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Potential predictive biomarker for diabetic peripheral neuropathy: serum neuron-specific enolase

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ABSTRACT

The early stages of diabetic peripheral neuropathy (DPN) are symptomless. A reliable dependable and sensitive biomarker is needed for the purpose of early identification of diabetic peripheral neuropathy. The main objective of the study was to evaluate the accuracy of serum neuron-specific enolase (NSE) as a biomarker for early identification of diabetic peripheral neuropathy. Patient samples were collected from the National Diabetes Center, Mustansiriyah University; a case control study was done from April 2022 to November 2022, in Baghdad, Iraq. One hundred sixty individuals between 30 to 60 years-old were included. Participants were divided into three groups: group one included 40 type 2 diabetic patients with peripheral neuropathy, group two consisted of 40 type 2 diabetic patients without peripheral neuropathy and group three included 80 apparently in good health as the control. Toronto Clinical Neuropathy Scoring System (TCSS) was used for clinical evaluation of peripheral neuropathy. Glycated hemoglobin (HbA1c) was measured by the CLOVER A1c system. In addition, serum NSE levels were measured by Enzyme Linked Immunosorbent Assay (ELISA) technique.

Age, sex, and other standard variables were used as a basis for comparisons between groups. Statistically, diabetic patients with peripheral neuropathy demonstrated higher level of NSE (28.42±6.93 ng/ml) than did either diabetic patients without peripheral neuropathy (21.07±2.0 ng/ml) or controls (12.54±2.34 ng/ml) with a high degree of significance ($p < 0.001$). In the context of Discrimination between DPN patients and diabetic patients without neuropathy, the area under curve for neuron-specific enolase was 0.812, 95% confidence interval [CI] = 0.716-0.909, $p < 0.001$. Cut-off value of serum neuron-specific enolase was 22.53 ng/ml, sensitivity and specificity were 70% and 77%, respectively. In the context of discrimination between DPN and controls, the area under curve for neuron-specific enolase was 1.00, 95% confidence interval was 1.0-1.0, $p < 0.001$. At a cut-off value of serum neuron-specific enolase = 18.3 ng/ml, both the sensitivity and specificity were 100%. Neuron-specific enolase could potentially be used as a biomarker to detect early diabetic peripheral neuropathy and prevent it from developing to an advanced state.

INTRODUCTION

Persistent hyperglycemia in diabetic patients is known to increase the chance of developing diabetic microvascular complications, such as diabetic neuropathy. Diabetic neuropathy (DN) is a typical microvascular problem associated with diabetes [1]. It is significant to note that DN is the primary risk factor for the emergence of diabetic foot ulcers, which may result in lower-extremity amputations [2]. Diabetes-related neuropathic pain affects between 3 and 13 million people globally [3]. In earlier epidemiological studies of neuropathy, obesity was determined to be the second-largest essential metabolic illness component after diabetes [4-6]. According to a recently released study, people with diabetic neuropathy had greater body mass indexes and waist circumferences than did people without the condition [7]. As concluded in Patel GR and colleagues' 2022

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study [8], Diabetic Peripheral Neuropathy is significantly and independently correlated with hyperglycemia, advanced age, longer duration of diabetes, and poor glycemic control. Numerous reports report that 50% of all diabetic peripheral neuropathies may be asymptomatic [9]. Thus, new sensitive and appropriate biomarkers that identify the severity or stage advancement are needed in conjunction with clinical symptoms and neurological observations for the early diagnosis of diabetic peripheral neuropathy. Neuron-specific enolase (NSE) is one of these biomarkers. NSE is a glycolytic enzyme and one of the isoenzymes of enolase. This isoenzyme has a biological half- life of roughly 24 hours. NSE is present in the cytoplasm of neurons and neuroendocrine cells. The mechanisms that lead to the demise of these cells cause an increase in the concentration of NSE in bodily fluids [10,11]. NSE is regarded as a helpful biomarker in evaluating functional damage to neurons [12-14]. However, there are limited studies from Iraq. Therefore, it was intended for this study to assess NSE's role in diabetic individuals who have peripheral neuropathy and to measure neuron-specific enolase levels in those who have the ailment compared to those who do not.

AIM

The main objective of the study was to evaluate the sensitivity of serum neuron-specific enolase (NSE) as a biomarker for the early identification of diabetic peripheral neuropathy.

MATERIALS AND METHODS

A case control study was done at the National Diabetes Center, Mustansiriyah University, Baghdad, Iraq. The study duration was from April 2022 to November 2022, in Baghdad, Iraq. One hundred sixty individuals between 30 to 60 years-old were included. The participants were divided into three groups: group one included 40 type 2 diabetic patients with peripheral neuropathy, group two consisted of 40 type 2 diabetic patients without neuropathy and group three took in 80 apparently in good health as the control. Each patient's medical history was recorded, including their gender, age, disease duration, BMI and any prior histories of other diseases. Ethical and scientific approval was gained from the college of medicine, Al-Nahrain University committee. Assessment of peripheral neuropathy was performed using Toronto Clinical Neuropathy Scoring System (TCSS). It is comprised of three elements: Symptoms, Sensory test and Reflex score. Herein, the greatest number of points is 19, and score ≥6 is diagnosed as Peripheral Neuropathy [15]. For both diabetic patient groups with and without peripheral neuropathy, clinical examination and biochemical tests such as HbA1c were measured. Five milliliters of blood specimen was drawn from the study subjects, which was employed for biochemical investigation, 2 ml of the blood was collected into EDTA tube for measuring glycated hemoglobin (HbA1c). Blood HbA1c levels were estimated by the CLOVER A1C system [16]. Serum NSE levels were measured by using ELISA techniques (sandwich method). Body Mass Index was calculated via utilizing (weight in kilograms/height in square meter) equation [17].

Statistical analysis

SPSS software version 25.0 was employed to perform the statistics. The quantitative description was summarized using mean and standard deviation, and three groups were compared by applying analysis of variance. Categorical variables, which were presented as numbers and percentages, were evaluated via the chi-square test. Serum neuron-specific enolase was evaluated utilizing the Receiver Operating Characteristic Curve (ROC) in the context of discriminating between diabetic patients, diabetics with peripheral neuropathy, and healthy controls. A statistically significant difference was determined to exist when the p-value was less than 0.05.

RESULTS

A total of 160 participants were involved in the final analysis. The mean age of the patients in DPN group was 49.1±5.44 years which did not differ significantly from that of diabetic patients without neuropathy (48.27±7.2 years). However, the two groups differed significantly from controls (42.06±6.21 years). Although the female ratio was higher in the DPN group (80%) than in either diabetic patients without neuropathy group (60%) or controls (62.5%), the differences were not significant. Similar to age, the BMI did not differ significantly between diabetic patients with and without DPN; however, both groups had significantly higher BMI than did controls ($p < 0.001$).

As a marker for diabetic control, HbA1c was higher in patients with DPN (10.56±2.64%) than in diabetic patients without neuropathy (8.79±2.53%), which in turn was higher than in controls (4.77±0.38%) with highly significant differences. The median duration of T2DM in patients with and without DPN was 7.0 years and 4.0 years, respectively with a highly significant difference (Table 1).

Table 1. Demographic characteristics of the population under study

Variable	Diabetic peripheral neuropathy (n=40)	Diabetes mellitus without peripheral neuropathy (n=40)	Controls (n=80)	p-value
Age (years) Mean±SD Range	49.1±5.44[a] 39-60	48.27±7.2[a] 30-60	42.06±6.21[b] 33-57	<0.001
Gender Males Females	8(20%) 32(80%)	16(40%) 24(60%)	30(37.5%) 50(62.5%)	0.101
BMI (kg/m²) Mean±SD Range	31.66±4.9[a] 23.65-44.5	30.59±3.5[a] 22.3-35.5	26.8±2.85[b] 20.5-32.5	<0.001
HbA1c Mean±SD Range	10.56±2.64[a] 7.0-15.6	8.79±2.53[b] 6.1-15.5	4.77±0.38[c] 4.0-5.8	<0.001
Disease duration (years) Mean±SD Median Range	8.26±4.69 7.0 1-18	5.25±4.38 4.0 1-22	–	0.001

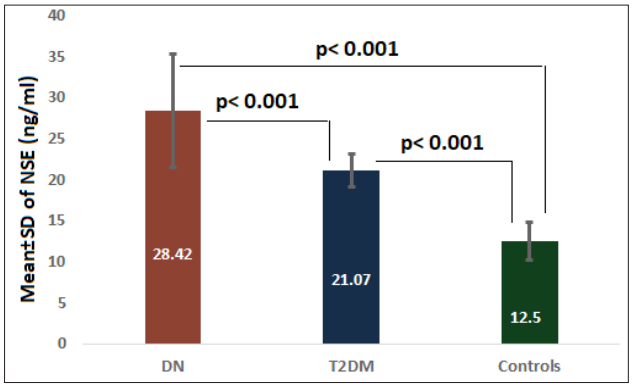
Different small letters (a,b,c) indicate significant differences between the groups, BMI – body mass index, SD – standard deviation, HbA1c – glycated hemoglobin

Patients with DPN demonstrated higher level of NSE (28.42±6.93 ng/ml) than did either diabetic patients without peripheral neuropathy (21.07±2.0 ng/ml) or controls (12.54±2.34 ng/ml), with highly significant differences (Figure 1,Table 2).

Table 2. serum levels of neuron-specific enolase in different groups

Variable	Diabetic peripheral neuropathy (n=40)	Diabetes mellitus without peripheral neuropathy (n=40)	Controls (n=80)	p-value
NSE (ng/ml)				
Mean±SD	28.42±6.93[a]	21.07±2.0[b]	12.54±2.34[c]	<0.001
Range	20.09-41.83	17.85-26.66	7.15-16.51	

Different small letters (a,b,c) indicate significant differences between the groups NSE – neuron- specific enolase



DN – diabetic neuropathy, T2DM – type 2 diabetes mellitus

Figure 1. Mean serum levels of neuron-specific enolase in DN, T2DM and controls

Neuron-specific enolase was evaluated for their diagnostic value using a Receiver Operating Characteristic (ROC) curve. In the context of Discrimination between Diabetic Peripheral Neuropathy and Diabetes Mellitus without Peripheral Neuropathy, the area under curve (AUC) for neuron-specific enolase was 0.812, 95% confidence interval [CI] = 0.716-0.909, p <0.001. Cut-off value of serum neuron-specific enolase was 22.53 ng/ml, sensitivity and specificity were 70% and 77%, respectively (Figure 2, Table 3).

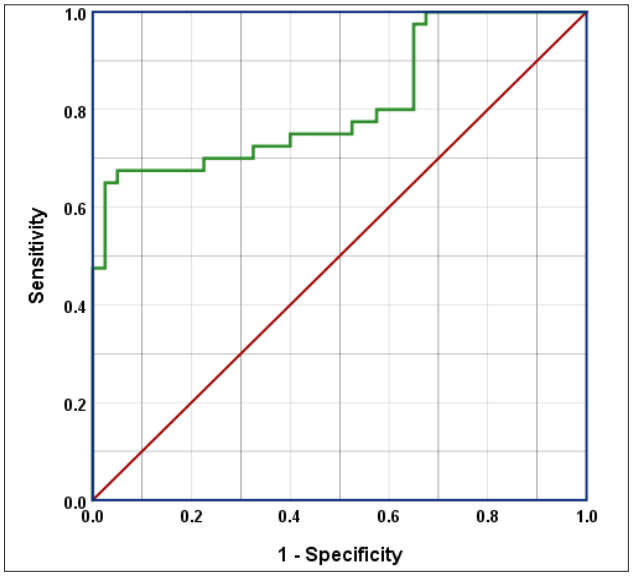


Figure 2. Receiver operating characteristic curve for neuron-specific enolase in the context of discrimination between diabetic peripheral neuropathy and diabetes mellitus without peripheral neuropathy

Table 3. Diagnostic value of neuron-specific enolase in the context of discrimination between diabetic peripheral neuropathy and diabetes mellitus without peripheral neuropathy

Markers	AUC	Sensitivity	Specificity	Cut off value
NSE	0.812	70%	77%	22.53 ng/ml

NSE – neuron specific enolase, AUC – area under the curve

In the context of discrimination between DPN and controls, the area under curve for neuron-specific enolase was 1.00, 95% confidence interval was 1.0-1.0, p <0.001. At a cut-off value of serum neuron-specific enolase = 18.3 ng/ml, both the sensitivity and specificity were 100% (Figure 3, Table 4).

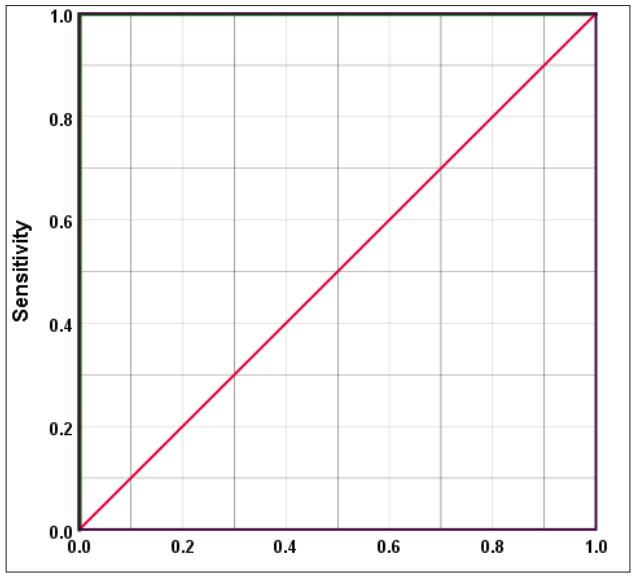


Figure 3. Receiver operating characteristic curve for neuron-specific enolase in the context of discrimination between diabetic peripheral neuropathy and controls

Table 4. Diagnostic value of neuron-specific enolase in the context of discrimination between diabetic peripheral neuropathy and control

Markers	AUC	Sensitivity	Specificity	Cut off value
NSE	1.0	100%	100%	18.3 ng/ml

NSE – neuron specific enolase, AUC – area under the curve

DISCUSSION

The most common microvascular consequence of diabetes mellitus is diabetic peripheral neuropathy. There are numerous diverse pathways that contribute to diabetic peripheral neuropathy, but oxidative stress, inflammation and mitochondrial dysfunction play a crucial part. Diabetes causes oxidative stress, altered metabolic pathways, inflammation and the activation of pro-inflammatory molecules [18]. In the current study, there are substantial differences in the age and BMI between the two groups – DPN and T2DM, and control. The average age of the patients in the DPN group was not statistically different from that of the diabetic patients, but the two groups differed significantly from controls. According to Albegali *et al*., people with T2DM who are over 50 years old and who exercise less, lose muscle mass and increase bulk mass with time are became more likely to experience long-term complications [19].

Similar to age, the BMI of diabetic patients with and without DPN did not differ substantially; nevertheless, both groups had considerably higher BMIs than did controls ($p < 0.001$). Of note, Zhou R *et al*., found that type 2 diabetes mellitus patients who were both general and abdominally obese had a higher probability of developing an incident DN, regardless of sex [20]. As a marker for diabetic management, the present study showed that HbA1c was higher in patients with DN than in diabetic patients without neuropathy, which in turn was higher than in healthy controls with highly significant differences. This result is in agreement with the results obtained by Hunaif *et al*., who found a relationship between the HbA1c level and the degree of diabetic neuropathy in Type 2 DM [21]. The present study also showed that the median duration of T2DM in patients with and without DN was 7.0 years and 4.0 years, respectively, with a highly significant difference. The current study is consistent with recent work by Jaya Kumar, who shown that having diabetes for a longer period of time and having a higher HbA1c level significantly increased the risk of neuropathy [22]. As a biomarker of hyperglycemia-induced nerve injury, NSE is regarded as a helpful biomarker in evaluating functional damage to neurons [12-14]. In the current study, patients with DPN demonstrated higher level of NSE than did either diabetic patients without neuropathy or controls, with highly significant differences. In contrast, patients with type 2 diabetes had NSE levels that were considerably greater than those in people without diabetes. The current study is in accordance with recent studies by Kandasamy S., which show that diabetic individuals with neuropathy had significantly higher NSE levels [23]. Other previous studies reported that 19.4% of diabetic patients had aberrant NSE concentration [24]. Hence, NSE can be used to detect diabetic peripheral neuropathy in at an early stage, and a severe stage of the condition can be avoided.

CONCLUSION

From the results of the present study, it can be concluded that diabetic peripheral neuropathy causes higher serum NSE levels. Consequently, this could potentially represent a reliable blood biomarker for diabetic neuropathy. By checking the NSE levels in diabetic patients, an early identification of the illness is therefore achievable.

LIMITATIONS OF THE STUDY

The study's limitation was the delay in collecting specimens from patients with peripheral neuropathy because every patient underwent a thorough medical investigation via the neurologist in accordance with the Toronto clinical system, which identified each patient as having peripheral neuropathy if the score following the investigation was ≥6, in order to avoid other disease complications from overlapping and having an effect on the outcomes. Future multicenter studies that examine other types of diabetic neuropathy are recommended.

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REFERENCES

1. Gouveri E, Papanas N. The emerging role of continuous glucose monitoring in the management of diabetic peripheral neuropathy: A narrative review. *Diabetes Ther.* 2022;13:931-52.
2. Bönhof GJ, Herder C, Strom A, Papanas N, Roden M, Ziegler D. Emerging biomarkers, tools, and treatments for diabetic polyneuropathy. *Endocr Rev.* 2019;40:153-92.
3. Young MJ, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH. A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. *Diabetologia*. 1993;36:150-4.
4. Andersen ST, Witte DR, Dalsgaard EM, Andersen H, Nawroth P, Fleming T, et al. Risk factors for incident diabetic polyneuropathy in a cohort with screen-detected Type 2 diabetes followed for 13 years: ADDITION-Denmark. *Diabetes Care*. 2018;41:1068-75.
5. Callaghan BC, Gao L, Li Y, Zhou X, Reynolds E, Banerjee M, et al. Diabetes and obesity are the main metabolic drivers of peripheral neuropathy. *Ann Clin Transl Neurol.* 2018 14;5:397-405.
6. Schlesinger S, Herder C, Kannenberg JM, Huth C, Carstensen-Kirberg M, Rathmann W, et al. General and abdominal obesity and incident distal sensorimotor polyneuropathy: Insights into inflammatory biomarkers as potential mediators in the KORA F4/FF4 cohort. *Diabetes Care*. 2019;42:240-7.
7. Oh TJ, Lee JE, Choi SH, Jang HC. Association between body fat and diabetic peripheral neuropathy in middle-aged adults with Type 2 diabetes mellitus: A preliminary report. *J Obes Metab Syndr.* 2019;28:112-7.
8. Patel GR, Samaria A, Kant C, Prajapati GR. Potential risk factors for peripheral neuropathy in patients with type 2 diabetes mellitus: a case control study from central Rajasthan. *Int J Adv Med.* 2022;9:448-55.
9. Pop-Busui R, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, et al. Diabetic neuropathy: A position statement by the American Diabetes Association. *Diabetes Care*. 2017;40:136-54.
10. Luescher T, Mueller J, Isenschmid C, Kalt J, Rasiah R, Tondorf T, et al. Neuron-specific enolase (NSE) improves clinical risk scores for prediction of neurological outcome and death in cardiac arrest patients: Results from a prospective trial. *Resuscitation*. 2019;142:50-60.
11. Wihersaari L, Tiainen M, Skrifvars MB, Bendel S, Kaukonen KM, Vaahersalo J, et al. FINNRESUSCI study group. Usefulness of neuron specific enolase in prognostication after cardiac arrest: Impact of age and time to ROSC. *Resuscitation*. 2019;139:214-21.
12. Haque A, Ray SK, Cox A, Banik NL. Neuron specific enolase: a promising therapeutic target in acute spinal cord injury. *Metab Brain Dis.* 2016;31:487-95.
13. Isgrò MA, Bottoni P, Scatena R. Neuron-specific enolase as a biomarker: Biochemical and clinical aspects. *Adv Exp Med Biol.* 2015;867:125-43.
14. Li J, Yan M, Zhang Y, Xie M, Yan L, Chen J. Serum neuron-specific enolase is elevated as a novel indicator of diabetic retinopathy including macular oedema. *Diabet Med*. 2015;32:102-7.
15. Bril V, Tomioka S, Buchanan RA, Perkins BA. Reliability and validity of the modified Toronto Clinical Neuropathy Score in diabetic sensorimotor polyneuropathy. *Diabet Med*. 2009;26:240-6.
16. Ibrahim AA, Baban RS, Khudair MS. Clinical and biochemical association between serum and urin level of the uromodulin protein with albuminuria in patients with type-2 diabetes mellitus. *Biochem Cell Arch*. 2019;19:2367-70.
17. Nuttall FQ. Body Mass Index: Obesity, BMI, and health: A critical review. *Nutr Today.* 2015;50:117-28.
18. Román-Pintos LM, Villegas-Rivera G, Rodríguez-Carrizalez AD, Miranda-Díaz AG, Cardona-Muñoz EG. Diabetic polyneuropathy in Type 2 diabetes mellitus: Inflammation, oxidative stress, and mitochondrial function. *J Diabetes Res.* 2016;2016:3425617.
19. Albegali AA, Shahzad M, Mahmood S, Ullah MI, Amar A, Sajjad O. Genetic polymorphism of eNOS (G894T) gene in insulin resistance in type 2 diabetes patients of Pakistani population. *Int J Diabetes Dev Ctries*. 2020;40:203-8.

20. Zhou R, Li F, Chen G, Fu Q, Gu S, Wu X. Associations between general and abdominal obesity and incident diabetic neuropathy in participants with type 2 diabetes mellitus. *J Diabetes.* 2021;13:33-42.
21. Hunaifi I, Agustriadi IGNO, Asmara IGY, Budyono C. The correlation between HbA1c and neuropathy disability score in type 2 diabetes. *Acta Med Indones.* 2021;53:164-8.
22. Kumar MJ, Ponnu S. Diabetic neuropathies-clinical and electrophysiological profile. *Int J Heal Clin Res.* 2022;5(3):769-72.
23. Kandasamy S, Krishnan B, Gopalakrishnan S, Ganesan H. Serum neuron-specific enolase as a biomarker in diagnosing diabetic peripheral neuropathy: A cross-sectional study. *Asian J Med Sci.* 2022;13:92-6.
24. Kelleher M, Singh R, O'Driscoll CM, Melgar S. Carcinoembryonic antigen (CEACAM) family members and inflammatory bowel disease. *Cytokine Growth Factor Rev.* 2019;47:21-31.