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Serum neuron-specific enolase as a biomarker in diagnosing diabetic peripheral neuropathy: A cross-sectional study

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ABSTRACT

Background: Diabetic peripheral neuropathy remains asymptomatic until the late stages. Hence, a need exists for a reliable and sensitive biomarker for the early diagnosis of diabetic peripheral neuropathy. **Aims and Objectives:** This study aimed to evaluate serum neuron-specific enolase (NSE) reliability as a biomarker in diagnosing diabetic peripheral neuropathy. **Materials and Methods:** In the department of biochemistry and internal medicine, a cross-sectional study was conducted at Government Villupuram Medical College from May 2018 to January 2020. One hundred diabetes patients with peripheral neuropathy were compared with 100 patients without peripheral neuropathy. A diabetic neuropathy symptom (DNS) questionnaire and diabetic neuropathy examination (DNE) score were used for diagnosis. In addition, vibration position sense and NSE values were compared between the two groups. For statistical analysis, R studio and coGuide were used. **Results:** Comparisons between groups were based on age, sex, and other baseline parameters. Statistically, significance was observed between the two groups in outcome parameters such as VPB (right), VPB (left), and DNS ($P<0.05$). There was a weak positive correlation between NSE and DNS (r_s value: 0.514, $P<0.001$). On the other hand, there was a strong positive correlation between NSE and DNE (r_s value: 0.937, $P<0.001$). The NSE of 6.50 and above had a sensitivity of 53% (95%CI 42.76–63.06%) and specificity of 57% (95% CI 46.71–66.86%). **Conclusion:** NSE acts as a biomarker for diabetic peripheral neuropathy. Therefore, NSE can be used for the early diagnosis of diabetic peripheral neuropathy, thereby preventing a severe form of the disease.

Key words: Biomarkers; Diabetic neuropathy; Neuron-specific enolase; Peripheral nervous system diseases; Type 2 diabetes mellitus

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INTRODUCTION

Diabetic peripheral neuropathy, a common and frequently occurring early complication in diabetics, is diagnosed only after the disease has progressed in severity.[1] Therefore, the early diagnosis of peripheral neuropathy and timely treatment is needed. The manifestations of peripheral neuropathy are pain, hyperesthesia, and gradual loss of sensation over the peripheries caused due to loss of nerve fiber. Due to reduced senses, trauma to the foot and irritations are not sensed by the patients, often leading to foot ulcers and gangrene, which frequently lead to amputation of the limb.[2] This can cause a potential decrease in quality of life and impose physical and mental trauma. It has been documented that a strict glycemic group 2 can delay the onset and progression of neuropathy.[3,4] However, it is impossible to prevent the condition completely. Only symptomatic management of the situation is being done due to the lack of a specific cure. It has been reported that 50% of diabetic peripheral neuropathy is asymptomatic.[5] Therefore, with clinical symptoms and neurological findings for the early detection of diabetic neuropathy, more sensitive and convenient biomarkers that detect the severity or stage progression are required. One such

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biomarker is neuron-specific enolase (NSE). NSE is a glycolytic enzyme that is located precisely in nerve tissue. NSE is widely used as a tumor marker for diseases such as small cell lung cancer and neuroblastoma.[6]

Li et al., investigated the relationship between blood NSE levels and diabetic neuropathy, because the synthesis of these enzymes may be altered during the degeneration and regeneration of peripheral nerves due to the oxidative stress caused by chronic hyperglycemia.[7] In addition, it has recently been published that the value decreased not only with the onset and progression but also with the improvement in neuropathy in response to treatment.[8] The previous studies have shown that the NSE levels in people with diabetes with neuropathy had mean NSE levels of 10.8±2.8 μg/L.[7] This result suggests that NSE may be a marker for predicting therapeutic effects for the early detection of diabetic neuropathy. Still, there is a lack of studies from India. Hence, this study was planned to evaluate the role of NSE in diabetic peripheral neuropathy patients and to compare the NSE levels with patients without peripheral neuropathy.

Aims and objectives

This study aimed to evaluate serum neuron-specific enolase (NSE) reliability as a biomarker in diagnosing diabetic peripheral neuropathy.

MATERIALS AND METHODS

In the biochemistry and internal medicine department, a cross-sectional study was done at Government Villupuram Medical College. The study duration was from May 2018 to January 2020. Two hundred patients with diabetes mellitus were recruited for the study. The Human Ethical Committee of the institution approved the proposal, and all patients signed informed written consents. After recruitment, clinical examinations were done. Peripheral neuropathy assessment was done using a diabetic neuropathy symptom (DNS) questionnaire and a diabetic neuropathy examination (DNE) score.[9] Those diagnosed with diabetic peripheral neuropathy were categorized as Group 1, and those without features of neuropathy were classified as Group 2. For both the groups, clinical examination, vibration position sense (VPS), and biochemical tests such as total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were measured. 5 ml venous sample was taken from the study population, which was used for biochemical analysis. Serum Total Cholesterol, Triacylglycerides, and HDL-C were estimated by the enzymatic method in the fully automated analyzer . LDL-C calculated by fried Ewald equation. From the sample obtained, HDL-C was estimated using direct HDL method using an automated analyzer.

NSE biomarker estimation was done for both groups. A pre-structured questionnaire with details of the study variables was used for data collection. NSE measured by enzyme chemiluminescence immunoassay closed system to pack by ROCHE diagnostics e411 instrument. VPS was measured using Bioesthsiometer (VPB).

Sample size calculation

The sample size was calculated assuming the expected proportion/prevalence of diabetic neuropathy as 37.67% and expected sensitivity of NSE as 66.3% as per the study by JIANBO LI[7] confidence levels of 95% and 7% precision for sensitivity which were considered. The following formulae proposed by Buderer[10] were used to separately calculate the sample size based on sensitivity and specificity. The sample size for expected sensitivity

$$n \geq \frac{Z^2_{1-\alpha/2} Sens(1-Sens)}{d^2 \times Prev}$$

Z: Z value for the given alpha (Type 1 error)=1.96, Sens: Expected sensitivity=0.663, Prev: Prevalence of the outcome in the population=0.3767, D: Marginal error rate=0.07. Based on sensitivity, as per the calculation mentioned above, the required sample size was 176 subjects. We decided to include 505 in the final study. To account for the non-participation rate of about 14%, another 24 subjects were added. Hence, the final required sample size was 200 subjects at the time of recruitment.

Statistical methods

The study group (Groups 1 and 2) was considered primary explanatory variables. VPB, DNS, DNE, and NSE were the primary outcome variables. The quantitative descriptive was summarized by mean and standard deviation. For categorical descriptive, frequency and proportions were used. The non-parametric statistical tests such as the Mann–Whitney U-test were used to find the difference between the case group and Group 2 group concerning biochemical indicators. Spearman's rank correlation was used to find the linear relationship between the continuous data variables statistical analysis were used by R studio and coGuide version [1.0] soft ware.[11]

RESULTS

A total of 200 subjects were included in the final analysis.

The difference in age between the study groups is found to be insignificant, with P=0.438. The difference in gender between the study groups is found to be insignificant with a P=0.087, with the majority of 63% female participants were having diabetic peripheral neuropathy. There was a statistically significant difference between the two groups

Table 1: Comparison of various parameters between the study groups (N=200)

Parameters	Study group		P value
	Group 1 (n=100)	Group 2 (n=100)	
Age (years)	52.87±10.39	54.14±12.63	0.438*
Gender			
Male	63 (63%)	51 (51%)	0.087†
Female	37 (37%)	49 (49%)	
Duration of diabetes (years)	9.07±5.54	9.00±5.52	0.929*
Investigation			
Sugar (mg/dL)	237 (164.25,295.25)	115 (102,153.25)	<0.001‡
Urea (mg/dL)	24 (21,31)	32 (27,38)	<0.001‡
Creatinine (mg/dL)	1 (1,1)	1 (1,1)	0.670‡
Cholesterol (mg/dL)	164.5 (134.5,194)	206 (193.25,219.75)	<0.001‡
Triglycerides (mg/dL)	189.5 (134.75,240.5)	162 (148,176)	0.001‡
VLDL (mg/dL)	42.32±21.37	33.04±5.33	<0.001*
HDL (mg/dL)	51 (44,63.5)	48 (43,54.75)	0.839‡
Outcome variables			
VPB (right)	1 (1,2)	0 (0,0)	<0.001‡
VPB (left)	1 (1,2)	0 (0,0)	<0.001‡
DNS	2 (0,2)	0 (0,0)	<0.001‡
DNE	11 (2,14)	3 (2,13.75)	0.714‡
NSE (ng/ml)	12 (1.25,24)	5 (2,16.75)	0.579‡

*Independent sample t-test, †Chi-square test, ‡Mann–Whitney U-test

in investigation parameters such as sugar, urea, cholesterol, TGL, and VLDL (P<0.05). No statistically significant difference between the groups in investigation parameters such as creatinine and HDL (P>0.05) was observed. The statistically significant difference between two groups in outcome parameters such as VPB (right), VPB (left), and DNS (P<0.05) was observed. No statistically significant difference between groups in DNE and NSE (P>0.05) was observed (Table 1).

There was a weak positive correlation between NSE and DNS (r_s value: 0.514, P<0.001) (Figure 1). There was a strong positive correlation between NSE and DNE (r_s value: 0.937, P<0.001) (Figure 2). The difference in the proportion of NSE between the study group was statistically not significant (P=0.157) (Table 2).

The NSE of 6.50 and above had sensitivity of 53% (95% CI 42.76–63.06%) in predicting cases, specificity was 57% (95% CI 46.71–66.86%), false-positive rate was 43% (95% CI 33.14–53.29%), false-negative rate was 47% (95% CI 36.94–57.24%), positive predictive value was 55.21% (95% CI 44.71–65.37%), negative predictive value was 54.81% (95% CI 47.82–62.02%), and the total diagnostic accuracy was 55.0% (95% CI 47.82–62.02%) (Table 3).

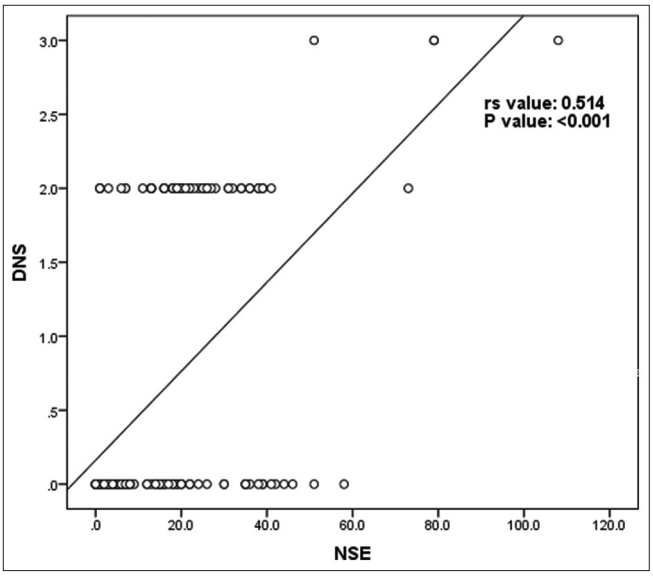

Figure 1: Scatter plot diagram of Correlation between the levels of NSE with DNS score in diabetic neuropathy (N=200). NSE: Neuron-specific enclose, DNE: diabetic neuropathy examination

DISCUSSION

With the findings of this study, it was evident that serum NSE acts as a potential biomarker of diabetic peripheral neuropathy. The study results show that NSE was significantly elevated in those with neuropathy. The elevated NSE levels were closely related to diabetic neuropathy, and this relationship was independent of coverable.

The previous study also had similar results to Li et al.,[7] where the authors observed that 214 diabetic subjects with neuropathy had elevated NSE levels. High blood glucose is attributed to elevated NSE levels in the body.[12]

Usually, the NSE levels are deficient in the serum (≤15.2 ng/mL).[13] However, due to nerve cell damage caused by ischemia, hypoxia, or hypoperfusion, NSE is released from the cells in large quantities and then

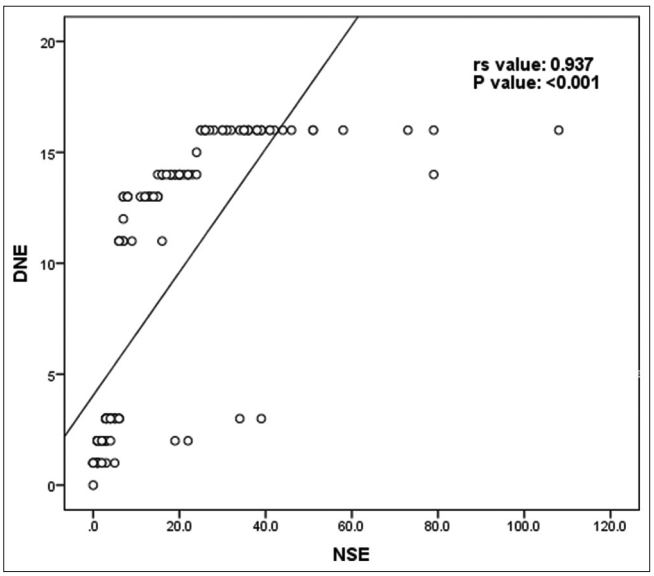

Figure 2: Scatter plot diagram of correlation between the levels of NSE with DNE score in diabetic neuropathy (N=200). NSE: Neuron-specific enclose, DNE: diabetic neuropathy examination

Table 2: Comparison of group with NSE (N=200)

NSE	Group		Chi square	P value
	Group 1 (N=100)	Group 2 (N=100)		
High (>=6.50)	53 (53%)	43 (43%)	2.003	0.157
Low (<6.50)	47 (47%)	57 (57%)		

Table 3: Predictive validity of NSE in predicting diabetic peripheral neuropathy (N=200)

Parameter	Value	95% CI	
		Lower	Upper
Sensitivity	53.00%	42.76%	63.06%
Specificity	57.00%	46.71%	66.86%
False-positive rate	43.00%	33.14%	53.29%
False-negative rate	47.00%	36.94%	57.24%
Positive predictive value	55.21%	44.71%	65.37%
Negative predictive value	54.81%	44.74%	64.59%
Diagnostic accuracy	55.00%	47.82%	62.02%
Positive likelihood ratio	1.23	0.83	1.613
Negative likelihood ratio	0.82	0.07	1.079

enters the circulation through the injured blood–brain barrier, leading to elevated NSE levels in the blood.[14] In addition, due to pathological changes, like demyelination and remyelination, associated with DPNP, NSE gets released from affected neurons and affected Schwann cells forming myelin; as suggested in one report, NSE was detected in oligodendrocytes as well as in neurons.[15] With high level of evidence, the systematic reviews and meta-analysis are available only for the role of NSE in traumatic brain injuries, cardiac arrest, and various cancers.[16-19] The level of evidence available for role of NSE in diabetics is low. In this study, there was no difference between the NSE levels between gender. However, there was a strong positive correlation between NSE and DNE. The present study findings add evidence to the previous studies and promote clinical examination scores as reliable diagnostic indicators of diabetic peripheral neuropathy.[20] NSE levels reliably predicted peripheral neuropathy in patients with early features. It also had corroboration with DNS and DNE scores, further strengthening the validity of NSE as a biomarker for neuropathy due to diabetes. Studies worldwide have highlighted the importance of NSE in identifying diabetic peripheral neuropathy; however, the test is yet to gain the confidence of diabetology practitioners.[21,22]

Limitations of the study

The limitations of this study are that the causal association cannot be established due to the study's cross-sectional nature. Multicenter studies that include more subcategories of diabetic neuropathy are recommended in the future.

CONCLUSION

The study findings prove that serum NSE levels are elevated in diabetes due to diabetic peripheral neuropathy. Therefore, this may act as a potential blood biomarker for diabetic neuropathy. Hence, early diagnosis of the condition is possible by measuring the NSE levels among diabetic patients.

Recommendations

The serum NSE levels can be utilized as a potential blood markers in diabetic patients to detect diabetic neuropathy.

ETHICAL CONSIDERATION

The Human Ethical Committee of the institution approved by Government Villupuram Medical College, Villupuram.

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Author's Contribution:
SG- has conceptualized the study and played a primary role in compiling, analyzing, and interpreting the data. All the drafts were prepared and reviewed, and the final draft was approved by Sangeetha Kandasamy, Babu Krishnan, Shivkumar Gopalakrishnan, and Harissh Ganesan; **SK, BK, SG, and HG-** have contributed to the proposal's fine tuning and data collection and entry. Reviewed the results and contributed to the preparation and review of drafts. All the authors have read and approved the final version of the manuscript. All the authors take complete responsibility for the content of the manuscript.

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