Original Article

Association of serum calprotectin with peripheral neuropathy in patients with type 2 diabetes mellitus

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Abstract

Introduction: Peripheral neuropathy is a common microvascular complication in patients with type 2 diabetes mellitus with a prevalence of around 50%. **Objectives:** This prospective observational cross-sectional study was done to assess serum calprotectin levels among diabetic patients with peripheral neuropathy as compared to those without neuropathy. **Methods:** This cross-sectional study was conducted in 126 diabetic patients attending the out-patient department of JIPMER Hospital, Pondicherry from July 2017 to January 2019. The subjects were divided into two groups (with and without peripheral neuropathy) and underwent nerve conduction study of both the lower limbs. Blood samples were collected and stored at −80°C for estimation of serum calprotectin. Serum calprotectin levels were compared between diabetic patients with and without peripheral neuropathy. **Results:** Serum calprotectin levels were increased in patients with diabetic peripheral neuropathy (DPN) as compared to those without DPN. However, there was no significant difference in the mean value of serum calprotectin among the various sub-groups of DPN. **Conclusion:** Serum calprotectin, an inflammatory biomarker is elevated in patients with diabetic peripheral neuropathy as compared to those without neuropathy.

Keywords: Calprotectin, diabetic peripheral neuropathy, nerve conduction study

Introduction

Diabetes mellitus can cause microvascular complications like nephropathy, retinopathy, and neuropathy. Clinical manifestations of diabetic peripheral neuropathy vary widely from being asymptomatic to painful neuropathic symptoms, loss of sensation, and diabetic foot ulcers.[1] Diabetic neuropathy is a common clinical condition that is observed by primary care physicians in the out-patient department. Its early diagnosis can help in reducing the morbidity among patients. Diabetic neuropathy is primarily diagnosed on the basis of neurologic symptoms and signs along with a nerve conduction study (NCS).

Many pathophysiologic mechanisms have been proposed for the development of diabetic peripheral neuropathy (DPN). A pro-inflammatory state in diabetic patients causes microvascular inflammation and damage to peripheral nerves. This is one of the widely accepted mechanisms for peripheral neuropathy in diabetic patients.[2] The diagnosis of DPN is based on the clinical symptoms and signs and neuroelectrophysiologic tests, like nerve conduction study.

Neuro-inflammatory serum biomarkers may have a role in the early diagnosis of DPN. Calprotectin is a heterodimer belonging

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to the S100 protein family. It is found in the cytoplasm of neutrophils and monocyte membranes and gets released in acute and chronic inflammatory states. Calprotectin plays a major role in DPN by causing activation of toll-like receptor 4 (TLR4) and advanced glycation end products (AGEs) receptors. Activation of these receptors leads to diabetic microvascular and macrovascular complications.

This study was done to assess diabetic peripheral neuropathy and its clinical and nerve conduction characteristics in a south Indian population and to find out an association between DPN and serum calprotectin.

Aims and Objectives

To assess serum calprotectin levels among diabetic patients with peripheral neuropathy and compare the same in patients without neuropathy.

Material and Methods

This cross-sectional comparative study was done in the Medicine OPD and Diabetes Clinic of JIPMER Hospital in Pondicherry, India, from July 2017 to January 2019 after obtaining clearance from the Institutional Ethics Committee (IEC Ref. No. JIP/IEC/2017/0315 and date of approval was 21/10/2017).

Assuming an expected difference in the mean serum calprotectin values between patients with and without diabetic peripheral neuropathy as 0.9 with a standard deviation of 1.8 at 5% level of significance and 80% power, the sample size was calculated as 126 with 63 subjects in each group. The convenience sampling technique was used to include diabetic patients attending the Medicine OPD and Diabetes Clinic of the hospital. Patients with known thyroid disease, history of alcohol consumption, chronic kidney disease (serum creatinine >1.5 mg/dL in males and >1.4 mg/dL in females), infected foot ulcers, and autoimmune diseases like rheumatoid arthritis were excluded from the study.

Data regarding age, gender and duration of diabetes were collected from subjects in both the study groups. Study subjects in both the groups underwent nerve conduction study (NCS) of both lower limbs which assessed the tibial, peroneal and sural nerves. Details of distal motor latency, compound muscle action potential (CMAP) and Sensory nerve action potential (SNAP) amplitudes, and conduction velocity were obtained from the NCS. The study participants were divided into 2 groups on the basis of history, clinical examination and NCS findings. Group 1 consisted of type 2 diabetic patients with peripheral neuropathy while group 2 included diabetic patients without peripheral neuropathy.

The study subjects in group 1 were having clinical symptoms or signs of peripheral neuropathy or a positive NCS. Symptoms of peripheral neuropathy reported by the subjects were tingling, burning sensation or pain in the lower limb. Signs of peripheral neuropathy that were noted on clinical examination were loss of touch and pain sensation or loss of vibration sense and proprioception in the lower limbs along with absent ankle reflex. Subjects without any clinical features of neuropathy and a normal NCS were included in group 2. Subjects in group 1 were further sub-divided into 4 groups as given below-

1. Possible neuropathy-Subjects with either clinical symptoms or signs of DPN
2. Probable neuropathy-Subjects with clinical symptoms and signs of DPN
3. Confirmed neuropathy-Subjects with clinical symptoms or signs of DPN and abnormal NCS
4. Sub-clinical neuropathy-Subjects with no clinical symptoms or signs of DPN, but having abnormal NCS.

Biochemical analysis

After obtaining informed written consent, 5 ml of venous blood sample was collected from all the study subjects in both the groups. 2 ml blood was collected in EDTA tube for estimation of glycosylated hemoglobin (HbA1c), while the remaining 3 ml sample was centrifuged and serum was stored at −80°C for estimation of serum calprotectin. Serum calprotectin level was estimated using a two-step capture ELISA kit.

Statistical analysis

Statistical analysis was done using SPSS software version 19.0. Data from the study was subjected to normality testing using the Kolmogorov-Smirnov test. Categorical data are expressed as frequencies or percentages. Comparison of categorical variables were done by using Chi-square or Fischer exact test. Continuous variables like duration of diabetes and serum calprotectin levels are expressed as mean with standard deviation.

Independent student's t-test or Mann Whitney U test was used for comparison of serum calprotectin levels among study subjects with and without peripheral neuropathy. ANOVA with post-hoc analysis was used for comparison of calprotectin levels among the four sub-groups of DPN.

Utility of serum calprotectin as a diagnostic biomarker for DPN was assessed by receiver operating characteristic (ROC) curve along with calculation of area under curve (AUC). ROC curve was used for the calculation of sensitivity and specificity of serum calprotectin along with the cut-off level in subjects with DPN. Correlation analysis was used to explore the linear relationship between the duration of diabetes and serum calprotectin in the study subjects.

All statistical analysis was done at 5% level of significance and *P* value below 0.05 was considered as significant.

Results

A total of 126 subjects were recruited in this study and were equally divided into two groups (with and without diabetic

peripheral neuropathy). The demographic characteristics among patients with and without peripheral neuropathy are given in Table 1. Subjects with DPN were found to have a longer duration of diabetes and had poor glycemic control as evidenced by their glycosylated hemoglobin (HbA1c) values.

The study group with DPN was sub-divided into 4 groups as possible, probable, confirmed, and sub-clinical neuropathy. This study found that 68% of the study subjects had confirmed neuropathy, 18% had probable neuropathy, 8% had possible neuropathy and 6% had sub-clinical neuropathy.

The analysis of clinical features and NCS findings among patients with DPN are given in Table 2. NCS was found to be positive in 74.6% of patients with DPN. Among subjects with peripheral neuropathy who had positive NCS, the most common pattern of nerve conduction abnormality was sensory-motor axonal neuropathy followed by sensory axonal neuropathy. The pattern of abnormality found on NCS is shown in Table 3.

Serum calprotectin was assessed and compared between the two study groups. The difference of serum calprotectin among both the groups was found to be statistically significant as shown in Table 4.

On analyzing the levels of serum calprotectin by ROC curve, it was found that the area under the curve was 0.92. Sensitivity of serum calprotectin was 88.9% and specificity was 85.7% at a cut-off level of 1722.83 ng/ml [Figure 1].

Serum calprotectin was compared among the various sub-groups of DPN in order to estimate the utility of calprotectin as an early biomarker. It was found that there was no significant difference in the mean value of calprotectin among the various sub-groups as shown in Table 5.

Serum calprotectin levels were compared with BMI of the study subjects to assess its correlation with obesity. It was found that there was no significant difference in mean calprotectin values among the various categories of BMI, as shown in Table 6.

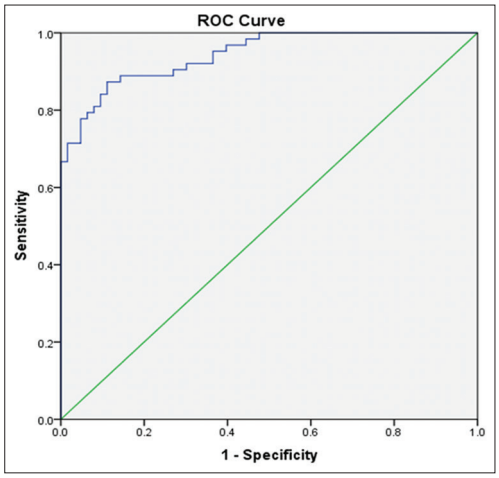

Figure 1: ROC curve of serum calprotectin

Duration of diabetes and serum calprotectin levels among the study subjects were analyzed with the help of scatter diagram. It was found that calprotectin levels showed an increasing trend as the duration of diabetes increased in the subjects [Figure 2].

Summary

1. Clinical symptoms of neuropathy were present in 87.3% of subjects with DPN
2. NCS was found to be positive in 74.6% of subjects with clinical features of diabetic peripheral neuropathy
3. Sensory motor axonal neuropathy was the most common pattern on NCS (38.09%) in the study subjects
4. Serum calprotectin was significantly elevated among subjects with DPN. However, serum calprotectin levels did not show any significant correlation among the various sub-groups of DPN

Discussion

Peripheral neuropathy is a common microvascular complication of diabetes mellitus that causes significant morbidity among

Table 1: Comparison of demographic characteristics among subjects with and without DPN

Parameter	DPN present (*n*=63)	DPN absent (*n*=63)	*P*
Age (mean years)	50.3	51.16	-
Duration of diabetes (mean years)	11.63±4.67	5.98±2.96	0.000 (<0.05)
BMI (mean kg/m^2)	24.85±3.22	25.22±3.66	0.58
HbA1C (%)			
<6.5	3	35	0.000
6.5-7.5	10	11	(<0.05)
>7.5	50	17	

Table 2: Clinical features and NCS findings in subjects with DPN

Clinical features and NCS findings	Subjects with DPN (*n*=63)
Subjects with symptoms of DPN	55 (87.3%)
Subjects with signs of DPN	36 (57.1%)
Subjects with positive NCS	47 (74.6%)

Table 3: NCS findings among study subjects

NCS finding	Subjects with DPN (*n*=63)
Sensory motor axonal neuropathy	24 (38.09%)
Sensory axonal neuropathy	15 (23.8%)
Motor axonal neuropathy	5 (7.93%)
Demyelinating neuropathy	3 (4.76%)
Normal study	16 (25.36%)

Table 4: Serum calprotectin levels among study subjects

Study groups	*n*	Serum calprotectin (ng/mL)	
		Mean±S.D	*P*
Subjects with DPN	63	2487.31±708.37	0.000
Subjects without DPN	63	989.61±615.58	

Table 5: Serum calprotectin levels among subgroups of DPN

Sub-group of DPN	*n*	Mean±S.D. (ng/ml)	*P*
Possible	11	2337.44±456.33	0.348
Probable	5	2688.32±429.91	
Definitive	43	2614.09±559.22	
Sub-clinical	4	2947.53±672.17	

Table 6: Correlation of BMI and serum calprotectin levels

BMI category	*n*	Mean±S.D. (ng/ml)	*P*
Underweight (<18.5 kg/m^2)	2	2701.25±168.38	0.317
Normal (18.5-22.9 kg/m^2)	28	1675.46±977.10	
Overweight (23-24.9 kg/m^2)	41	1886.24±1015.58	
Obese (>25 kg/m^2)	55	1625.35±1007.99	

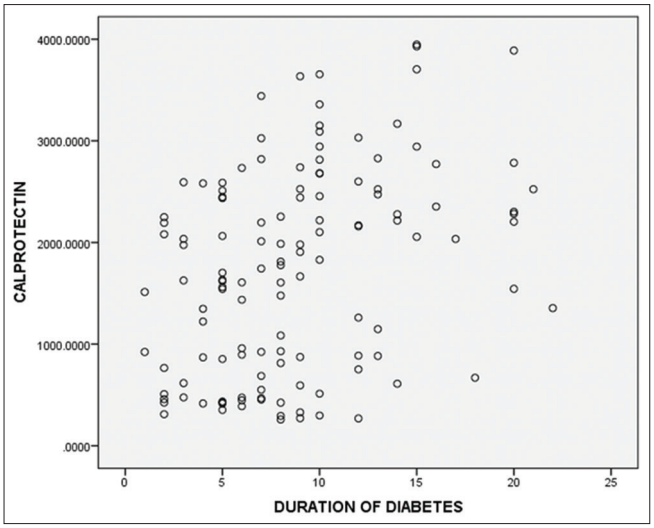

Figure 2: Correlation analysis of serum calprotectin level and duration of diabetes. Pearson's correlation r=0.365

diabetic patients. It can remain asymptomatic for a long time and can be responsible for non-healing foot ulcers. Diabetic peripheral neuropathy is diagnosed by history and clinical examination along with nerve conduction study. Since neuro-inflammation plays an important role in the pathogenesis of DPN, several inflammatory markers are being studied to search for a potential biomarker that can help in the diagnosis of DPN.

Our study found that patients with DPN had diabetes for a longer duration as compared to those without neuropathy. A study done by Oguejiofor OC, *et al.*, from the United Kingdom showed that the prevalence of DPN was highest among patients with longer duration of diabetes (>15 years).[3] In another study done from south India, it was found that patients with peripheral neuropathy had long-standing diabetes.[4]

Poor glycemic control as demonstrated by elevated values of glycosylated hemoglobin has a major role in the development of peripheral neuropathy in diabetic patients. This study showed that 79.4% of patients with DPN had poor glycemic control, while only 27% of patients without DPN had poor control. These findings correlate with a study done by Nisar *et al.*, which found that 88.6% of patients with DPN had glycosylated hemoglobin value above 6.5%, while the corresponding figure was 11.4% for patients without DPN.[5]

Nerve conduction study (NCS) is an important diagnostic tool in the diagnosis of peripheral neuropathy in diabetic patients. This study found that 6% of the subjects had sub-clinical peripheral neuropathy which was identified by NCS. In a study done from Haryana in 2018, NCS was helpful in diagnosing sub-clinical neuropathy in neurologically asymptomatic diabetic subjects. Parameters like F wave latency, low amplitude (CMAP or SNAP), and slow nerve conduction velocity were important findings in the diagnosis of sub-clinical neuropathy based on NCS.[6] In another study done by de Souza RJ, *et al.*, NCS showed diffuse changes in diabetic patients without DPN. Electrophysiologic parameters showed a correlation with neuropathic pain, clinical signs, and glycosylated hemoglobin.[7]

The most common pattern of NCS abnormality among the subjects of this study was sensory-motor axonal neuropathy. This was followed by sensory and motor axonal neuropathy in the descending order. Similar findings were obtained from a large retrospective analysis done in Boston, Massachusetts from 2205 to 2007. A total of 63,779 electro-diagnostic tests done on diabetic patients were analyzed and it was found that sensory-motor axonal neuropathy was found in 52.6% of the study subjects while 14.8% and 13.3% of subjects in this study had sensory axonal and motor axonal neuropathy respectively based on NCS parameters.[8]

Though demyelination is not commonly found in patients with diabetes, 4.76% of subjects had demyelinating pattern on NCS in this study. This was in contrast to a study done by Dunnigan SK, *et al.*, where it was seen that 46% of diabetic subjects had axonal type of nerve injury, 32% had conduction slowing and 22% had a combined pattern of nerve injury on NCS.[9]

This study found that the mean serum calprotectin level was significantly elevated in diabetic subjects with peripheral neuropathy compared to those without neuropathy. In a study done by Tabur S, *et al.*, it was found that diabetic patients irrespective of their neuropathy status had increased levels of serum calprotectin and hs-CRP (high sensitivity c-reactive protein) as compared to healthy subjects. Also, it was found that serum calprotectin levels were higher in the subgroup of diabetic patients with peripheral neuropathy than in those without neuropathy.[10]

This study also found that there was no correlation between the various categories of BMI and serum calprotectin levels in diabetic subjects. A study done by Mortensen OH, *et al.*, showed that serum calprotectin was significantly elevated in obese as compared to non-obese non-diabetic subjects. However, this difference was not found between obese and non-obese patients with type 2 diabetes.[11] In another study done by Pedersen L, *et al.*, it was

found that serum calprotectin value was higher among diabetic subjects than in non-diabetic subjects. On further analysis among the diabetic subjects, it was found to be higher among diabetic subjects with metabolic syndrome compared to those without.[12]

Clinical symptoms and nerve conduction study are useful in the diagnosis of peripheral neuropathy in patients with type 2 diabetes mellitus. Serum calprotectin is an inflammatory biomarker that is elevated in diabetic peripheral neuropathy may add to the diagnostic utility in these patients.

Limitations of the study

1. Majority of the study subjects in the group with peripheral neuropathy had a longer duration of diabetes which could have influenced the association of serum calprotectin with peripheral neuropathy.
2. There were fewer number of study subjects in the groups with probable and sub-clinical neuropathy. Hence, the role of serum calprotectin as an early biomarker of diabetic peripheral neuropathy cannot be deduced from this study, as it requires a longer period of follow-up and a larger study population.
3. There was no control group of non-diabetic subjects in the study to compare serum calprotectin values between diabetic patients and non-diabetic subjects.
4. Patients with nephropathy were excluded from the study based on serum creatinine value instead of microalbuminuria which is the gold standard for the diagnosis of nephropathy.

Conclusion

The level of serum calprotectin which is an inflammatory biomarker was higher among patients with diabetic peripheral neuropathy as compared to those without neuropathy. Longer duration of diabetes and poor glycemic control were important risk factors for the development of peripheral neuropathy in patients with type 2 diabetes mellitus.

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Conflicts of interest

There are no conflicts of interest.

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