 20 years of DM duration.

An inclusion criteria was the diagnosis of DM, both type 1 and type 2, by definition given by the International Diabetes Federation (IDF) (12) and the American Diabetes Association (ADA) (13), according to which a patient is considered diabetic if he/she has a glycaemia above 11.2 mmol/L at any time (14). Exclusion criteria were: participants who withdrew their consent given in writing, women who became pregnant during the examination, participants who experienced any changes during the examination period that they could not reasonably explain.

Control subjects, without DM diabetes, were recruited from the systematic preventive examination of healthy employees of the schools.

Each participant signed an informed consent to participate in the study. Ethical approval was obtained from the Ethical Committee of the General Hospital Tešanj, Bosnia and Herzegovina.

Methods

Blood samples were taken during a patients' hospitalization or a regular follow up.

All measurements were performed in the Biochemical Laboratory of the General Hospital, Tešanj, with fresh or frozen serum samples (-60^{o}C). The whole blood sample was centrifuged and the serum was prepared and used for IL-6 determination by ELISA test (Quantikinine, Becton Dickinson, USA).

For all patients an analysis of IL-6 level (reference value 3- 477.30 pg/mL), glycosylated haemoglobin (HbA1c), C- reactive protein (CRP), uric acid, erythrocytes sedimentation rate (ESR) and leucocytes (Le) in the blood was performed at the time of admission.

Monofilament test for peripheral neuropathy (15) was performed by using the single - fiber nylon thread (a filament of nylon). It was placed on the patient's skin (preferable on the feet). The patient was asked whether he/she felt the touch of the filament or not.

Sonic fork test was used for the examination of deep sensitivity (Michigan Neuropathy Screening Instrument, MNSI) (16). The fork was applied to the patient's bone (preferable on distal part of radius) and activated. The patient was asked to signal when she no longer felt the vibration. It was monitored how long the examinee felt the vibration. The test was described as normal, decreased or absent sensation of vibration (17).

Statistical analysis

All variables were tested for normal distribution using the Kolmogorov-Smirnov test and presented descriptively using appropriate measures of central tendency (arithmetic mean and median). Quantitative variables were compared using Student's t-test with correction for unequal variance where needed. The relationships between the variables were tested using the parametric Pearson correlation. The variance test (ANOVA) was used to analyze the results of IL-6 measurements between the groups. All tests were performed with an accuracy level of 95% ($p<0.05$).

RESULTS

The patients and individuals in the control group (n=120) were between 38 and 82 years old, with mean age of 61.61±20.2 years.

IL-6 showed significantly lower levels in the groups with DM than in the control group. In the group A, IL-6 mean level was 47.23±6.15 pg/mL, in the group B, mean IL-6 level was 43.31±5.69 pg/mL, in the group C, 70.39±10.64 pg/mL, while in the control group it was 180.32±94.20.08 pg/mL (Table 1).

The control group had a significantly different level of IL-6 than the groups A, B, and C (p=0.0001).

A statistically significant difference in IL-6 values between all analyzed groups (p=0.0001), as well as between group A and group K (p=0.0001) was found. Group B in relation to group K also showed a statistically significant difference (p=0.0001), as well as group B in relation to group C (p=0.041). Group C compared to group K also showed a statistically significant difference in IL-6 values (p=0.0001).

The relationship between polyneuropathy and IL-6 with statistical significance was only found in the group A (p=0.038). Other groups did not show statistically significant ifference in results.

In group A, an impairment measured by a decrease in deep sensitivity showed statistical signifi-

Table 1. Followed parameters in four groups

Variable	Reference value	Groups*			
		A	B	C	K
Age (years)		59.44±9.38	67.81±7.32	70.04±6.96	47.91±10
DM duration (years)		3.59±2.56	13.91±2.36	24.17±4.52	-
HbA1C (%)	4.5-6.0	7.62±1.27	8.7±1.66	9.2±2.17	-
CRP (mg/dL)	0-10	18.51±39.03	18.5±37.52	31.22±42.56	-
Uric acid (μmol/L)	360-400	353.24±146.36	313.67±88.94	419.19±114.24	150.3
ESR (mm)	<20	34.95±24.24	32.75±27.28	44.70±34.20	-
Le (106/mm3)	4.0-10.0	8.18±1.86	7.98±3.11	8.93±3.15	-
IL-6 (pg/mL)	3- 477.30	47.23±34.8	43.31±33.17	70.39±59.26	180.32

*according to diabetes mellitus duration: A, <10 years; B, 10-20 years; C, > 20 years; K, control group (healthy participants)
DM, diabetes mellitus; HbA1c, glycosylated haemoglobin; CRP, C- reactive protein; ESR, erythrocytes sedimentation rate; Le, leucocytes;

cant difference with a decrease in peripheral sensation (p=0.0001). Total impairment, expressed by MNSI score, showed a significant correlation with IL-6 (p=0.038).

DISCUSSION

Risk factors for diabetic neuropathy were an important issue of many studies during the last few years, concluding that DM control, duration, type, therapeutic modality used in the treatment and comorbidities all affect the development of diabetic neuropathy (18-20). Additionally, hyperglycaemia is one of the most important causes of oxidative stress (21-24). Many studies published in recent years have shown that IL-6, with other inflammatory cytokines, is a part of this inflammatory process. In this study it was shown that IL-6 level is much higher in the control group than in the groups with diabetes. It can be considered that the inflammatory response is more powerful in healthy subjects than in patients with DM (25). Magrinelli et al. showed that IL-6 and IL-10 were related with large nerve fibres damaging, but not with small nerve fibers and polyneuropathy (26). Korkmaz et al. showed that IL-6 and fibrinogen had higher values in infected than in non-infected diabetic foot ulcer (27), suggesting that IL-6 and fibrinogen are certain markers of infection (consequently inflammation too) (19,28). Cox et al. suggested the idea of treatment of peripheral neuropathy with low-dose pulsatile IL-6, assuming that IL-6 will show a potentially anti-inflammatory effect (29). However, the anti-inflammatory effect of IL-6 has not been confirmed up to date. In fact, the idea was to imitate natural response of the human body to physical activity, by excreting IL-6 in low dose and intervals, like a myokine (30). In contrast, our study showed that IL-6 was significantly higher in the control group (K) than in all three groups of patients with DM. This interesting finding suggests that younger and healthy people have stronger immunological response than older ones and patients with DM, even if DM induced some tissue damage. In this study we also included DM patients with amputation. There were not many analyses in the recent literature related to IL-6 and the Michigan Neuropathy Score (monofilament test, test with sonic fork and subjective evaluation of symptoms included) (15). In our study no significant correlation between IL-6 level and diabetic polyneuropathy was shown, but changes of cytokine levels can be seen in a long term follow-up (31).

It is known that reparation of tissues in patients with long lasting DM is impaired (32); it is necessary to conduct studies with at least 600-800 participants in order to establish the cause of it. Also, for some conclusion about details of these processes it is necessary to analyze the patients with DM type 1 and DM type 2 separately, because the therapy can influence the behaviour of inflammatory cytokines. This fact could be the main limitation of this and similar studies. We did not separate patients with and without statins therapy.

As the inflammation is one of the main immunological barriers, it should be considered that an inappropriate immune response can be seen in serious metabolic disorders, like in diabetes (33). Infection of the foot is very common in patients with DM, with very strong influence of cytokine synthesis and release (34). The Further research should consider comorbidities and their therapy for possible influence to immune response. It is known that statins, very often used as hypolipemic therapy in patients with DM, can influence inflammation as the anti-inflammatory substances (35).

In conclusion, there are so many factors that influence damaged nerve tissues with a lot of overlapping with oxidative stress in diabetes. Interleukins, like IL-6 could be used as a parameter of tissue damage and inflammatory response in patients with DM. It is very important to point out that the damage of nerve tissue was in a statistically significant correlation with IL-6 in group A (younger diabetics). The higher levels of IL-6 in the control group than in the diabetic groups is a sign of a stronger inflammatory answers in younger and healthy people than in patients with DM.

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TRANSPARENCY DECLARATION

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