Original Article

Serum Calprotectin Level in Type 2 Diabetic Patients with and without Diabetic Peripheral Neuropathy: A Comparison Study

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Abstract

Background: Peripheral neuropathy is one of the microvascular complications that affects patients with diabetes mellitus and involves both sensory and motor nerves. The development and the progress of diabetic peripheral neuropathy (DPN) were ascribed to the inflammatory activity of the immune cells in the nerves. Calprotectin (CLP) is a heterodimer protein found in the membranes of monocytes and other inflammatory cells and the cytosol of neutrophils and released from them up to activation. **Aim of Study:** Evaluation of serum CLP level as a potential inflammatory biomarker for the occurrence of DPN in type 2 diabetic patients. **Patients and Methods:** one hundred and twenty-six patients diagnosed with type 2 diabetes mellitus were randomly selected from those who attended the National Diabetic Center between December 2022 and July 2023. Michigan Neuropathy Screening Instrument (MNSI) and nerve conduction study (NCS) were used for grouping the patients. Enzyme-linked immunosorbent assay technique has been used to measure serum CLP levels. **Results:** Serum levels of CLP showed no significant differences among patients with and those without diabetic peripheral neuropathy according to their NCS findings and the total scores for each and for both in combination according to the subgroups ($P > 0.05$). **Conclusions and Recommendations:** The serum level of CLP in type 2 diabetic patients was not affected by the occurrence of diabetic peripheral neuropathy. Further studies are required on newly diagnosed patients and a larger sample size.

Keywords: Calprotectin, diabetic peripheral neuropathy, type 2 diabetes mellitus

Introduction

Calprotectin (CLP) is a heterodimer protein consisting of two monomers (S100A8 and S100A9) in which each monomer consists of two Ca^{+2} binding sites in both N- and C-terminal EF hands that are connected by hinge region and it belongs to the S100 protein family and exists in the cytosol of neutrophils in addition to the membranes of monocytes and other inflammatory cells and releasing from them on activation or adhesion to the endothelium.[1-3] It has been found that CLP blood levels are elevated in several chronic low-grade inflammatory conditions including obesity, and insulin resistance due to its over-expression and this makes it an inflammatory marker.[4-6] Increased CLP levels in plasma and urine have been linked to type 2 diabetes mellitus (T2DM) and its consequences, including hypertension and renal failure.[7] CLP has different biological roles, intracellularly it can act as an indicator of differentiation for phagocytes while extracellularly it acts as a damage-associated molecular pattern molecule that interacts with different receptors.[8,9]

CLP is involved in the pathogenesis of micro- and macrovascular complications of diabetes mellitus (DM) through the activation of key signaling pathways through the activation of Toll-like receptor-4 (TLR4) and the receptors of advanced glycation end products (AGEs).[3,4] Thus, according to those mechanisms, it may be involved in the pathogenesis of diabetic peripheral neuropathy (DPN) that is associated with irreversible nerve injury.[10,11] It was suggested that AGEs played a significant part in the development of DPN through inflammatory cascade

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activation, the elevation of pro-inflammatory cytokines, and neuroimmune communication pathways which result in structural and functional nerve damage.[12,13] AGEs bind to advanced glycation end products receptors (RAGE) and activate the nuclear factor-kappa enhancer of B-cell (NF-κB) signaling pathway, which has been linked to the occurrence of diabetic neuropathy in mouse models through controlling immune and inflammatory responses, TLR4 binding cans also leads to activation of NF-κB leading to an increase in the variety of inflammatory mediators expression.[10] Following inflammatory stimulation, CLP secretion from granulocytes occurs, and after that, CLP acts as a cytokine-like protein by attaching to surface receptors (RAGE, TLR4, and CD33) and activating pathways involved in the escalating immune response.[3,14] Michigan Neuropathy Screening Instrument (MNSI) is a method used for screening the presence of DPN and it involves two sections: a self-assessment questionnaire of 15 (yes/no) equations in addition to a physical examination of the lower limbs using instruments like (10 g monofilament, tuning fork 128 [Hertz] Hz, and neurological hummer that aid in filling the form of physical examination).[15] The total score of the MNSI is obtained from the combination of the questionnaire and the physical examination scores, and only when the total score is above or equal to 9½, it is considered as possible diabetic peripheral neuropathy.[16] A nerve conduction study (NCS) is usually done as a gold standard to confirm the diagnosis of DPN since it is regarded as one of the gold standards that fits its diagnosis.[17] It involves the stimulation of motor nerves, sensory nerves, or both across the skin using an electrical current (a small pulse) and recording the generated electrical responses.[18] It is important to note that DPN cannot be initially diagnosed using a simple and accurate diagnostic procedure so this study aimed to estimate the serum levels of CLP in type 2 diabetic patients with DPN and compare it to those without DPN to determine whether serum CLP level as an inflammatory biomarker is affected by the occurrence of DPN, and thus, it could be used to indicates the occurrence of DPN.

Patients and Methods

One hundred and twenty-six patients diagnosed with T2DM who visited the National Diabetic Center of Mustansiriyah University between December 2022 and July 2023 who fitted the inclusion criteria and signed a written consent were enrolled in this cross-sectional study design. The patients were subjected to the MNSI and NCS at the NCS unit at Al-Yarmouk Teaching Hospital. DPN was either confirmed or eliminated in each patient based on (nerve conduction velocity and amplitude) of the sural, ulnar, median, and common peroneal nerves. The results were compared to the normal values utilized in the Al-Yarmouk Teaching Hospital's EMG/NCS unit. Based on the findings of both MNSI and NCS they were grouped into different groups based on MNSI score and NCS each one alone, the same patients were subgrouped using both scores and NCS at the same time, as shown in Figure 1. Waist circumferences and body mass index (BMI) were obtained. Three ml from the obtained fasting blood specimens were collected using a gel tube to obtain serum after clotting and centrifugation at 3000 rpm for 10 min for the analysis of glycemic profile using Glucose HK by Roche Diagnostics and lipid profile using TRIGL for triglyceride (TG), CHOL2 for cholesterol, and HDLC4 for high-density lipoprotein (HDL-c) kits made by Roche Diagnostics while serum CLP was estimated using a human CLP enzyme-linked immunosorbent assay kit (Catalog No: YLA2079HU) by Shanghai YL Biont, China. The remaining 2 ml were collected in EDTA tubes for assessing hemoglobin A1C (HbA1c) using the A1C-3 kit by Roche Diagnostics. The homeostasis model assessment of insulin resistance (HOMA-IR), very low-density lipoprotein (VLDL-c), low-density lipoprotein (LDL-c), and BMI were determined mathematically.

The exclusion criteria included the patients how had other types of diabetics (type 1 and gestation), other caused of neuropathy (renal and liver disease). malignancies and smoking. It is worth mentioning that the included patients were on treatment using oral medication and or insulin therapy

Ethical approval

The scientific committees of the local Health Care Department at Al-Karkh in Baghdad province approved this research according to the document with the number of 432 on December 21, 2022. The research's goals were explained for each patient to obtain their approval to involve in it. They were made aware of their freedom to leave the study whenever they wanted.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp) was used in the analysis of the data of this study. Independent Student's *t*-test and analysis of variance analysis were employed. The corrections were determined using Pearson's linear correlation coefficient. GraphPad Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) was used for the visualization of data beside the SPSS 26 version.

Results

As shown in Table 1, there were no significant differences ($P > 0.05$) in the mean level of serum CLP (mean ± standard deviation) between type 2 diabetic patients according to their MNSI total score: below 9.5 (37.94 ± 1.69) and above and equal to 9.5 (35.30 ± 2.17) and according to presence of DPN based on the finding of NCS: absence of DPN (36.91 ± 1.87) and presence of DPN (36.75 ± 1.87). According to Table 2 and Figure 2, the mean level of CLP also showed no significant differences among the four subcategories ($P > 0.05$). Among type 2 diabetic patients and within each subcategory, CLP exhibited no association with age, disease duration, BMI, glycemic profile, and lipid profile (triglyceride, cholesterol, HDL-c, LDL-c, and VLDL-c), as shown in Figure 3 and Table 3, respectively.

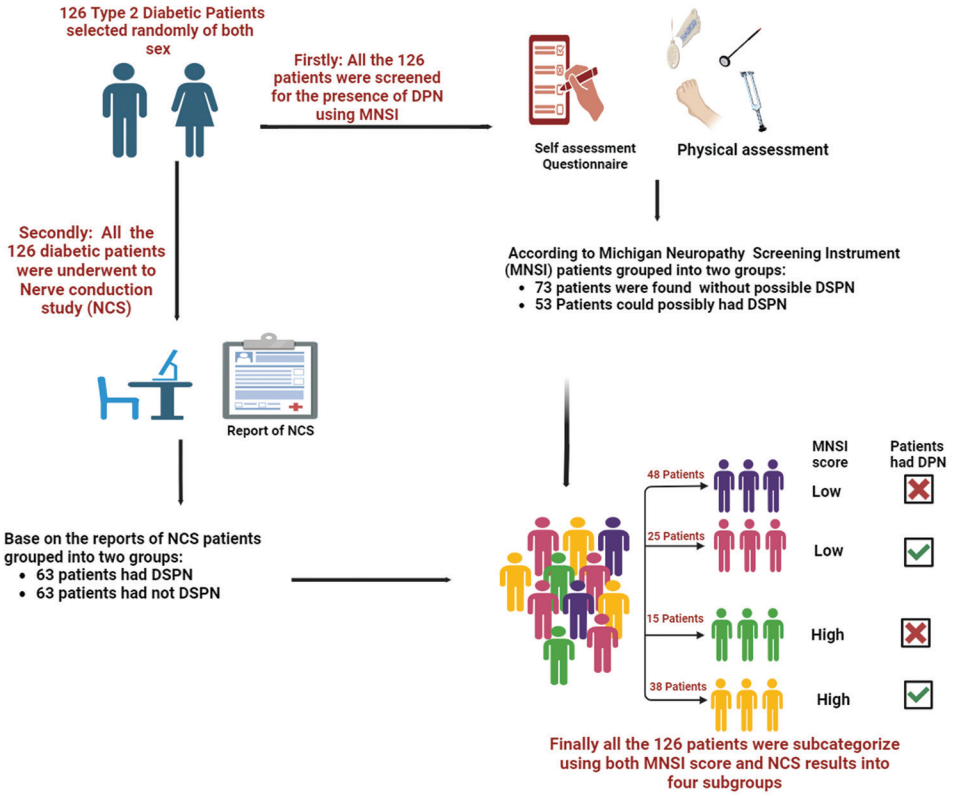

Figure 1: A diagram showing the study's conception and development

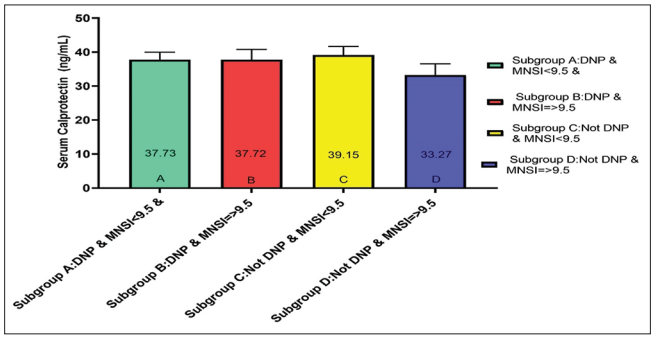

Figure 2: The mean and standard deviation of serum calprotectin by the subgroups

Table 1: Comparison of serum calprotectin among the studied group

Groups	*n*	Mean±SE	*P*
MNSI total score			
<9.5	73	37.94±1.69	0.327
≥9.5	53	35.30±2.17	
DPN			
No	63	36.91±1.87	0.952
Yes	63	36.75±1.87	

MNSI: Michigan Neuropathy Screening Instrument, DPN: Diabetic peripheral neuropathy, SE: Standard error

Table 2: Comparison of serum calprotectin among the studied subgroups

	DPN (+/−)	MNSI total score	*n*	Mean±SE	*P*
CLP (ng/mL)	Positive	<9.5	25	37.73±2.24	0.658
		≥9.5	38	37.72±3.06	
	Negative	<9.5	48	39.15±2.49	
		≥9.5	15	33.27±3.26	

CLP: Calprotectin, MNSI: Michigan Neuropathy Screening Instrument, DPN: Diabetic peripheral neuropathy, SE: Standard error

Discussion

The finding of this study shows no significant statistical differences in the mean of serum CLP between the diabetic patients with and without DPN ($P > 0.05$), as shown in Table 1. This finding was consistent with the finding of Ghaib *et al*.[19] which showed nonsignificant statistical differences in serum CLP levels between the patients with and without DPN ($P > 0.05$), while the differences were statistically significant when comparing the DPN patients to healthy individuals.

The findings of the current study were inconsistent with the findings of a previous study by Taur *et al*[10] which studied patients patients who showed the symptoms of DPN without being confirm and it was also inconsistent with study of El-Hafez *et al*[20] which studied patients who newly diagnosed with DPN, the both Taur et al[10] and El-Hafez[20] studies demonstrated that elevation of serum CLP could be implicated

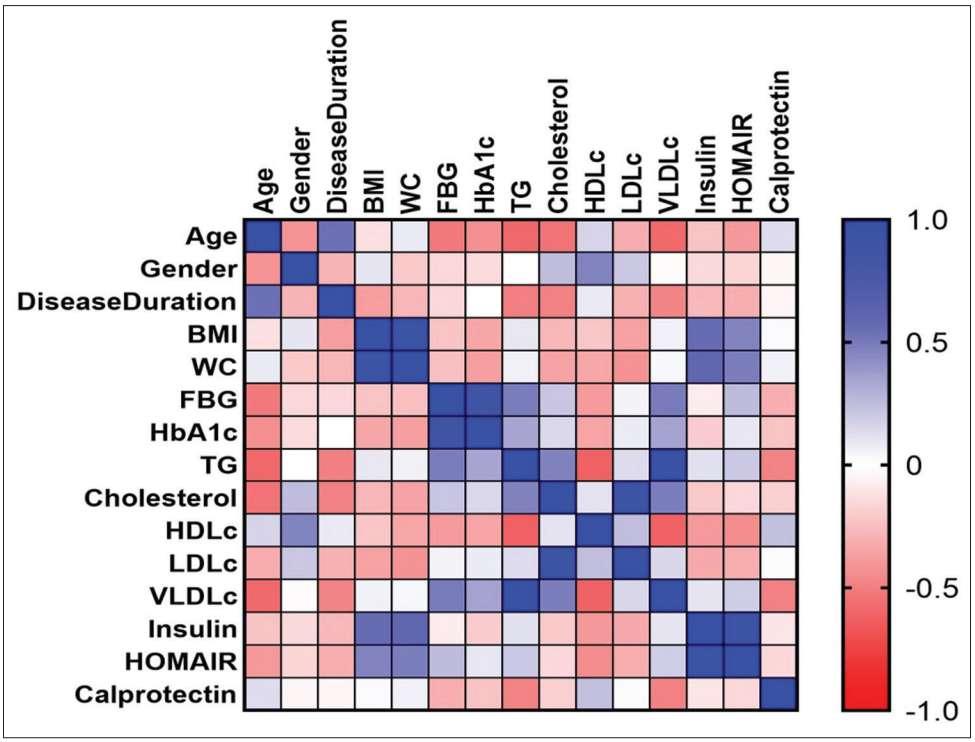

Figure 3: The correlation of serum calprotectin with the studied parameters among all type 2 diabetic patients

in the neuroinflammation pathogenic changes of DPN. The probable reason for the inconsistency of the findings of those studies with the current study could be due to the differences in sample size and the criteria for studied patients. In addition, the current study finding was inconsistent with the finding of Velayutham *et al*.[2] who demonstrated that serum CLP in patients with DPN was significantly elevated compared to patients without DPN; however, no significant differences were found among DPN subcategories in their study that include those of possible, probable, and confirmed and finally those with subclinical DPN ($P > 0.05$). One probable reason behind this inconsistency is that Velayutham *et al*. relayed the presence of the signs and symptoms of DPN or the finding of NCS in classifying the patients into patients with DPN and patients without DPN, while in this current study, the presence and absence of DPN in the patients were based on the finding of NCS alone. Moreover, in the study of Velayutham *et al*., the disease duration was significantly higher in those with DPN compared to those without DPN while in this current study, the disease duration was similar in both patients with and without DPN which could be an additional possible cause of inconsistency, additionally, the study of Velayutham *et al*. did not mention the gender of the studied patients. Moreover, the finding of the current study was inconsistent with the finding of Afify *et al*.[9] who found that the level of CLP was significantly higher in patients with DPN compared to those without DPN, it is also suggested that CLP could be a potential marker for the occurrence, severity, and development of DPN. However, it did not rely on NCS to diagnose the presence of DPN in newly diagnosed patients instead the diagnosis was made based on the symptoms of DPN. This could probably be the reason behind the inconsistency with the findings of this current study.

According to this current study finding, the mean serum level of CLP was not significantly changed in diabetic patients according to their MNSI total score, as indicated in Table 1. By comparing the mean of serum CLP levels among diabetic patients' subgroups to determine the value of serum CLP as an early indicator of DPN. This study indicated the absence of significant differences in the mean serum levels and CLP levels among them ($P > 0.05$), as shown in Table 2 and Figure 2.

Among diabetic patients, as shown in Figure 3, this current study showed no association between CLP and BMI among diabetic patients ($P > 0.05$); this finding was consistent with the findings of Mortensen *et al*.,[21] Tabur *et al*.,[10] and Velayutham *et al*.[2] Besides that, CLP shows no association with (age, disease duration, fasting blood glucose, insulin, HOMA-IR, and lipid profile) ($P > 0.05$). Moreover, no association has been found in this study between CLP and HbA1c ($P > 0.05$) which conflicts with the finding of Tabur *et al*.[10] who found a positive link between CLP and HbA1c among diabetic patients attributing that to the effect of glucose in the regulation of CLP during diabetic complication since HbA1c represented a marker of chronic hyperglycemia among early diagnosed patients this inconsistency is probably due to differences in sample size, patients' inclusion criteria and statistically using spearman correlation to assess the correlation.

In this study, serum CLP also showed no association with the other studied parameters according to patients' subgroups,

Table 3: The correlations of serum calprotectin with studied parameters according to patient's subgroups

	Subgroup A	Subgroup B	Subgroup C	Subgroup D
Age (year)				
R	0.040	−0.181	0.166	0.259
P	0.851	0.277	0.259	0.351
Disease duration (year)				
R	0.254	−0.004	−0.133	0.211
P	0.221	0.979	0.366	0.451
BMI (kg/m^2)				
R	0.040	0.225	−0.009	0.033
P	0.851	0.174	0.949	0.908
Waist circumference (cm)				
R	0.020	0.173	0.214	−0.021
P	0.926	0.300	0.144	0.942
FBG (mg/dL)				
R	−0.156	0.170	−0.066	−0.210
P	0.457	0.308	0.657	0.453
HbA1c%				
R	−0.209	0.239	−0.025	−0.111
P	0.316	0.149	0.867	0.693
Triglycerides (mg/dL)				
R	−0.327	−0.011	−0.108	−0.444
P	0.110	0.946	0.464	0.098
Cholesterol (mg/dL)				
R	−0.072	0.069	0.094	−0.314
P	0.733	0.680	0.527	0.254
HDL-c (mg/dL)				
R	0.170	0.293	−0.075	0.124
P	0.416	0.074	0.612	0.659
LDL-c (mg/dL)				
R	0.078	0.027	0.217	−0.070
P	0.712	0.871	0.139	0.804
VLDL-c (mg/dL)				
R	−0.339	−0.027	−0.129	−0.424
P	0.097	0.872	0.382	0.116
Insulin (μIU/mL)				
R	0.149	−0.032	0.030	0.118
P	0.478	0.849	0.837	0.676
HOMA-IR				
R	0.077	0.031	0.052	−0.029
P	0.716	0.855	0.723	0.918
CLP (ng/mL)				
R	1	1	1	1
P				

Subgroup A: Patients with DPN and MNSI total score of <9.5, Subgroup B: Patients with DPN and MNSI total score of ≥9.5, Subgroup C: patients with no DPN and MNSI total score of <9.5, Subgroup D: Patients with no DPN and MNSI total score of ≥9.5. MNSI: Michigan Neuropathy Screening Instrument, DPN: Diabetic peripheral neuropathy, HOMA-IR: Homeostasis model assessment of insulin resistance, HDL-c: High-density lipoprotein-cholesterol, LDL-c: Low-density lipoprotein-cholesterol, VLDL-c: Very LDL-c, FBG: Fasting blood glucose, BMI: Body mass index, HbA1c: Glycated hemoglobin, CLP: Calprotectin

as shown in Table 3. It is worth mentioning that no previous studies subgrouped the patients according to their MNSI and NCS findings at the same time. The onset of T2DM appears to be significantly affected by low-grade chronic systemic inflammation and the elevation in the level of inflammatory biomarkers is a predictor of the occurrence of T2DM.[22] In addition, low-grade chronic systemic inflammation has also been implicated in the development of peripheral neuropathy in T2DM.[23] However, obesity, control of glucose blood level, and medical therapy were also linked to low-grade chronic systemic inflammation.[24] Furthermore, in DPN it had been found that genetic variation could be crucial in its development.[25] Thus, the inflammatory process could not be the only responsible for the development of peripheral neuropathy in diabetic patients,

this could explain why serum CLP levels did not change in diabetic patients whether they suffered from DPN or not.

Conclusion

serum CLP in type 2 diabetic patients was not affected by the presence of DPN. Thus, based on the results of this study it cannot be used as an indicator for early detection of DPN.

Recommendations

Further studies are required on newly diagnosed patients and a larger sample size.

Limitations

Since its cross-sectional study design, it could not find the causal association between the studied variables. Other limitations involved the sample size and the majority of studied patients in this study had long disease duration.

The strength of this study

Two different methods have been used to confirm the occurrence of DPN in the patients whose serum CLP levels were estimated in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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