European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 08, Issue 02, 2021

Detection Of Serum Calprotectin Level Changes For Early Diagnosis Of Diabetic Peripheral Neuropathy In Type 2 Diabetic Patients

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ABSTRACT

Background: Calprotectin was identified as an endogenous Toll-liken receptor 4 (TLR4) activator and as a receptor for progressive glycation end products (RAGE),
Elevated Calprotectin plasma levels have been recorded in various chronic inflammatory conditions, expecting microvascular alterations in patients with type 2 diabetes (T2DM).
Objective: To make an up to date review of Serum level of Calprotectin as a predictor for early diagnosis of peripheral neuropathy in type 2 diabetes patients.
Patients and methods: A total number of 90 subjects were included in the study after fulfillment of the inclusion and exclusion criteria. Calprotectin was measured for all the 90 subjects.
Results: Serum calprotectin levels were significantly higher in group III "diabetics with neuropathy" compared to group II "diabetics without neuropathy "and group I "healthy controls"
Conclusion: In Type 2 diabetes patients with peripheral neuropathy, elevated levels of calprotectin have been identified.
Key words: Calprotectin, diabetes mellitus, neuroinflammation, peripheral neuropathy.

1. INTRODUCTION

The prevalence of Diabetes mellitus remains one of the most daunting health problems in the 21st century and in developing countries is higher. Diabetes mellitus is a chronic endocrine condition that may arise if the pancreas doesn't secrete enough insulin, or when the human body can't effectively use insulin. In the case of developing countries the growth rate is estimated to be 170 % compared to 42% in developed countries [(2)].

Diabetic neuropathy with a prevalence of 50-60% is the most common micro vascular complication of type 2 diabetes. A decreased nerve function and nerve perfusion with chronic nerve damage can result from neuropathy. The development of a foot-ulceration risk is increased by diabetic peripheral neuropathy, which can lead to lower limb amputations.

Peripheral diabetic neuropathy has important morbidity and mortality contributions in diabetic patients [3] [4].

Although hyperglycemia was forecast to be an important factor in diabetic neuropathy development, the related mechanisms were not fully clarified. There are growing opinions that inflammatory processes may play a role in diabetic neuropathy pathogenesis. Patients with type 2 diabetes experienced increased immune mediator levels with peripheral neuropathy in previous studies [5] [6].

Calprotectin, also called MRP8/14, is an inflammatory myeloid-related protein complex consisting of two intracellular calcium-binding proteins S100A8 (MRP8) and S100A9 (MRP14), primarily expressed in activated human neutrophils and macrophages. Inflammatory myeloid-relevant protein complex the phagocyte's stress response actively distinguishes calprotectin. [7], and was found to be associated with inflammation more than 20 years ago [8].

Calprotectin was identified as an endogenous activator of Toll-like receptor 4 (TLR4) and as receptor for advanced glycation end products (RAGE) [9]. It is assumed that calprotectin acts as both a phagocytic intracellular differentiation marker and an extracellular protein complex (a molecular pattern (DAMP) molecule associated with damage) [10]. In a number of chronic inflammatory conditions including rheumatoid arthritis, rejection of allograft, inflammatory bowell disease and lung cancer, elevated plasma levels have been reported for Calprotectin. [11]. In patients with type 2 diabetes (T2DM), high levels of calprotectin have been reported to predict microvascular alterations. [12].

2. PATIENTS AND METHODS

A total number of 90 subjects were included in the study after fulfillment of the inclusion and exclusion criteria, this study was conducted from March 2019 through December 2019 in Endocrinology clinic of Zagazig university hospital and Endocrinology clinic of Shbeen El Kom Teaching hospital and followed by Case- control study, group I: 15 healthy control subject, group II: 15 diabetic patients without peripheral neuropathy, group III: Group III: 60 diabetic patients with early peripheral neuropathy. Calprotectin was measured for all the 90 subjects, blood samples were collected, centrifuged and stored at −20°C until Calprotectin levels were measured. C reactive protein (CRP), Random blood glucose (RBG), HbA1c, High density lipoprotein- cholesterol (HDL C), Low density lipoprotein cholesterol (LDL C), Total cholesterol, Triglyceride (TG) also were measured.

Our Inclusion criteria were: Gender made no bias; both male and female patients aged between 20- 75 years with type 2 diabetes patients with and without peripheral neuropathy are included.

Our exclusion criteria were: Patients with Infectious diseases, inflammatory diseases, liver failure, malignancies, neurodegenerative diseases, renal failure, Cerebrovascular diseases, B12 vitamin deficiency, medical history of serious trauma to the limbs, use of neurotoxic medication, excessive alcohol consumption and smokers were all excluded from both study and control group.

3. METHODS:

This study was approved by the local ethical committee of Zagazig university hospitals. Written knowledgeable consent was taken from patients or their first-degree relatives. On admission: all patients in this Case- cohort study were subjected to the following: Clinical assessment: including detailed history taking, full examination (general and neurological), and laboratory investigations including Calprotectin: Blood samples were collected, centrifuged and stored at −20°C until Calprotectin levels were measured, Complete

blood count, HbA1c, C-reactive protein (CRP), Random blood glucose (RBG), liver functions, kidney functions, Albumin creatinine ratio in urine, eGFR and lipid profile.

The serum concentration of calprotectin was expressed in ng/ mL. The calibration curve was organized ranging from 1–30 ng/ mL.

4. ETHICAL APPROVAL:

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national). Institutional Review Board (IRB) of the Faculty of Medicine, Zagazig University approved the study protocol. An informed consent was obtained from all participants or their first-degree relatives and they were told about the aim of the study, and were informed that the data would be used for scientific purposes only.

5. STATISTICAL ANALYSIS

All data were collected, tabulated and statistically analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium).Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) and Fisher exact was used to calculate difference between qualitative variables as indicated. Quantitative data were expressed as mean ± SD (Standard deviation) for parametric and median and range for non-parametric data. Independent T test and Mann Whitney test were used to calculate difference between quantitative variables in two groups for parametric and non-parametric variables respectively. One way ANOVA test was used to compare between more than two dependent groups of normally distributed variables. Pearson's and Spearman's correlation coefficient were used for correlating normal and non-parametric variables respectively.

6. RESULTS

Demographic data and laboratory studies of the three studied groups: Regarding mean values ± & standard deviation (±SD) of age, sex, BMI, systolic & diastolic Bp showed: that difference between the three groups regarding age & diastolic BP was non-significant. Difference between the three groups regarding BMI was highly significant. Difference between the three groups regarding systolic BP was significant**.**

Diabetes parameters of the three studied groups: A comparison between data of the three studied groups regarding mean values ± & standard deviation (±SD) of RBS, HbA1c and DM duration was highly significant.

Renal parameters of the three studied groups. Difference between the three studied groups regarding serum creatinine, urea, ACR and eGFR was non-significant.

Lipid profile of the three studied groups. Lipid profile data of the three studied groups regarding mean values ± & standard deviation (±SD) of TC, TG, LDL and HDL was highly significant. Difference between the three groups regarding HDL was significant. Difference between the three studied groups regarding CRP mean values ± & standard deviation (±SD was highly significant (**Table 1).**

Table (1): Demographic data and laboratory studies of the three studied groups

		ntrol =15)	M =15)	PN =60)	χ^2	
e (years) ean ± SD		.2 ± 9.24	.8 ± 13.61	.53 ± 10.52	06	14
x	ale	(66.7%)	53.3%)	(36.7%)	04	49
	male	33.3%)	46.7%)	(63.3%)		
MI (kg/m^2) ean ± SD		.41 ± 1.35	.88 ± 1.49	.11 ± 1.62	.44	.001**
stolic blood pressure ean ± SD		1.33 ±19.13	4.64 ±13.02	3.75 ±15.04	.65	48*
astolic blood pressure ean ± SD		.67 ± 13.69	.07 ± 9.13	.77 ± 6.47	41	297
S (mg/dL) ean ± SD		2.13 ± 25.82	3.57 ± 27.82	7.04 ± 58.23	.88	.001**
A1c (%) ean ± SD		.9 ± 0.533	8 ± 0.307	9 ± 0.783	.56	.001**
M duration (years) ean ± SD		-	± 3.28	.87 ± 5.31	08	.001**
Cr (mg/dL) ean ± SD		10 ± 0.116	13 ± 0.119	67 ± 0.149	89	44
ea (mg/dL) ean ± SD		.23 ± 8.29	.17 ± 9.67	.44 ± 7.39	77	37
CR (mg/g) ean ± SD		.83 ± 18.49	.1 ± 91.49	3.87 ± 93.3	94	73
FR (mg/24h) ean ± SD		.19 ± 14.42	.91 ± 10.89	.22 ± 13.22	01	.20
(mg/dL) ean ± SD		4.53 ± 13.31	5.6 ± 17.31	1.2 ± 32.15	.561	.001**
(mg/dL) ean ± SD		4.67 ± 17.53	0.93 ± 37.38	2.33 ± 33.48	.156	.001**
L (mg/dL) ean ± SD		.73 ± 9.2	.33 ± 12.07	3.37 ± 21.05	89	,001**
L (mg/dL) ean ± SD		.86 ± 8.23	.07 ± 9.09	.54 ± 14.05	13	07*
= P < 0.001 Highly significant *= P ≤ 0.05 Significant P > 0.05 Non-significant						
S. Cr: Serum Creatinine ACR: Albumin/creatinine ratio			*TC: Total cholesterol* *TG: Total triglycerides* *LDL: Low-density lipoprotein*			

eGFR: estimated glomerular filtration rate	*HDL: High-density lipoprotein*

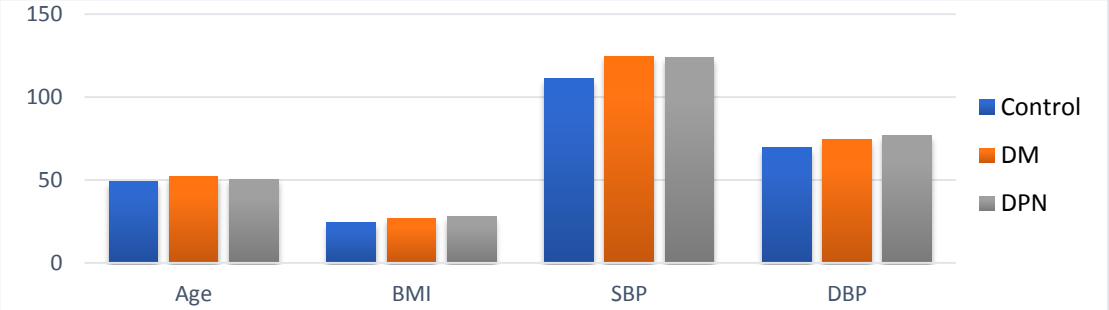


Figure (1): Patients characteristics between three studied groups

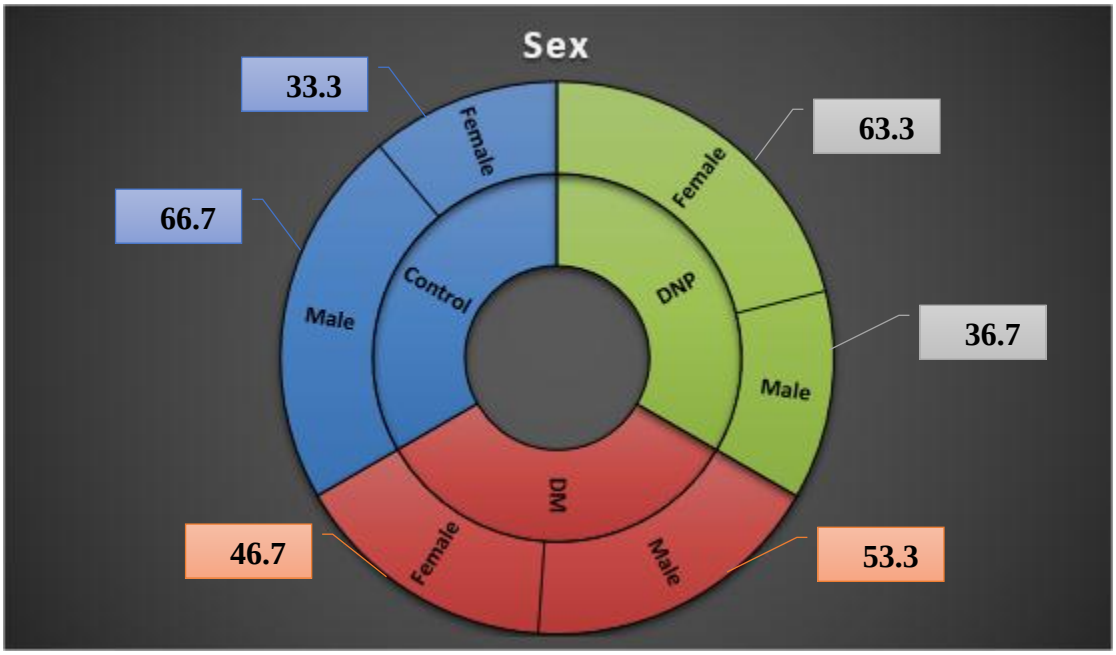


Figure (2): Sex distribution among the three studied groups.

LSD regarding CRP, RBS: between control group and diabetic group with neuropathy was highly significant, and between diabetic group without neuropathy and diabetic group with neuropathy was highly significant**. LSD regarding HBA1C**: between control group and diabetic group with and without neuropathy was significant, and between diabetic group with and without neuropathy was significant. **LSD regarding TC**: between control group and diabetic group without neuropathy was significant, and between control group and diabetic group with neuropathy was highly significant. **LSD regarding TG**: between control group and diabetic group without neuropathy was significant, and between control group and diabetic group with neuropathy was highly significant. **That LSD regarding LDL**: between control group and diabetic group with neuropathy was highly significant, and between diabetic group without neuropathy and diabetic group with neuropathy was significant. **That LSD regarding HDL**: between control group and diabetic group with neuropathy was significant, and between diabetic group without neuropathy and diabetic group with neuropathy was significant **(Table 2)**.

Table (2). Post hoc (LSD) test, to indicate the difference in the three studied groups according to different parameters levels of significant difference

Variable	up	up	Mean Difference -J)	S.E.	g.	95% Confidence Interval	
						Lower Bound	Upper Bound
HB	ntrol		.89333	0.63248	163	0.3732	.1598
			0.03333	0.54774	952	1.1302	.0635
			0.92667	0.54774	096	2.0235	.1702
CRP	ntrol		6.88000	5.57703	222	18.0478	.2878
			33.43333**	4.82985	**0.001**	43.1049	23.7617
			26.55333**	4.82985	**0.001**	36.2249	16.8817
RBS	ntrol		**104.90667****	15.96568	**0.001**	136.8774	72.9359
			81.43333**	13.82669	**0.001**	109.1208	53.7459
			3.47333	13.82669	095	4.2141	1.1608
HBA1C	ntrol		**0.50667***	0.22699	**030**	0.9612	0.0521
			4.11333*	0.19658	**007**	0.5070	.2803
			.39333*	0.19658	**05**	0.0003	.7870
TC	ntrol		**21.07333***	9.25810	**027**	39.6124	2.5343
			36.67333**	8.01775	**0.001**	52.7286	20.6181
			15.60000	8.01775	057	31.6553	.4553
TG	ntrol		**26.267***	9.656	**009**	45.60	6.93
			37.667**	8.362	**0.001**	54.41	20.92
			11.400	8.362	178	28.14	.34
LDL	ntrol		10.600	6.132	089	22.88	.68
			21.633**	5.311	**0.001**	32.27	11.00
			11.033*	5.311	**042**	21.67	0.40
HDL	ntrol		3.210	4.341	463	11.91	.49
			11.679*	3.823	**003**	19.34	4.01
			8.469*	3.737	**027**	15.96	0.98
Urea	ntrol		5.20667	3.22274	112	11.6601	.2468
			3.94000	2.79098	163	9.5288	.6488
			.26667	2.79098	652	4.3222	.8555
eGFR	ntrol		.69000	4.86880	730	8.0596	11.4396
			5.27867	4.21651	142	14.7221	.1647
			7.96867	4.21651	064	16.4121	.4747
Creatinine	ntrol		0.05333	0.04598	251	0.1454	.0387
			.00300	0.03982	940	0.0767	.0827
			.05633	0.03982	163	0.0234	.1361
ACR	ntrol		45.60000	40.56854	266	126.8371	5.6371
			3.76667	35.13338	697	56.5867	4.1201
			9.36667	35.13338	097	10.9867	29.7201

****= P < 0.001 Highly significant *= P ≤ 0.05 Significant P > 0.05 Non-significant**

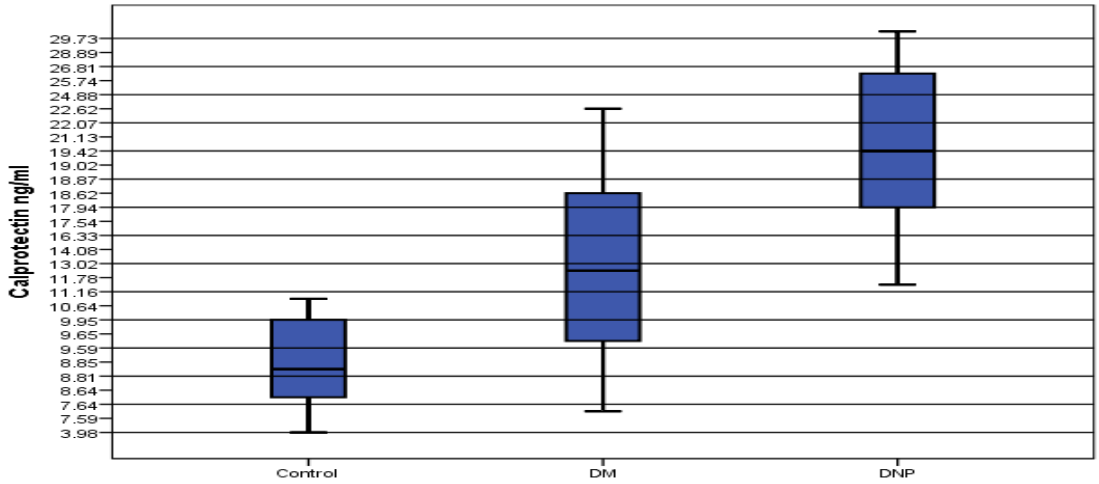
Table 3: Calprotectin of the three studied groups

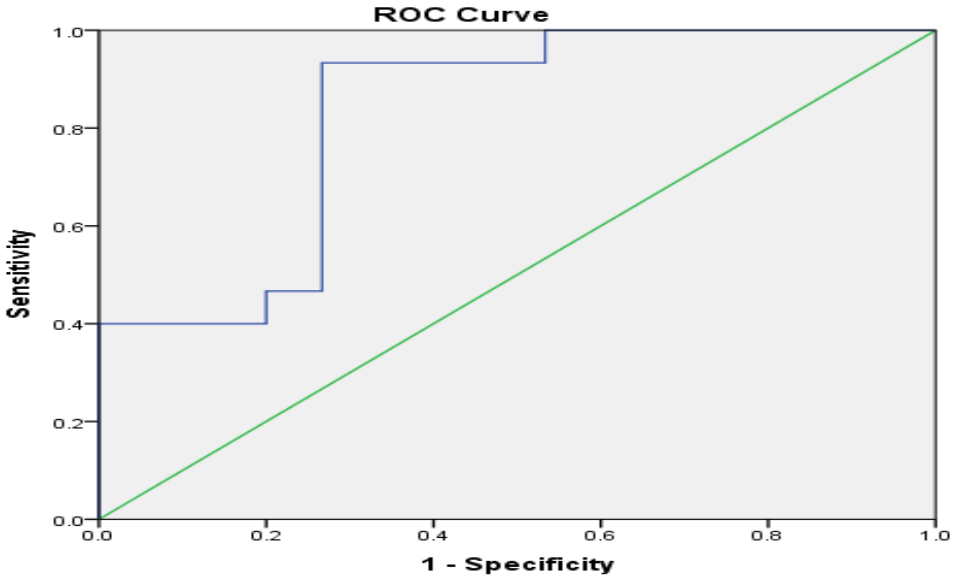
	ntrol *=15)*	***M*** *=15)*	***PN*** *=60)*		
lprotectin *(ng/ mL)* *ean ± SD*	4 ± 1.91	.71 ± 5.38	.57 ± 5.43	**.419**	**.001****
= P < 0.001 Highly significant		***= P ≤ 0.05 Significant**		**P > 0.05 Non-significant**	

Difference between the three studied groups regarding Calprotectin mean values ±&standard deviation (±SD) (P value < 0.001) was highly significant **(Table 3)**.

Figure (3): Calprotectin levels between three studied groups.

Figure (4): ROC of calprotectin as a marker of neuropathy in type 2 diabetic patients





7. DISCUSSION

Calprotectin is a protein of S100. It has two proteins (S100A8 and S100A9), a high calcium affinity and a strong proinflammatory signalling impact. Calprotectin complex is suggested to be a biomarker for inflammation and to control disease activity. In chronic inflammatory illnesses such as inflammatory bowel disorder, atherosclerosis, and rejection of allograft, the calprotectin levels are high. The level of calprotectin is high. Unlike the link entre high calprotectin and insulin resistance and low inflammation in type 2 dabetesi, there has been an association between high calprotectin and glucose metabolism in few studies to date. Calprotectin may therefore be used as an inflammatory marker and plays a function in diabetic peripheral neuropathy pathogenesis [13].

We aimed at detecting Serum level of Calprotectin as a marker for early diagnosis of peripheral neuropathy in type 2 diabetes patients by measuring Calprotectin serum level, Nerve conduction velocity studies (NCVS), Modified neuropathy disability score (MNDS) and Monofilament test. To achieve that we compared the absolute levels of calprotectin in between the three groups of patients.

In this study, we found no statistical differences between the three groups in age. That assures that no age related variation can affect the results. The distribution of gender in the studied groups showed no statistical difference, which prevents to get biased data due to difference in hormonal profile, variable risks, or physical activity in our study.

In our study, Patients of group I (normal healthy people) showed lower systolic blood pressure than other groups (diabetics), and that means that Hypertension in addition to Atherosclerosis and Insulin resistance may contribute in the pathogenesis of peripheral neuropathy in type 2 diabetes patients. ***Sowers., et al*** reported that more than half of diabetic patients presented with coexisting hypertension and that hypertension is potent risk factor to both micro and macro vascular diseases in diabetic patients [14- 15].

In our study, we concluded that diabetic group with peripheral neuropathy results of Calprotectin levels was higher than diabetic group without neuropathy and healthy controls. ***Tabur., 2015,*** supporting our study, reported also that calprotectin level in patients with diabetic peripheral neuropathy is higher than that in diabetic patients without neuropathy, and higher than that in healthy people [16].

In our study, we concluded that calprotectin is an inflammatory mediator that increased in inflamed tissues and probably has a role the neuroinflammatory conditions as it directly proportionate with CRP in patients with DPN. Like to our study, positive correlation between Calprotectin and CRP described also by other studies [17-18].

In our study, we found a positive correlation between calprotectin and HbA1c in patients with DPN. This point suggests that levels of glucose or glycation end products may affect metabolism of high calprotectin levels in diabetics**.** Agreed with our study **Tabur., 2015** also reported the same positive correlation between calprotectin and HbA1c " the marker of long-term elevation of blood sugar"[16].

In our study, BMI was found to be significantly higher in both diabetic groups "with and without neuropathy" compared to healthy group, while BMI was higher in diabetic neuropathy patients compared to diabetic patients without neuropathy. The high BMI in diabetic with and without neuropathy also explained by ***(Avila., et al 2004)*** that BMI increased in diabetic patients with and without neuropathy than normal healthy people [19].

Regarding lipid profile: TG, TC, HDL, LDL levels. Our study found their levels significantly high in both diabetic groups with and without neuropathy compared to healthy group; however they were higher in diabetic neuropathy patients compared to diabetic

patients without neuropathy, this may explain atherosclerotic changes as a risk factor of diabetes alone or if also associated with neuropathy. ***Peng., et al 2011*** concluded also that type 2 diabetic patients with atherosclerotic disease have increased serum level of calprotectin [(17)].

8. CONCLUSION

Increased serum calprotectin levels are correlated with diabetic neuropathy.

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