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INVESTIGATING NEURON-SPECIFIC ENOLASE AS A DIAGNOSTIC MARKER FOR EARLY DETECTION OF DIABETIC PERIPHERAL NEUROPATHY IN PATIENTS WITH TYPE II DIABETES MELLITUS

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Abstract
Diabetic peripheral neuropathy is one of the serious complications of diabetes, often goes undetected until the advanced stages, resulting in serious health consequences such as loss of sensation, diabetic foot, limb amputations, kidney damage, and eyesight losses.
A total of 148 Type II diabetes mellitus patients were included in the study.Patients were equally divided into, two groups based on MSNI (Michigan Neuropathy Screening Instrument) 74 cases with positive symptoms of peripheral diabetic neuropathy and 74 control diabetic patients without symptoms of peripheral diabetic neuropathy. Vital signs and basic lab parameters were determined for all the studied groups.
The neuron-specific enolase (NSE) levels was measured by the Enzyme-linked immunosorbent assay (ELISA) method. The results of our study revealed that levels of NSE were elevated with high HbA1C, abnormal lipid profile among the patients with diabetic peripheral neuropathy symptom as compared to control group (0.69 ± 0.31 vs 0.48 ± 0.28; p-value <0.001) odds of diabetic complication were increased with the raise in HbA1C aOR 50.25, 95% CI 6.82-369.86. Similarly, odds of having diabetic complications increased with the raise in LDL and triglyceride aOR 1.03, 95% CI 1.00-1.07 and aOR 1.01, 95% CI 1.00-1.03. NSE level were found significant in univariate analysis which shows the odds of having diabetic complication increased with the increase in NSE level COR 9.79, 95% CI 3.08 – 31.16. Results of our study suggested that NSE may serve as a biomarker for the early detection of diabetic peripheral neuropathy.

Keywords: Neuron specific Enolase, Diabetic peripheral neuropathy, enzyme-linked immunosorbent assay, diabetic foot, limb amputations.

1. Introduction

Diabetes mellitus (DM) is one of the most prevalent metabolic diseases, and its occurrence is rapidly increasing worldwide [1].Diabetes mellitus is characterized by polyuria, polydipsia, polyphagia, and hyperglycemia [2].The core issue lies in either defect in insulin production and insulin function lead to long-term damage and dysfunction in several organs of the body [3, 4]. The growing prevalence of diabetes mellitus presents a significant global health challenge. Diabetes mellitus significantly impair quality of life [5]. As per World health organization prediction, diabetes will be seventh leading cause of death by 20230 [3]. With the rising prevalence of diabetes, it is crucial to understand its various types along with their causes and the effects they have on the body's physiology and biochemistry. Diabetes is a complex metabolic disorder consisting of two main types, type 1, comprising nearly 5% of diabetes, and type 2, comprising 90%–95%[6].The incidence of Type 2 diabetes (T2D) is predicted to rise significantly, with projections estimating that 590 million people could be diagnosed by 2035 [7].The development of Type 2 diabetes is often linked to being overweight and consuming diets high in surplus calories with low physical activity, as around 90% of individuals found overweight at the time of diagnosis [8]. Approximately 463 million people worldwide are affected by Type 2 diabetes, making it the most prevalent form of diabetes [9] [10]. It is also estimated that within the next 30 years, about 1.3 billion people men, women, and children of all ages will have diabetes. Currently, over half a billion people worldwide suffer from diabetes [11]. According to estimations from the International Diabetes Federation, by 2045, approximately 783 million adults, or 1 in 8 people, are projected to have diabetes. Pakistan has highest rates of diabetes in the world [12]. Nearly 33 million adults in Pakistan are living with diabetes, representing a prevalence rate is 30.8 % among adults aged 20-79 years[13-15]. Persistent high blood sugar can increase the body's susceptibility to certain diseases. Among diabetic complications, diabetic peripheral neuropathy (DPN) is particularly notable, impacting diabetic patients and causing sensory and motor impairments [16]. Almost 50% of persons with diabetes will eventually develop diabetic peripheral neuropathy [17] It is linked to significant morbidity, such as foot ulceration, and lower limb amputation. Diabetic peripheral neuropathy affects up to 50% of patients with diabetes and is a main reason of morbidity and rise mortality [18].

Pakistan currently has the third-highest number of diabetes worldwide. Pakistan is part of the IDF Middle East and North Africa (MENA) region, which comprises 21 countries and territories. Globally, 537 million individuals have diabetes, with 73 million in the MENA Region. [19] [20]. Early diagnosis of diabetic peripheral neuropathy (DPN) can significantly enhance patient outcomes and overall quality of life, while it also helping to lower healthcare expenses. Nerve conduction studies, which are the gold standard for diagnosing DPN, are known for their accuracy. However, these studies are often costly and time consuming. Despite the lack of treatments specifically for nerve damage, the most effective strategy to improve patient outcomes is through the prevention of DPN, consequently, the discovery of a reliable biomarker for the early identification of DPN is essential [21, 22]. Biomarkers play a crucial role by providing clear diagnostic picture and predictive information, which helps to identify underlying issues more accurately. For the early detection of diabetic neuropathy, it is crucial to have more sensitive and convenient biomarkers that can accurately detect the severity and stage of the disease, in addition to clinical symptoms and neurological findings [22]. One capable approach is measuring neuron-specific enolase (NSE) levels, a protein mainly found in neurons and neuroendocrine cells. Elevated NSE levels can offer valuable insights into the extent of nerve damage, helping clinicians identify complications sooner and potentially improve patient outcomes by initiating timely interventions. This method may serve as an important tool in assessing nerve health in diabetic patients. When nerve cells are damaged or under stress, neuron-specific enolase (NSE) can leak into the bloodstream or cerebrospinal fluid, signaling neuronal injury. NSE is an isoform of enolase, an enzyme that plays a key role in the glycolytic pathway by catalyzing the conversion of 2-phosphoglycerate into phosphoenolpyruvate. This biochemical function is critical for energy production in cells, but its presence outside neurons can be a marker of nerve cell damage or dysfunction.[23]. Level of NSE fluctuate in reactive astrocytes undergoing various morphological

changes[24]. Central nervous system's neuron specific enolase activity may be impacted by hyperglycemia induced oxidative stress, which could have an effect on the survival and death of neuronal cells during neurodegenerative processes [25]. Study found diabetic patients with peripheral neuropathy had much higher serum NSE levels than either patients without neuropathy, and this difference can be distinguished with high accuracy[26].Elevated levels of NSE may predict the formation or progression of neuropathy, while its dynamic reactivity to acute neuronal insult gives a real-time representation of neuropathological processes [27, 28].
There is a vital need for the identification of more sensitive and precise biomarkers for subset of individuals with different underlying pathogenesis of diseases. Therefore, further disease progression can be prevented and effective treatment can be done for diabetes mellitus.

2. Aim of the study

The purpose of this study was to evaluate neuron specific enolase (NSE) as a probable biomarker for the early detection of peripheral diabetic neuropathy and the complications that accompany it.

3. Methodology:

3.1. Study setting

This study was conducted at two tertiary care hospitals in Karachi,Pakistan. The Indus Hospital & Health Network (IHHN) and Medicare Cardiac and General Hospital.

3.2. Study design and sampling technique

This study was conducted at two tertiary care hospitals in Karachi. After screening participants based on eligibility criteria, informed consent was obtained, and the participants were divided into two groups: cases and controls. This study was conducted in accordance with the Ethical approval of Sohail university with study protocol No. 000204/22. The health history of patients related to his or her disease was taken. After detailed history of patients, 5 ml fasting blood samples were taken from the patient. During data collection and blood sampling, precautions were strictly followed. Blood samples were used for biochemical estimation of neuron-specific enolase (NSE) by enzyme-linked immunosorbent assay (ELISA) methods and basic parameters including HbA1c, lipid profile, BMI, vital signs and Michigan neuropathy screening instrument was used for assessment.

3.3. Inclusion criteria

For the case group. All individuals with Type II diabetes who were above 20 year of age were included, regardless of gender. Positive symptoms of diabetic peripheral neuropathy and diabetic foot and other major diabetic complications. On the basis of symptoms and the Michigan Neuropathy Screening Instrument (MNSI) scoring from the diabetic OPD [29, 30].

For the Control group. On the Basis of Michigan Neuropathy Screening Instrument (MNSI) [30] all male and female patients with type II diabetes who were above 20 years of age were diagnosed with the diabetes without any neuropathy symptoms.

3.4. Exclusion criteria

All the non-diabetic patient, who are clinically not diagnosed and all diabetic patient who had already diagnosed with diabetic neuropathy.

3.5 .Study duration

This study had been completed in 1 year.

3.6. Sample size

A total of 148 diabetic patients, (74 cases and 74 controls) were included in the study. The sample size was calculated through OpenEpi software, (The confidence interval is 99 % and the power of

study 85 %) with the help of a reference study. NSE mean Diabetes without neuropathy 9.1 ±1.5 and NSE mean Diabetes with neuropathy 10.8 ±2.8.[31]

3.7. Dependent variables

Diabetic peripheral neuropathy related symptoms, such as tingling, numbness, or pain in the extremities, diabetic foot ulcer, loss of sensation in lower extremities, retinopathy, nephropathy and other major diabetic induce complications cardiac issues etc.

3.8. Independent variables

Age, gender, occupation, marital status, duration of diabetes diagnosis, Neuron Specific enolase (NSE), HbA1c, lipid profile, random blood sugar, vital signs, Michigan neuropathy screening instrument (MNSI), and enzyme-linked immunosorbent assay (ELISA) body weight and blood pressure.

3.9. Measurement of Human Neuron-specific enolase (NSE)

The neuron-specific enolase was measured through the Sandwich ELISA. Sandwich enzyme-linked immunosorbent assay (ELISA) is a sensitive and widely used technique for the detection and quantification of specific proteins.[32] In the process, capture antibodies were immobilized on a solid surface to form the base layer. A sample containing the target protein was added, which caused the target protein to bind to the capture antibody.

After washing to remove unbound material, an enzyme-conjugated detection antibody was introduced and binds to the captured protein to form a "sandwich." After another wash, the substrate was added where the enzyme activity produced a measurable signal. This quantitative method is important in various fields such as diagnostics and research.

3.10. Measurement of HbA1c

Diabetic patients visiting diabetic OPD routinely advised for HbA1c to assess their diabetes control. We got access the results of latest HbA1c tests at the time of data and sample collection with consent of patient. HbA1c values were compared with Neuron specific enolase and other parameters.

3.11.Michigan neuropathy screening instrument (MNSI) measurement.

The Michigan Neuropathy Screening Instrument (MNSI) is a reliable assessment instrument for diabetic neuropathy symptoms assessment. Peripheral neuropathy can be evaluated with the Michigan Neuropathy Screening Instrument (MNSI). It consists of a history questionnaire and physical examination to assess neuropathy-related symptoms and indicators. Higher scores indicate a higher chance of neuropathy.[33]

4. Results:

In this study we enrolled 148 patients; 74 patients with diabetic peripheral neuropathy symptoms and 74 having diabetes without diabetic peripheral neuropathy symptoms. The mean age of patient of cases was 53.15 ± 11.54 years while control group 50.89 ± 13.17 years.

However mean duration of diabetes and HbA1C in cases vs control group was 12.28 ± 6.09 months vs 6.91 ± 4.65 months and 9.44 ± 1.55 % vs 7.27 ± 0.89 % respectively. Details of demographic and clinical characteristics are shown in Table 1. However, in most of the cases female were predominant 55.41% on the other hand in control group male were predominant 55.41% [Figure 1].

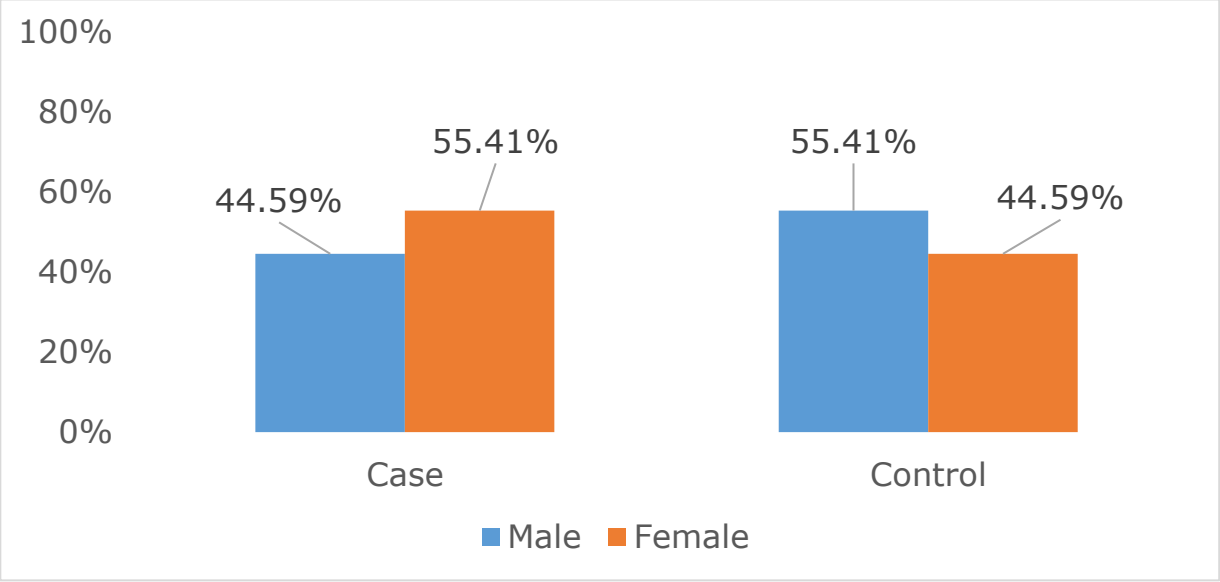

Figure 1: Distribution of Gender between groups.

Table 1 Distribution of demographic and clinical characteristics between groups among diabetic patients.

Variable	**Case**	**Control**	**p-value**
	Mean ± SD	**Mean ± SD**	
Age (Years)	53.15 ± 11.54	50.89 ± 13.17	0.270
HbA1C (%)	9.44 ± 1.55	7.27 ± 0.89	<0.001*
RBS (mg/dl)	263.32 ± 69.9	176.45 ± 72.54	<0.001*
Weight (Kg)	70.26 ± 13.77	67.81 ± 12.09	0.253
SBP (mmHg)	125.7 ± 12.91	124.36 ± 9.86	0.480
DBP (mmHg)	76.08 ± 7.51	79.04 ± 9.31	0.035*
Duration of Diabetes	12.28 ± 6.09	6.91 ± 4.65	<0.001*
RBS: Random Blood Sugar; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; *Significant p-value.			

Moreover, LDL, cholesterol and triglyceride were higher in case group as compared to control group. In addition, HDL was lower in cases as compared to control group [Table 2]. Besides, NSE level were higher in cases as compared to control group (0.69 ± 0.31 vs 0.48 ± 0.28; p-value <0.001).

Table 2 Distribution of Lipid Profile between groups among diabetic patients.

Variable	**Case**	**Control**	**p-value**
	Mean ± SD	**Mean ± SD**	
LDL	131.69 ± 20.37	110.57 ± 35.75	<0.001*
HDL	38.51 ± 7.61	46.55 ± 15.85	<0.001*
Triglyceride	238.49 ± 70.99	173.38 ± 51.64	<0.001*
Cholesterol	229.89 ± 53.57	181.38 ± 48.7	<0.001*

Furthermore, the predictive validity of NSE level as assessed by area under the ROC curve was 0.712 (95% CI 0.630 to 0.794). In addition, the cut-off value of 0.32 pg/ml shows sensitivity and specificity of 81.1% and 45.9% respectively. Figure 2.

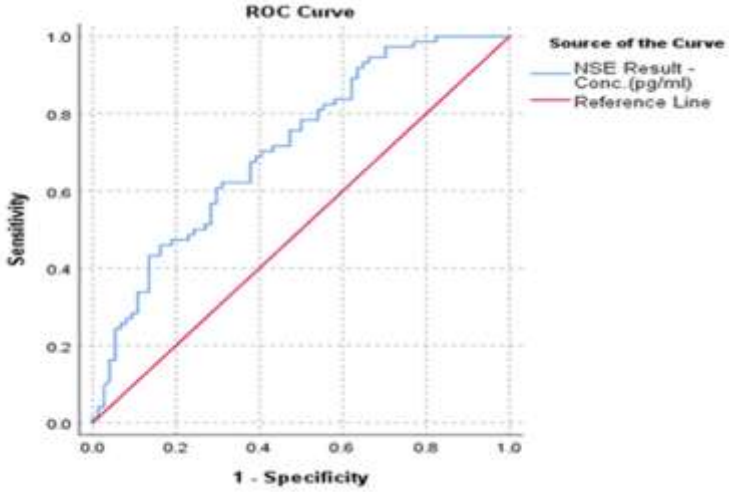

Figure 2: ROC analysis to assess the predictive validity of NSE level

Table 3. shows the association of complications with baseline characteristics. A significant association was found between HbA1C, RBS, and duration of diabetes, LDL, triglyceride and diabetic complications. Findings of this study shows that the odds of diabetic complication were increased with the raise in HbA1C [aOR 50.25, 95% CI 6.82-369.86]. Similarly, odds of having diabetic complications increased with the raise in LDL and triglyceride [aOR 1.03, 95% CI 1.00-1.07 and aOR 1.01, 95% CI 1.00-1.03]. Furthermore, NSE level were found significant in univariate analysis which shows the odds of having diabetic complication increased with the increase in NSE level [COR 9.79, 95% CI 3.08 – 31.16]. However, NSE level were insignificant after adjusting the other cofounding variables in multivariable analysis.

Table 1: Association of baseline characteristics with complication in patient with Diabetes

Independent Variables	**COR (95% CI)**	**aOR (95% CI)**
Age	1.01 (0.98-1.04)	0.99 (0.92-1.06)
Gender		
Male	Ref	Ref
Female	1.54 (0.80-2.95)*	0.38 (0.06-2.32)
HbA1C (%)	11.75 (4.89-28.21)**	50.25 (6.82-369.86)**
RBS (mg/dl)	1.01 (1.01-1.02)**	1.01 (1.00-1.02)**
Weight (Kg)	1.01 (0.98-1.04)	
SBP (mmHg)	1.01 (0.98-1.03)	
DBP (mmHg)	1.04 (1.00-1.08)**	
Duration of Diabetes	1.23 (1.13-1.34)**	1.29 (1.06-1.57)**
LDL (mg/dl)	1.02 (1.01-1.04)**	1.03 (1.00-1.07)**
HDL (mg/dl)	0.94 (0.90-0.97)**	
Triglyceride (mg/dl)	1.01 (1.01-1.02)**	1.01 (1.00-1.03)**
Cholesterol (mg/dl)	1.02 (1.01-1.02)**	0.99 (0.98-1.01)
NSE (pg/ml)	9.79 (3.08-31.16)**	18.49 (0.84-404.87)
COR: Crude Odds Ratio; aOR: Adjusted Odds Ratio; NSE: Neuron Specific Enolase; *p-value <0.25; **p-value ≤0.05; Ref: Reference		

5. Discussion

The aim of this case-control study was to explore the potential of serum neuron-specific enolase (NSE) as a predictive biomarker for the early diagnosis of diabetic peripheral neuropathy (DPN) and

its related complications in type II diabetes patients. Diabetic peripheral neuropathy complications extend beyond just nerve damage in the feet and legs. While the most immediate effects include loss of sensation, which can result in unnoticed injuries, foot ulcers, and potentially severe infections requiring amputation, DPN also has broader implications for overall health [34]. DPN cause chronic pain and muscle weakness, as well as it damages other organs and leading vision loss or impaired due to diabetic retinopathy, where the blood vessels in the eyes are affected, potentially resulting in blindness [35] [36]. In our study, we enrolled a total of 148 patients, divided into two equal groups: one group consisting of patients with symptoms of diabetic peripheral neuropathy, and a control group of patients with diabetes but without neuropathy symptoms.

The gender distribution between these groups revealed an interesting pattern, with a higher percentage of females in the group with neuropathy symptoms, while the control group had a greater proportion of males. Furthermore, the higher HbA1C levels observed in the case group. The elevated HbA1C levels in these patients are indicative of prolonged periods of hyperglycemia, which is known to contribute to the development of diabetic neuropathy through mechanisms such as advanced glycation end products (AGEs) formation, oxidative stress, and chronic inflammation [37].

Our study findings suggest a significant association between elevated HbA1C levels and an increased likelihood of diabetic complications, as reflected in the adjusted odds ratio (aOR) of 50.25 with a 95% confidence interval (CI) ranging from 6.82 to 369.86. it strongly indicates that as HbA1C levels increase risk of developing complications related to diabetes worsens markedly [38-39]. Previous studies also support our result, during the 3-year monitoring of diabetic patient glycemic control the DPN group's mean HbA1c levels were 7.2 ± 1.0%, while the control groups were 6.9 ± 1.1% [40]. DPN records were substantially correlated with elevated 3-year mean HbA1c levels (adjusted odds ratio: 1.23, 95% confidence interval 1.06–1.42) [41]. this result agreed with other studies [42-44]. In addition to the glycemic control, our research also investigated the lipid profiles of patients presenting with symptoms of diabetic peripheral neuropathy (DPN). The findings were quite informative, indicating that case group had markedly abnormal lipid profiles compared to their counterparts without DPN symptoms.

Specifically, individuals with DPN symptoms showed significantly higher levels of low-density lipoprotein (LDL), total cholesterol, and triglycerides, which are known risk factors for cardiovascular and other metabolic complications, Our finding are in agreement to the previously reported study [45].These elevated lipid levels were accompanied by a concerning decrease in high-density lipoprotein (HDL), a lipoprotein typically regarded as protective against cardiovascular disease [46]. The differences in lipid profiles between the case and control groups were not just statistically significant but also clinically relevant, as outlined in the mean differences presented in Table 2. This table illustrates how lipid abnormalities are more noticeable in case group, suggesting a strong link between dyslipidemia and the development of diabetic neuropathy symptoms[47].

Moreover, our study finding revealed as levels of LDL and triglycerides level play significant role in the risk of developing diabetic complications. This relationship was quantitatively supported by adjusted odds ratios (aOR) of 1.03 for LDL (95% CI 1.00-1.07) and 1.01 for triglycerides (95% CI 1.00-1.03), indicating that even slight elevations in these lipid parameters can significantly heighten the likelihood of complications. These findings are in alignment with the existing body of literature, which has consistently reported similar trends. As many authors studies finding supporting our finding individuals with diabetes had elevated triglyceride levels accompanied by decreased HDL levels [48-52]. Our study results demonstrated a significant elevation in Neuron-specific enolase (NSE) levels among patients with diabetic peripheral neuropathy symptoms. This observation is consistent with the findings reported by other researchers found significantly elevated levels of NSE in patients with complications, indicating potential neurological involvement in diabetic complications [28, 53, 54]. In our study NSE levels were significant in univariate analysis which shows the odds of having diabetic complication increased with the increase in NSE level [COR 9.79, 95% CI 3.08 – 31.16]. Our univariate analysis further reinforced this connection, revealing that higher NSE levels are significantly associated with an increased likelihood of diabetic complications.

Specifically, our analysis showed that the odds of developing complications increases substantially with elevated NSE levels, as evidenced by a compelling odds ratio (COR) of 9.79 and a 95% confidence interval ranging from 3.08 to 31.16. This finding indicates that patients with higher NSE levels are nearly 10 times more likely to experience diabetic neuropathy development risk as compared to those with lower levels, underscoring the potential of NSE as a predictive biomarker. However, when we adjusted other confounding variables in our multivariable analysis, the significance of NSE levels diminished. This suggests that while NSE levels may initially appear to be a strong indicator of diabetic neuropathy development due to other factors also play crucial roles in the progress of these complications, and the predictive power of NSE alone may be limited when these factors are considered. To further evaluate the utility of NSE as a predictive marker, we conducted a Receiver Operating Characteristic (ROC) analysis, which provided valuable insights into its predictive validity. The ROC analysis revealed a moderately strong ability of NSE to predict diabetic neuropathy, with an Area Under the Curve (AUC) of 0.712. This AUC value indicates that NSE has a reasonable level of accuracy in distinguishing between patients who are likely to develop complications and those who are not. However, it is important to note that while the predictive ability is moderate, it is not sufficient as a standalone diagnostic tool. Our study also determined an optimal cut-off value for NSE levels at 0.32 pg/ml. At this threshold, the sensitivity was 81.1%, meaning that the test could correctly identify 81.1% of patients at risk for diabetic neuropathy development. However, the specificity was 45.9%, indicating that nearly half of the patients without complications might still be identified as at risk. We agree with the previous studies, as comparing with studies [31] results 10.10 mg/L was the ideal cutoff point for serum NSE levels, with 66.3% sensitivity, 72.5% specificity, and a maximum AUC of 0.73 (0.68–0.77, P = 0.000) and comparison with [55] study result found AUC of 0.812 with a sensitivity of 70% and specificity of 77% at a cut-off value of 22.53 ng/ml, demonstrating a stronger discriminative power of NSE in prediction of diabetic neuropathy. One of study conducted by [56] found NSE test derived a cut-off point of >2.794 ng/ml from the ROC curve, showing an impressive sensitivity of 95.2% and specificity of 87.5%. This high level of accuracy suggests that NSE is highly effective at distinguishing between healthy individuals and those with a disease condition at this specific threshold. Values above 2.794 ng/ml were considered abnormal, indicative of a disease condition, while values below this threshold were deemed representative of a healthy state. In contrast, our study identified a lower cut-off point of 0.32 pg/ml with a sensitivity of 81.1% and specificity of 45.9%, reflecting a more moderate predictive ability.
The considerable difference in cut-off values and the corresponding sensitivity and specificity highlights how the effectiveness of NSE as a biomarker can vary depending on the population being studied and the specific disease conditions being targeted. These comparisons highlight the importance of context when interpreting NSE levels and suggest that while NSE has potential as a biomarker, it should be used in conjunction with other diagnostic tools and risk factors to provide a more comprehensive assessment of a patient's risk for diabetic complications. while our study identified NSE as a potentially useful marker for predicting diabetic complications, its significance diminishes when accounting for other variables, and its moderate predictive ability suggests that it should not be relied upon in isolation.

6. Conclusion

The study's findings showed that serum NSE could serve as a biomarker for the early detection of diabetic peripheral neuropathy. As compared to the control group, those experiencing symptoms of diabetic neuropathy had considerably higher NSE levels.Therefore, assessing NSE levels in diabetes individuals can aid in the early detection of the diabetic peripheral neuropathy.

7. Funding Information:

This study did not receive any external funding or grants. The primary purpose of this research is academic, aiming to contribute to scientific knowledge and improve patient outcomes.

8. Conflicts of Interest:
The authors declare no conflicts of interest.

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