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Evaluation of Interleukin-6 (IL-6) Level in Serum of Type 2 Diabetes Patients and Correlation with Neuropathy

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Abstract

Background Diabetic polyneuropathy (DPN) is a prevalent long-term consequence of type 2 diabetes mellitus (T2DM). The cause of DPN is still a subject of controversy, but it is possible that proinflammatory cytokine mediators such as interleukin-6 (IL-6) play a role.

Objective To assess the correlation between pro-inflammatory marker (Il-6) and the development of DPN in T2DM patients.

Methods A case-control study, involved randomly selected 140 T2DM patients attended to Al-Numan Teaching Hospital at Baghdad during the period from October 2023 to January 2024. Their age ranged from 25 to 80 years, 57 males and 83 females. The patients have been divided into two groups; those with DPN and diabetic patients without neuropathy. For both groups' patients, whole blood sample was centrifuged and serum was prepared and used as biological material for IL-6 levels determination by enzyme-linked immunosorbent assay (ELISA) technique. This study compared IL-6 levels between the two groups.

Results The mean age of study samples was 58.69±10.51 years. The female to male ratio 1.45:1, as well as females were dominant among cases and that of controls group, but such differences were not statistically significant. The mean value of IL-6 was found to be significantly higher among cases group than that of controls group (117.68±27.38 vs. 57.3±13.24 pg/ml) respectively. The optimal cutoff value of IL-6 for detection of diabetic patients with high risk of neuropathy development was 72.46 pg/ml with sensitivity of 97.1%, and specificity of 87.1% with good area under the ROC curve (AUC) (P <0.001). The mean level of glycated hemoglobin (HbA1c) was significantly higher among cases group than that of controls group (8.27±1.92 vs. 7.19±1.61) %.

Conclusion Current study concluded that IL-6 is significantly elevated levels in T2DM patients with DPN, the proinflammatory cytokine could be used as a useful indicator for development of DPN.

Keywords Diabetic neuropathy, IL-6, type 2 diabetes mellitus

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List of abbreviations: AUC = Area under the curve, BMI = Body mass index, CRP = C-reactive protein, DPN = Diabetic peripheral neuropathy, ELISA = Enzyme-linked immuno -sorbent assay, IL = Interleukin, T2DM = Type 2 diabetes mellitus, TNF-α = Tumor necrosis factor-alpha

Introduction

Diabetic peripheral neuropathy (DPN) is the most prevalent microvascular consequence in patients with diabetes mellitus [(1)]. DPN is a significant public health issue that contributes to illness, death, and healthcare costs. DPN is linked to foot ulcers, which can result in lower-limb amputation and

higher mortality rates [2]. Both small and large peripheral nerve fibers can be affected by DPN. Paresthesia, sensory loss, muscle weakness, pain, anesthesia, foot ulcer, and autonomic symptoms are consequences of large and tiny nerve fiber injury, respectively [3].

People with type 2 diabetes mellitus (T2DM) can develop DPN, and about 25% of them experience painful DPN [4]. The pathophysiology of DPN includes oxidative stress, overactivation of the polyol pathway, and neuroinflammation, these systems are inherently interconnected [5]. Polyol pathway is linked to elevated levels of inflammatory markers and impaired endothelial function. Neuropathic pain is linked to heightened inflammation and indicators of endothelial dysfunction, although the nerve axon reflex remains intact [6]. Inflammation is a crucial factor in the development of diabetes and related metabolic issues. Therefore, there is a growing focus on targeting inflammation as a means to prevent and manage diabetes [7]. A proinflammatory state may be the common denominator of pain and peripheral neuropathy in people with diabetes mellitus may share a proinflammatory state, while the specific inflammatory profiles appear to vary [8]. Various complications of T2DM, like diabetic neuropathy and diabetic nephropathy, often show elevated levels of certain acute phase indicators of inflammation, such as C-reactive protein (CRP), serum amyloid A, fibrinogen, and interleukin-6 (IL-6), IL-10, and IL-11. It is valuable to investigate the correlation between inflammatory factor levels in patients with DPN and the severity of the condition. Overexpression of pro-inflammatory cytokines indicates an inflammatory and stressful condition in the body. Many studies hypothesize that the production of inflammatory adipocytokines like IL-6 and tumor necrosis factor-alpha (TNF-α) was important in the etiology of the diabetes mellitus [9]. Other articles have shown that IL-6 crucial in the development of DPN [10].

IL-6 is a multifunctional cytokine that is typically generated in reaction to tissue damage or infection [11]. IL-6, a versatile cytokine, is being considered as a potential treatment, although IL-6 is traditionally recognized as a pro-inflammatory cytokine in the immune system, recent research indicates that it also functions as a myokine and neurocytokine, triggering anti-inflammatory and regenerative reactions. [12]. DPN is usually diagnosed at late stage and the early detection of diabetic neuropathy still a challenge.

The aim of the study was to compare the level of IL-6 as an immunological parameter among T2DM patients with and without neuropathy, and to assess the role of IL-6 as biomarker for detection of DPN.

Methods

Study design and studies groups

This is a case-control study, involved randomly selected 140 T2DM patients attended to Al-Numan Teaching Hospital during the period from October 2023 to January 2024. Their age ranged from 25 to 80 years; 57 of them were males and 83 were females, The patients have been divided into two groups; those with diabetic neuropathy and diabetic patients without neuropathy.

Inclusion criteria

The inclusion criteria for patients with DPN is having T2 DM for more than 6 months with clinically approved diabetic neuropathy, while for control group is a Known case of T2 DM for more than 6 months without neuropathy.

Exclusion criteria

Patient with T1DM, those who had any of the acute complications of diabetes at the time of inclusion (hypoglycaemia, ketoacidosis etc.), and patients who have other neurological disease.

Laboratory data

Blood sampling was performed by venipuncture of the cubital vein, 5 ml per

sample. Samples were taken during hospitalization; the collected blood was immediately transformed in to jel plain tube and left to clot for 15 min in room temperature. Then, it was centrifuged from 2500 to 3000 rpm for 10 min period to isolate serum, then serum was put into 5 Eppendorf tubes and kept in deep freezer (-80°C) until enzyme-linked immunosorbent assay (ELISA) analysis for assessment of IL-6 and for glycated hemoglobin (HbA1c) was used as a measure of glycemic control.

Ethical consideration

The study was approved by the Medical Ethics Committee at College of Medicine, Al Iraqia University. With a full understanding of the study, each participant signed the informed consent form voluntarily.

Clinical evaluation of DPN

The neurological examination has been carried out with clinical evaluation. The assessment examined every abnormality seen in the foot's appearance, such as deformities, dry skin, calluses, infections, or fissures. It also assessed foot ulceration, the presence of ankle reflex, and the vibratory threshold using a tuning fork. All the examinations performed by expert physician.

Statistical analysis

Data were entered, checked as well as analyzed using computer software programs of statistical package of social science (SPSS) version 27 and STATISTICA version 9. Descriptive statistics of frequency distribution tables, number and percentage were used for qualitative data, whereas mean, standard deviation and range were used for quantitative data. Unpaired t-test, one way analysis of variance (ANOVA) test, and Chi-square test were used to identify the significant differences between study groups of cases and controls regarding different quantitative and categorial parameters respectively. A logistic regression model and receiver operating characteristic (ROC) curve were used to identify the optimal cut off value of immunological parameter as predictive noninvasive marker for development risk of neuropathy complication among diabetic patients. A P value of <0.05 was used for determining statistical significance throughout study.

Results

Baseline characteristics of study's sample

A total 140 of 1:1 ratio of collected cases and controls samples were investigated respectively following inclusion and exclusion criteria. The age of study samples was normally distributed and ranged from 25 to 80 years with a mean of 58.69±10.51 with the most of the sample at the age group of 47-57 years old (34.3%). The mean age of cases group was 60.86±9.64 years old mostly at the age group of 47-57 years old (38.6%), and that of controls was 56.53±10.96 years old mostly at the age group of 58-68 years old (35.7%) with significant mean differences among them (P = 0.014).

However, the entire study's sample were female predominant (59.3%) with female to male ratio 1.45:1, as well as females were dominant among cases (61.4% vs. 38.6%) and that of controls group (57.1 vs. 42.9%) respectively, but this difference was not statistically significant (P >0.05) which reflecting the matching purpose of samples collection (Table 1).

The mean value IL-6 was found to be significantly higher among cases group than that of controls group (117.68±27.38 vs. 57.3± 13.24) respectively (P <0.00) (Table 2).

IL-6 as predictive diagnostic marker for developing risk of neuropathy complication among diabetic patients

Among a 70-study cases sample, the optimal cutoff value of IL-6 for detect diabetic patients with high risk of neuropathy development was 72.46 pg/ml with sensitivity of 97.1%, and specificity of 87.1% and correctly predicted by the regression model of 92.1% with excellent

area under curve (AUC) of 0.984 (P <0.001) (Table 3 and Figure 1).

Table 1. Baseline characteristics of the two study groups

Characteristics	Cases (n=70)	Control (n=70)	Total (n=140)	P value
Age (years)				
Mean±SD	60.86±9.64	56.53±10.96	58.69±10.51	0.014[a]
Range (min-max)	39 (41-80)	48 (25-73)	55 (25-80)	
Age (In groups)				
≤35, n (%)	---	2 (2.9)	2 (1.4)	
36-46, n (%)	3 (4.3)	12 (17.1)	15 (10.7)	
47-57, n (%)	27 (38.6)	21 (30.0)	48 (34.3)	0.023 [b]
58-68, n (%)	21 (30.0)	25 (35.7)	46 (32.9)	
>68, n (%)	19 (27.1)	10 (14.3)	29 (20.7)	
Sex				
Female	43 (61.4)	40 (57.1)	83 (59.3)	0.606 [b]
Male	27 (38.6)	30 (42.9)	57 (40.7)	

a: Unpaired t-test, b: Pearson Chi-Square test

Table 2. Comparison of interleukin-6 between two study groups

Characteristics	Cases (n=70) Mean±SD	Control (n=70) Mean±SD	P value
IL-6 (pg/ml)	117.68±27.38	57.3±13.24	<0.001

Table 3. Predective value of IL-6 as a marker for developing neuropathy risk among diabetic cases sample

Paramter	Sensitvity	Specificity	Accuracy	Area Under the curve	P value
IL-6	97.1	87.1	92.1	0.984	<0.001

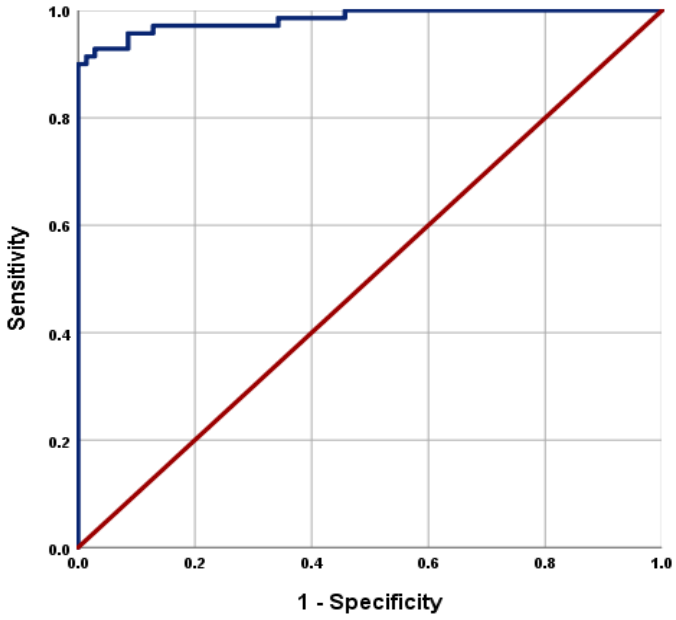

Figure 1. ROC Curve of neuropathy development risk predicted by immunological parameter of IL-6 among diabetic cases sample

Association between disease duration and IL-6 plasma level among cases group

Regarding association between the plasma level of IL-6 and disease duration among cases group, no significant differences were identified between the means of IL-6, among diabetic patients with neuropathy and its several duration periods of either less than 8, 8-15, 16-23, or more than 23 years respectively (P >0.05) (Table 4).

Comparison of HbA1c levels among study groups

The mean level of HbA1c was significantly higher among cases group than that of controls group (8.27±1.92 vs. 7.19±1.61%) respectively (P <0.001) (Figure 2).

Table 4. Comparison of IL-6 among cases group according to duration of disease

Disease duration (n=70)	IL-6 (Mean±SD)	P value [a]
<8 years (n=32)	123.17±28.55	0.144
8-15 years (n=17)	121.17±26.775	
16-23 years (n=16)	108.4±25.15	
24-31 years (n=5)	103.75±20.36	

a: One-Way-ANOVA Test

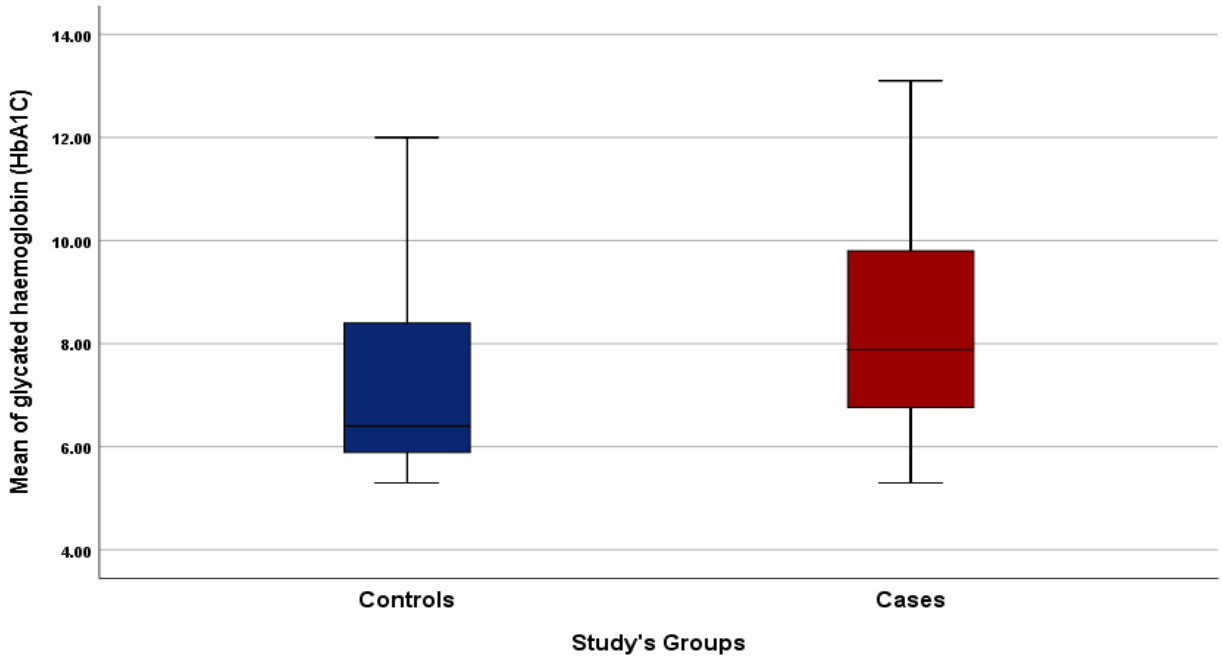

Figure 2. Comparison of HbA1C among study's groups (n=140)

Discussion

Diabetes mellitus has emerged as a significant worldwide public health concern. Gaining insight into the processes of diabetic neuronal damage is crucial in order to avoid the progression of DPN and to devise innovative therapeutic approaches [5].

In this study, IL-6 was found to be significantly higher among cases group than that of controls group. This result in agreement with study by Chanda et al. (2022) [12], which was a cross-sectional observational study to assess IL-6 level and its relation to painful PDN patients compared to patients with diabetes with painless neuropathy or normal control groups, the result revealed no significant difference in serum IL-6 levels between healthy controls and patients with T2DM without neuropathy. However, they noted a significant increase in serum IL-6 levels among patients with painful DPN compared to control groups. Interestingly, serum IL-6 levels were higher in patients with painful DPN than patients with painless DPN. Current result disagreed with Mussa et al. (2021) [13] who found that levels of IL-6 did not differ between patients without and with DNP prior to or after adjustment of sex, age, body mass index (BMI) and HbA1c.

In the case group of this study, the optimal cutoff value of IL-6 for detect diabetic patients with high risk of neuropathy development was 61.85 with excellent sensitivity, very good specificity, and correctly predicted by the regression model of 92.1%, this finding is consistent with the research conducted by Fadel et al. (2024) [14] on the early identification of DPN. The study included categorizing the sample into four groups based on the degree of neuropathy. The results revealed that IL-6 has a strong discriminate capacity between diabetic patients without neuropathy (group 1) and individuals with mild neuropathy (group 2). The AUC (area under the curve) for IL-6 was 0.896, whereas the best cutoff value for IL-6 was 34 pg/ml. This is substantially lower than the findings of the present research, the likely reason for this disparity is because the designated threshold is tailored specifically for mild neuropathy, as shown by the study's design.

According to the result of current study the sensitivity was high in prediction of DPN but relatively of low specificity about (65.7%), this

result is similar to Jin and Park study (2018) [15], they reported that IL-6 increased DPN development, and elevation of the levels of these systemic cytokines is not specific to DPN, but is observed in cardiovascular disease, obesity and other diabetic complications, such as diabetic nephropathy. Therefore, it should confirm the difference in systemic cytokine change between DPN and other low-grade inflammatory disease, they found elevated plasma level of IL-6 with high-sensitivity.

Regarding association between IL-6 level and disease duration among case group, no significant differences were identified between the means of interulkin-6 in diabetic patients with neuropathy (P >0.05). This finding is in line with recent study by Sher et al. (2023) [4], which designed to compare values of the cytokines and inflammatory markers between these groups and control group to assess the correlations between these markers with duration of DM, it found that IL-6 values did not differ significantly between the groups with different durations of diabetes.

Regarding HbA1c, it is an accurate and easy-to-administer test and can be an effective biomarker in establishing the diagnosis of diabetes, the mean level of HbA1C was significantly higher among cases group than that of controls group. Similarly to the study of Casadei et al. (2021) [16]; they found that there is a strong correlation between an increase in HbA1c variability and the development of DPN in people with diabetes. The findings of this study align with similar research conducted by Nozawa et al. (2022) [17], where they also reported comparable results, the average HbA1c values over the 3-year observation period were 7.2±1.0% in the group with DPN and 6.9±1.1% in the control group. There was a significant association between elevated average HbA1c values over a period of three years and the presence of DPN records.

In conclusions, the pro-inflammatory marker IL-6 level is highly and significantly elevated in patients with DPN, this proinflammatory cytokines could emerge as a useful indicator for predicting the incidence of DPN, IL-6 plays a crucial role in the development and progression of neuropathy, which should be confirmed in larger sample studies.

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Author contribution

All authors contributed in writing and reviewing this study and have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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