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Research Article

# IL-1β in Correlation to the Common Diabetic Complications

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## Abstract

The aim of this research study was to compare interleukin 1β (IL-1β) levels in participants with diabetes mellitus 2 (DM2) depending on the duration of disease and comorbidity.

**Methods:** A total number of 150 participants were observed by two different ways. In a first observation, all participants were grouped into four different groups (A, B, C, K), with criteria duration of DMT2: A- less than 10, B- 10-20, C- 20-30 years of DMT2 duration. Second observation was conducted with criteria accompanied comorbidities of DMT2, and all participants were grouped into 5 different groups (1: DMT2+ polyneuropathy (PNP) + hypertension, 2: DMT2 + PNP, 3: DMT2 + hypertension, 4: DM, 5: control). Each group included 30 participants, except group A in first observation that included 60 participants. Control group included 30 healthy participants. An enzyme-linked immunosorbent assay (ELISA) was performed to measure IL-1β levels in each group.

**Results:** In the first evaluation, IL-1β of the group C (98.43 pg/ml ± 5.72) was at a significantly lower level compared to group A 113.49 pg/mL ± 5.29 and B 114.53 pg/ml ± 5.69, and was not significantly different than control group K 98.88 pg/ml ± 14.42 ($p = 0.002$). IL-1β in group A was significantly different to group K, $p = 0.0001$, and group B was significantly different to group K, $p = 0.003$. IL-1β did not show a significant correlation with diabetic polyneuropathy.

In the second evaluation, IL-1β (pg/ml) was significantly different in groups ($p < 0.001$) with average ranges per groups: group 1: 93.84, group 2: 63.76, group 3: 86.69, group 4: 69.42 and group 5: 47.97. Groups 1 and 2 were significantly different (40.09 vs. 27.29, $p = 0.007$), groups 1 and 5 were significantly different (45.97 vs. 22.03, $p < 0.001$) and groups 3 and 5 show significant difference between each other (38.96 vs. 22.94, $p < 0.001$).

**Conclusion:** Hypertension has bigger impact to IL-1β ranges than diabetic neuropathy, but showed that hypertension and neuropathy are correlated and will probably be risk factors for the manifestation of another comorbidity in diabetic participants.

**Keywords:** Interleukins; Inflammation; Diabetes Mellitus; Hypertension; Diabetic Neuropathy

## Introduction

Diabetes mellitus (DM) and hypertension are very often presented together. Complications of DM mostly depend on regulation of glycaemia and duration of DM [1], which complication will be manifested depends on other factors too (nutritive factors, sedentary lifestyle, genetic factor, epigenetic factor and comorbidity) [1]. Common complication of DM is diabetic polyneuropathy [2]. Diabetic polyneuropathy is more frequent in DM1 (54-59%) than in DM2 (45%) [3].

Hyperglycaemia is the main factor related to diabetic polyneuropathy [4]. Actually, explanation is oxidative stress as a result of non-enzymatic glycosylation of physiological structures [4]. Inflammatory cytokines have the main control in these processes because of their inflammatory promoting activity. The cytokines included in this process are Interleukin 1β (IL-1β), IL-6, tumor necrosis factor α (TNF-α), transforming growth factor β (TGF-β) except chemokine, IL-8 and nuclear factor (NF-κβ) [4].

IL-1β is recognized as the main promoter of this inflammatory reaction. Higher expression of IL-1β is described in human monocytes, macrophages, pancreatic islets, myocardium and aortic endothelium, which is associated with serious complications of DM [5,6]. Healthy people have limited levels of IL-1β, but after some microbial stimuli, tissue damaging or other cytokines like TNF-α, IL-18 or IL-1β monocytes, macrophages or dendritic cells start to produce IL-1β as an auto-inflammatory response [5].

Damage of peripheral nerve tissue is accompanied with inflammatory response manifested as increased secretion of inflammatory cytokines (IL-1β, IL-6 and TNF-α), especially by Schwann cells that produce IL-1β, and activate the macrophages. Activation of macrophages leads to a cascade of different events, and one of them is increased expression of nerve growth factor by fibroblast. This is a very important step in regeneration of nerve tissue but accompanied with hyperalgesia. Few studies show that adult sensory neurons express receptors for cytokines, such as LIF, IL-6 and IL-1β, and during the nerve damage these cytokines are increasingly produced by dorsal root ganglia (DRG). IL-1β can regulate axonal regeneration during injury processes [5,7].

## Purpose of the Study

The purpose of this study was to measure the values of IL-1β in groups of participants with DM, to check if there is any dependence on glycaemia, DM duration, and could it be a diagnostic marker for diabetic polyneuropathy (I). In addition, the aim was to compare IL-1β ranges depending on comorbidities (hypertension and diabetic and to check what influences make comorbidities between each other (II).

## Research Methods

### Research study design and data collection

The study had a quantitative research prospective character and included 150 participants with DM (type 1 and type 2), ages 18-80, males and females, who were examined at the Department of Internal Medicine, General Hospital Tešanj, over the period of 18 months (during the year of 2018 and 2019) and 30 healthy participants as control group.

Study inclusion criteria was the diagnosis of DM both type 1 and type 2, by the definition given by the International Diabetes Federation (IDF) and American Diabetic Association (ADA), according to which a patient is considered diabetic if it has a glycaemia level above 11.2 mmol/L at any time [7]. Study exclusion criteria were: participants who withdrew their consent given in writing, women who became pregnant during the examination, participants who experienced changes during the examination period that they could not reason meaningfully.

Study groups were formed with two criteria: duration of DM and presented comorbidities. The first evaluation included grouping by DM duration: group A consisted of 60 participants with DM duration of less than 10 years, group B consisted of 30 participants with DM duration of 10 - 20 years (30) and group C consisted of 30 participants with more than 20 years of DM duration (30). Control group consisted of 30 healthy participants.

After the first evaluation, the second criteria for grouping were presented comorbidities (II). A total number of 150 participants were divided into 5 groups, depending on comorbidity. Group 1, consisted of 31 DM participants, with hypertension and diabetic neuropathy. Group 2, consisted of 33 DM participants with diabetic neuropathy. Group 3, consisted of 29 DM participants with hypertension. Group 4, consisted of 27 DM participants without comorbidity and group 5, consisted of 30 healthy participants.

Institutional Review Board Approval (IRB approval) was obtained from the Ethical Committee of General Hospital Tešanj. Each participant signed an informed consent form to participate in the study.

### Research method

Participants were analysed to interleukin 1β levels (reference values 0.2 - 11.1 pg/ml) at admission and were tested by Michigan Neuropathy Score Instrument (that included monofilament test and tunic fork test) for polyneuropathy examination. Blood samples were taken in early morning hours, during patient hospitalization or regular check-ups. The whole blood samples were centrifuged, a serum was prepared and used as biological material

for interleukin determination. An enzyme-linked immunosorbent assay test (ELISA) (Human Interleukin 1beta ELISA kit, MyBiosource, San Diego, USA, 2019) was used for IL-1β beta ELISA determination. All measurements were performed in the biochemical laboratory of the General Hospital in Tešanj, with fresh and frozen serum samples (-80°C).

For the group of control subjects, the absence of DM was determined by a control laboratory test of fasting glucose from a whole blood sample and urine sample as part of a systematic examination, which the participants previously had, not older than 3 months.

## Data analysis

Statistical analysis was done using SPSS Windows software program (version 21.0). All variables were tested for normal distribution using the Kolmogorov-Smirnov test. All variables are presented descriptively using appropriate measures of central tendency (arithmetic mean and standard deviation, SD). 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. All tests were performed with an accuracy level of 95% ($p < 0.05$).

## Results

Participants in this study (150) were ages between 38 and 82, 72 males and 78 females, average age of participants was 61.61 ± 20. Participants without DM were grouped to control group (K). Research groups in this study were described with descriptive statistics, table 1.

| Group in the study | A (Average Mean ± SD) | B (Average Mean ± SD) | C (Average Mean ± SD) | K (Average Mean ± SD) |
|---|---|---|---|---|
| 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 | - |
| $HbA_1C$ (%) | 7,62 ± 1,27 | 8,7 ± 1,66 | 9,2 ± 2,17 | - |
| CRP (mg/dl) | 18,51 ± 39,03 | 18,5 ± 37,52 | 31,22 ± 42,56 | - |
| Acidum uricum (µmol/L) | 353,24 ± 146,36 | 313,67 ± 88,94 | 419,19 ± 114,24 | 150.3 |
| TAS (mmHg) | 135,56 ± 28,56 | 140,41 ± 22,8 | 131,76 ± 16,29 | 120 |
| TAD (mmHg) | 84,44 ± 20,4 | 82,32 ± 10,78 | 80,59 ± 10,29 | 80 |
| Cholesterol (mmol/L) | 5,56 ± 1,56 | 5,33 ± 1,56 | 5,47 ± 2,12 | 5,17 ± 0,97 |
| Triglyceride (mmol/L) | 4,82 ± 6,82 | 3,03 ± 2,06 | 2,66 ± 1,85 | 1,28 ± 0,41 |
| Creatinine (µmol/L) | 98,94 ± 19,09 | 107,28 ± 38,23 | 107,26 ± 38,91 | 86,45 ± 12,99 |

**Table 1:** Descriptive statistics of the study groups.

Interleukin 1β (IL-1β) showed significantly lower levels in groups C and K than in the groups A and B. In group A, IL-1β average mean level was 113.49 pg/ml ± 5.29. In group B, the average mean IL-1β level was 114.53 pg/mL ± 5.69. In group C, the average mean value was 98.43 pg/mL ± 5.72 while in the control group the average mean value was 98.88 pg/mL ± 14.42 (Figure 1).

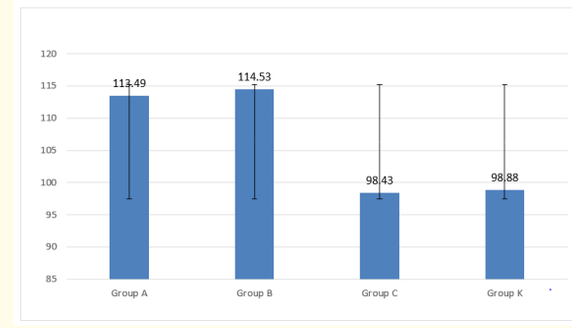


**Figure 1:** Series of Il-1β (pg/ml) per groups.

Statistical analysis showed that groups A, B, C and K are statistically significantly different between each other in IL-1β levels, p = 0.002. Meanwhile group A and group K are significantly different, level p = 0.0001. Group B and group K are significantly different, p = 0.003.

Correlation between polyneuropathy and IL-1β was not statistically significant in any group. Correlation between HbA1C and IL-1β was not statistically significant in any group.

IL-1β was significantly different in groups ($X^2 = 24.475$; $p < .001$) with mean ranges per group: group 1: 93.84, group 2: 63.76, group 3: 86.69, group 4: 69.42 and group 5: 47.97 (Figure 2).

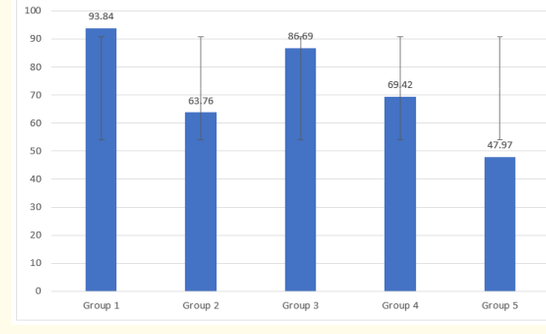


**Figure 2:** Mean ranges of IL-1β per groups.

Group 1 and 2 were significantly different (40.09 vs. 27.29, p = 0.007), group 1 and 5 were significantly different (45.97 vs. 22.03,

$p < 0.001$) and group 3 and 5 show significant difference between one another (38.96 vs. 22.94, $p < 0.001$).

## Discussion

It has been known that hyperglycaemia has an influence to some big comorbidities such as hypertension, arrhythmias, diabetic polyneuropathy, but not all details of this processes were known [2,8]. For years, the accepted idea was that β pancreatic islets produce IL-1β along with insulin. But in 2017 it was discovered that α pancreatic cells produce IL-1β [9]. IL-1β affects β pancreatic islets by inhibiting glucokinase expression [10], and inhibiting glucokinase expression on the β pancreatic islets, leads to glycolysis decrease. According to these facts, it is expected to have accompanied correlation of haemoglobin $A_1C$ (HbA1C) and IL-1β. Some studies suggested it in the past [11]. This research study, however, revealed the opposite results, even if measured levels of HbA1C were higher in longer duration DM than in shorter duration of disease- the levels of IL-1β were lower in longer duration of DM. IL-1β levels are limited among healthy people, and its levels increase by stimuli. In this study, it is shown that the inflammatory response is more powerful in shorter duration of DM participants than in longer duration DM participants. Stimulus in this case is oxidative stress caused by hyperglycaemia, and the reparative mechanism of the body is inflammatory response (producing of IL-1β). This is somewhat logical explanation of facts from clinical perspective, the wound healing process among older participants with diabetes is usually slow or absent all together. The fact that their body can not induce adequate response by producing IL-1β and stimulate reparation of tissues, could be an explanation of these results. This study is important in understanding the repairing processes of tissues and can serve as the basis for the future studies in this field.

As a result of increased levels of IL-1β, the correlation with diabetic polyneuropathy was expected. But, the fact that endogenous production of IL-1β was decreased in nerve tissues in DM1, could be one of potential reasons of diabetic neuropathy and plasticity [12]. In this research study, the measured levels of IL-1β were higher among group of diabetic participants with hypertension than in group of diabetic participants with polyneuropathy. It is probably because of atherosclerosis that makes this inflammatory ambient. Potentially, this is a more serious problem, especially if take in consideration the fact that cell apoptosis caused by the stress of endoplasmic reticulum in myocytes is the cause of diabetic cardiomyopathy. The promotion of endoplasmic reticulum stress is caused by elevated IL-1β [13]. Furthermore, Monnerat., *et al*. noticed that IL-1β induces spontaneous contractile events like arrhythmias. In the study it is shown that DM-induced arrhythmias can be successfully treated by inhibiting the IL-1β, possible with IL-1β receptor antagonist [14]. Blocking IL-1β will improve infarct healing and probably reduce post-MI remodelling [15].

The absence of statistically significant correlation between IL-1β and diabetic polyneuropathy is probably due to the results of the earlier research studies. IL-1β levels are decreased in diabetic polyneuropathy participants, and IL-1β is not specific in nerve reparation process. Its increased levels are more specific to cardiovascular comorbidities of DM. But, the fact that IL-1β is stimulus by its own (already realised molecules of IL-1β will be a stimuli for new ones), leads to the conclusion that hypertension in diabetic participants will probably induce diabetic neuropathy and reverse. Diabetic neuropathy will induce hypertension and cardiovascular neuropathy, even more serious cardiovascular complications (infarct, ischemia, Ictus cerebrovascularis). Limitation of this quantitative research study, except the number of participants in some groups, is in imprecise graduation of hypertension and other cardiovascular diseases, but the therapies participants used in a treatment of it were not observed too. Some of the medicines used in daily treatment of participants for hypertension, DM and PNP have cardio-protective and anti-inflammatory effects. The future research studies should observe hypertension by grades, check the possibilities of atherosclerosis inhibiting, and an influence of medicines to IL-1β. This quantitative research study could be basis for future research studies in this field.

## Conclusion

Glycaemia and duration of DM do not have any influence on IL-1β levels and IL-1β can not be any prognostic marker for diabetic polyneuropathy. Much bigger influence to IL-1β levels in plasma has hypertension than diabetic polyneuropathy. IL-1β has positive feedback control and increased levels of it caused by polyneuropathy will probably induce hypertension and reverse.

## Funding Support

No specific funding was received for this study.

## Competing Interests

None to declare.

## Appendix

### Informed consent form

Faculty of Pharmacy

University of Tuzla

### INFORMED CONSENT FORM

Informative consent by which I, _____ (Name of participant) confirm by my wish and understanding goals and methods, participate in the research study.

"CORRELATION OF CONCENTRATION OF INTERLEUKIN IL-1, IL-6, TNF-α, TGF-β WITH HBA1C AND THE DEGREE OF DIABETIC POLYNEUROPATHY". I also confirm that it has been explained to me that all the information that I will give as a respondent in the interrogation will remain anonymous and will not be able to recognize my identity in any way.

Signature of researcher Signature of participant

_____ _____

Date:

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