Wiley
International Journal of Clinical Practice
Volume 2025, Article ID 5569034, 8 pages
https://doi.org/10.1155/ijcp/5569034

WILEY

Research Article

Serum Neuron-Specific Enolase Levels in Patients With Diabetic Peripheral Neuropathy

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Received 20 September 2024; Revised 19 October 2025; Accepted 11 November 2025

Academic Editor: Jui An Lin

Background: Based on previous reports that elevated NSE levels may predict diabetic neuropathy, we aimed to validate this association in a well-characterized cohort. Using strict exclusion criteria, standardized clinical scales, and nerve conduction studies, we aimed to evaluate the clinical utility of NSE levels in diabetic patients with and without neuropathic pain.
Methods: A total of 144 Type 2 diabetic patients were included in this prospective cross-sectional study. Neuropathic pain symptoms were assessed using the Douleur Neuropathique 4 questionnaire (DN4) and the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS). The diagnosis of diabetic peripheral neuropathy (DPNP) was established based on electrophysiological findings. Patients were divided into three groups as follows: those having neuropathic pain with DPNP (Group 1, $n = 60$) and without DPNP (Group 2, $n = 56$). The patients without neuropathic pain ($n = 28$) were defined as Group 3. Serum NSE levels were derived via electrochemiluminescence immunoassay.
Results: There was no significant between-group difference in age or gender (both $p > 0.05$). The disease duration was significantly longer in patients having neuropathic pain, compared to those without neuropathic pain ($p = 0.004$). The serum levels of NSE ($p < 0.001$) and the scores on the DN4 ($p = 0.001$) and LANSS ($p = 0.016$) instruments were higher in Group 1 than in Group 2. The NSE level was positively correlated with the LANSS score ($r = 0.260$, $p = 0.005$) and independently associated with the presence of DPNP in a multivariate model (OR 1.33, 95% confidence interval 1.12–1.58, $p = 0.001$). On the ROC analysis, an NSE cutoff of 9.51 ng/mL predicted the presence of DPNP.
Conclusion: Elevated serum NSE levels were significantly associated with DPNP in diabetic patients, indicating that NSE may serve as a potential biomarker for large-fiber involvement. However, this finding warrants confirmation in larger, multicenter studies.

Keywords: diabetic peripheral neuropathy; DN4 questionnaire; electroneuromyography; LANSS score; neuron-specific enolase

1. Introduction

Diabetic peripheral neuropathy (DPNP) is one of the major microvascular complications of diabetes and is associated with limb amputations and severe neuropathic pain [1–3]. Distal symmetric polyneuropathy, the most common form of DPNP, causes minimal-to-severe neuropathic pain in 13%–26% of diabetic patients [2, 4]. The main risk factors for DPNP are diabetes of long duration, dysregulated hyperglycemia, older age, hypertension, dyslipidemia, obesity, smoking, insulin resistance, and hypoinsulinemia [2].

The pathogenesis of DPNP involves oxidative stress, microvascular changes, nerve degeneration and regrowth, and inflammation [5, 6]. Excess glucose renders the polyol

and hexosamine pathways hyperactive, associated with increased production of reactive oxygen species and inflammation that in turn cause mitochondrial damage. Glycation of many structural and functional proteins creates advanced glycation end-products (AGEs) that, in turn, trigger loss of or changes in protein function and interactions with the AGE-specific receptor that affects gene expression and intracellular signaling and promotes the release of proinflammatory molecules and free radicals [7]. Several biomarkers of diabetic neuropathy have been suggested, including C-reactive protein, tumor necrosis factor alpha, Interleukin 6, toll-like Receptor 4, transforming growth factor Beta 1, antinuclear and antiganglioside antibodies, brain-derived neurotrophic factor, kynurenine, and tetrahydrobiopterin [6, 8]. Additionally, neuron-specific enolase (NSE) may serve as a useful marker. NSE is an intracellular protein that maintains the excitability of the neuronal membrane [9, 10]. NSE is mainly expressed in mature neurons of the central nervous system, but it has also been found in glial cells as microglia, astrocytes, and oligodendrocytes [11]. That is, NSE is released into the cerebrospinal/endoneurial fluid and systemic circulation after damage of both neuronal and glial cells [12]. Upon central pathologies, including traumatic brain injury, neuroendocrine tumors, hypoxic encephalopathy, stroke, and cognitive disorders, NSE expression is markedly increased [9, 13]. However, there is a lack of data on peripheral pathologies in which the mechanisms primarily involve glia cells [14, 15].

In clinical practice, diabetic patients with neuropathic pain underwent electroneuromyography (ENMG) for the confirmation of DPNP. However, some diabetic patients were intolerant to this painful procedure. Circulating markers can serve as an alternative to the ENMG testing. Our previous study demonstrated that NSE mRNA levels were similar among prediabetic patients with and without peripheral neuropathy and the healthy control group [10]. Past limited data found high levels of NSE and suggested that NSE is a potential predictive biomarker for diabetic neuropathy [12, 16, 17]. In the light of previous evidence, we sought to verify these findings in our own well-characterized patient cohort. To ensure more reliable results, we carefully applied stringent exclusion criteria, enrolling only individuals with Type 2 diabetes, and strengthened our evaluation through standardized clinical scales and ENMG assessments. Accordingly, we aimed to evaluate the clinical utility of NSE levels in diabetic patients with and without neuropathic pain.

2. Methods

2.1. Study Population. A total of 144 Type 2 diabetic patients, recruited from endocrinology and metabolism outpatient clinic, were included in this prospective cross-sectional study. Demographic data, including age, gender, educational level, occupation, duration of diabetes mellitus, and the antidiabetic medications taken, were obtained for the patients. Patient groups were as follows.

i. Group 1 ($n = 60$): patients having neuropathic pain *with* DPNP by ENMG
ii. Group 2 ($n = 56$): patients having neuropathic pain *without* DPNP by ENMG
iii. Group 3 ($n = 28$): patients without neuropathic pain

Group 1 patients, where there were enough patients for analysis, were further divided into three subgroups, thus those on oral antidiabetic drugs (OADs) ($n = 29$), insulin ($n = 11$), and OADs plus insulin ($n = 20$).

Patients under 40 years of age, with diseases that may affect NSE levels (cerebrovascular conditions, dementia, cerebral tumors, systemic infection and inflammation, a malignancy, and rheumatological diseases), and those receiving drugs to control neuropathic pain were excluded.

This study was conducted at Kirsehir Training and Research Hospital (Kirsehir, Turkey) between March 2023 and December 2023. All procedures conformed to the Declaration of Helsinki. Ethical approval for this study was obtained from Kirsehir Ahi Evran University Local Research Ethics Committee (approval date 03/21/2023; approval number 2023–04/27).

2.2. Assessment of Neuropathic Pain. Douleur Neuropathique 4 questionnaire (DN4): This scale includes 10 questions exploring neuropathic pain symptoms such as burning and freezing, and examination findings (hypesthesia and allodynia). Each question is answered "yes" or "no." The maximum score is 10, with a score of 4 or more indicating neuropathic pain [18].

Leeds Assessment of Neuropathic Symptoms and Signs (LANSS): This instrument includes a pain questionnaire and a sensory examination; the maximum score is 24; scores of 12 and above indicate neuropathic pain [19]. The validity and reliability of the Turkish version were confirmed by Yucel et al. [20].

ENMG: Diabetic patients with neuropathic pain ($n = 116$) underwent ENMG that evaluated bilateral median, ulnar, sural, tibial, and peroneal sensory, motor, and antidromic nerve conduction. Those with DPNP were defined as Group 1 ($n = 60$) and those without DPNP as Group 2 ($n = 56$) by the Nihon–Kohden MEB-9200K (Nihon Kohden Corp., Tokyo, Japan; 2010 model) ENMG device.

Severity of neuropathy: The Baba Diabetic Neuropathy Classification was used to divide the severity of neuropathy into five categories [21]. *Normal:* Sural sensory nerve action potential (SNAP) amplitude > 10 μV, and the nerve conduction (NC) velocities for both sural and tibial nerves > 40 m/s (reference ranges of our laboratory). *Mild:* Sural SNAP amplitude > 5 μV and a delay in the tibial motor NC velocity, the sural sensory NC velocity. *Moderate:* Sural SNAP amplitude < 5 μV and tibial compound muscle action potential (CMAP) amplitude ≥ 5 mV. *Moderate to severe:* Sural SNAP amplitude < 5 μV and tibial CMAP amplitude ≥ 2 to < 5 mV. *Severe:* Sural SNAP amplitude < 5 μV and tibial CMAP amplitude < 2 mV.

2.3. Blood Samples. After an overnight fast, venous blood samples were collected into serum gel tubes without anticoagulants and tubes with K2EDTA; the tubes were centrifuged at 2000 × g for 10 min, and the serum NSE, vitamin D, and vitamin B12 levels were analyzed. The hemoglobin A1c (HgbA1c) levels were determined in samples containing the anticoagulant. Sera were stored at −80°C prior to the analysis of NSE levels. The serum levels of NSE, 25-OH vitamin D, and vitamin B12 were measured via electrochemiluminescence immunoassay (Cobas 801 platform; Roche Diagnostics, Mannheim, Germany), and whole-blood HgbA1c levels were determined (by the same instrument) using an inhibitory, turbidimetric immunoassay method.

2.4. Statistical Analysis. The IBM Statistical Package for the Social Sciences (SPSS; Ver. 25.0, IBM Corp., Armonk, NY, USA) was employed. The Kolmogorov–Smirnov test was used to verify that quantitative data were normally distributed. Categorical and continuous variables are presented as numbers (percentages) and means ± standard deviations or medians (interquartile ranges), respectively. The independent samples *t*-test and the Mann–Whitney *U*-test were used to compare continuous variables, and the chi-squared ($\chi 2$) test to compare categorical variables between diabetic patients with and without neuropathic pain (Table 1) and with and without DPNP (Table 2). Pearson correlations (among normally distributed parameters) and Spearman rho correlations (among non-normally distributed variables) were derived when examining the relationships between the demographic and laboratory data of diabetic patients with neuropathic pain (Table 3). One-way analysis of variance (for normally distributed parameters) and the Kruskal–Wallis test (for non-normally distributed variables) were used to compare quantitative data on the antidiabetic medications taken by diabetic patients having neuropathic pain with DPNP (Table 4). Binary logistic regression was used to identify predictors of DPNP in diabetic patients having neuropathic pain. For each factor, an odds ratio (OR) with a 95% confidence interval (CI) was calculated. The NSE data were subjected to receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) calculated (with the 95% CI). NSE cutoff values were determined, and their statistical utilities calculated (with 95% CIs). A p value < 0.05 was considered significant.

According to a similar study in the literature [12], serum NSE levels were found as 10.8 ± 2.8 in diabetic patients with neuropathy ($n = 214$) and 9.1 ± 1.5 in those without neuropathy ($n = 218$). In the G∗Power 3.1 program, as a result of 0.80 power, 0.05 margin of error, and 0.50 effect size, it was calculated that a total of 144 patients should be included in the study.

3. Results

Demographic and laboratory data for all participants are summarized in Table 1. The disease duration was significantly longer in the diabetic patients with neuropathic pain (Group 1 and Group 2) than in those without (Group 3) ($p = 0.004$). Age, gender, and laboratory parameters including HgbA1c, 25-OH Vitamin D, Vitamin B12, and serum NSE levels were similar among these groups ($p > 0.05$).

Demographic and laboratory data of Group 1 and Group 2 are listed in Table 2. The HgbA1c ($p = 0.008$) and NSE ($p < 0.001$) serum levels, and the DN4 ($p = 0.001$) and LANSS ($p = 0.016$) scores were higher, but the 25-OH Vitamin D level lower ($p = 0.036$) in Group 1 than in Group 2. In Group 1, neuropathy was moderate in 36.7%, moderate-to-severe in 43.3%, and severe in 20%. In Group 2, 87.5% were normal and 12.5% had mild neuropathy.

In the correlation analysis (Table 3), HgbA1c levels demonstrated a negative correlation with age ($r = -0.218$, $p = 0.019$) and a positive correlation with the duration of diabetes ($r = 0.368$, $p < 0.001$). Serum 25-hydroxyvitamin D levels showed a positive correlation with age ($r = 0.219$, $p = 0.018$), while NSE levels exhibited a weak but statistically significant positive correlation with the LANSS score ($r = 0.260$, $p = 0.005$). In the multivariate model, only HgbA1c (OR 1.54, 95% CI [1.07–2.21], $p = 0.020$) and NSE (OR 1.33, 95% CI [1.12–1.58], $p = 0.001$) levels were identified as independent factors associated with DPNP in diabetic patients having neuropathic pain. On ROC analysis, the NSE AUC was 0.70 (95% CI [0.61–0.79], $p < 0.001$) (Figure 1). An NSE cutoff of 9.51 ng/mL predicted DPNP in diabetic patients with a sensitivity of 62.7% and a specificity of 63.3%.

The laboratory data and neuropathic pain scale scores by the antidiabetic medications of Group 1 are shown in Table 4. The HgbA1c level was higher ($p < 0.001$), and the vitamin D level ($p = 0.029$) and LANSS score ($p = 0.020$) were lower in patients on *OADs plus insulin* than the other groups.

4. Discussion

Four main findings emerge. First, the serum NSE level and the DN4 and LANSS scores were higher in diabetic patients having neuropathic pain with DPNP than without DPNP. Second, the NSE level positively correlated with the LANSS score. Third, the multivariate analysis revealed that serum NSE levels were independently associated with the presence of DPNP, with a 1.3-fold increase in risk for each unit rise in NSE. Taken together, this may suggest a direct correlation between neuronal damage and symptomatic neuropathy.

DPNP is both disabling and irreversible and affects about half of all diabetic patients [22]. The pathophysiology is complex; the underlying mechanisms remain elusive. Hyperglycemia and insulin deficiency or resistance impose oxidative and/or nitrosative stress on mitochondria and the endoplasmic reticulum, initiated by reactive oxygen species produced by hyperactive polyol and hexosamine pathways, inflammation, and cellular damage. Subsequently, proinflammatory cytokines and chemokines are released from macrophages, triggering further inflammation [7, 22]. The major DPNP risk factors include the duration of diabetes, dysregulated hyperglycemia, and advanced age [2]. In line

Table 1: Comparison of demographic and laboratory data between diabetic patients with and without neuropathic pain ($n = 144$).

	Diabetic patients with neuropathic pain ($n = 116$)	Diabetic patients without neuropathic pain ($n = 28$)	p
Age	61.5 (56–68)	59.0 (46.2–69.7)	0.230
Female gender	68 (58.6)	18 (64.9)	0.585
Duration of diabetes (years)	15 (7.2–20)	9 (1.2–15)	**0.004**
Education level			
Illiterate	13 (11.2)	0 (0)	
Primary school	64 (55.2)	17 (60.7)	
Secondary school	17 (14.7)	3 (10.7)	0.185
High school	11 (9.5)	4 (14.3)	
University	11 (9.5)	4 (14.3)	
Occupation			
Self-employment	4 (3.4)	2 (7.1)	
Employee	14 (12.1)	3 (10.7)	
Government official	8 (6.9)	5 (17.9)	0.443
Retired	22 (19)	3 (10.7)	
Housewife	68 (58.6)	15 (53.6)	
Medication			
OADs	68 (58.6)	21 (75)	
Insulin	19 (16.4)	3 (10.7)	0.113
OADs plus insulin	29 (25)	4 (14.3)	
Laboratory			
HgbA1c (%)	7.6 (6.6–9.1)	7.6 (6.9–9)	0.836
Vitamin D (ng/mL)	14 (10–22.7)	16 (12–24.7)	0.214
Vitamin B12 (pg/mL)	364 (283.2–513.2)	330.5 (271–497.7)	0.513
NSE (ng/mL)	10.9 ± 5.9	8.7 ± 4.6	0.062

Note: Values are expressed as n (%), mean ± SD, or median (interquartile range). HgbA1c: hemoglobin A1c. The bold p values indicate statistical significance ($p < 0.05$).
Abbreviations: OADs, oral antidiabetic drugs; NSE, neuron-specific enolase.

Table 2: Comparison of data between with and without diabetic peripheral neuropathy (DPNP) in diabetic patients having neuropathic pain ($n = 116$).

	With DPNP ($n = 60$)	Without DPNP ($n = 56$)	p
Duration of diabetes (years)	15 (10–25)	10 (6–20)	0.078
Medication			
OADs	29 (48.3)	39 (69.6)	
Insulin	11 (18.3)	8 (14.3)	**0.015**
OADs plus insulin	20 (33.3)	9 (16.1)	
Laboratory			
HgbA1c (%)	8.3 (6.9–9.9)	7.2 (6.4–8.4)	**0.008**
Vitamin D (ng/mL)	12 (8–18.7)	15 (11–25)	**0.036**
Vitamin B12 (pg/mL)	394.5 (267.7–595.5)	343.5 (291.5–474.7)	0.319
NSE (ng/mL)	13.4 ± 6.6	8.2 ± 3.4	**< 0.001**
Neuropathic pain scale			
DN4 score	8 (6–9)	6 (5–7.7)	**0.001**
LANSS score	15 (13–18)	13 (12–16)	**0.016**
Severity of neuropathy*			
Normal	0 (0)	49 (87.5)	
Mild	0 (0)	7 (12.5)	
Moderate	22 (36.5)	0 (0)	**< 0.001**
Moderate-to-severe	26 (43.3)	0 (0)	
Severe	12 (20)	0 (0)	

Note: Values are expressed as n (%), mean ± SD, or median (interquartile range). HgbA1c: hemoglobin A1c, DN4: Douleur Neuropathique 4 questionnaire. The bold p values indicate statistical significance ($p < 0.05$).
Abbreviations: LANSS, Leeds Assessment of Neuropathic Symptoms and Signs; OADs, oral antidiabetic drugs; NSE, neuron-specific enolase.
*Neuropathy severity was based on the Baba classification.

with the literature, we found that the duration of diabetes was longer in the group with than without neuropathic pain.

Many recent studies have sought biomarkers of DPNP pathogenesis and progression [23]; NSE is promising in this regard. NSE catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate [24]. Elevated NSE levels in serum and cerebrospinal fluid are generally associated with neuronal injury [24, 25]. The serum NSE initially increased in

Table 3: Correlation analysis of demographic and laboratory data in diabetic patients having neuropathic pain ($n = 116$).

	Age	Duration of diabetes	HgbA1c	Vitamin D	Vitamin B12	NSE	DN4 score	LANSS score
Age	.	NS	**r = -0.218 $p = 0.019$**	**r = 0.219 $p = 0.018$**	NS	NS	NS	NS
Duration of diabetes	NS	.	**r = 0.368 $p < 0.001$**	NS	NS	NS	**r = 0.228 $p = 0.014$**	NS
HgbA1c	**r = -0.218 $p = 0.019$**	**r = 0.368 $p < 0.001$**	.	NS	NS	NS	NS	NS
Vitamin D	**r = 0.219 $p = 0.018$**	NS	NS	.	NS	NS	NS	NS
Vitamin B12	NS	NS	NS		.	NS	NS	NS
NSE	NS	NS	NS	NS	NS	.	NS	**r = 0.260 $p = 0.005$**

Note: HgbA1c: hemoglobin A1c, DN4: Douleur Neuropathique 4 questionnaire.
Abbreviations: LANSS, Leeds Assessment of Neuropathic Symptoms and Signs; NSE, neuron-specific enolase.

Table 4: Comparison of data according to antidiabetic medication in diabetic patients with diabetic peripheral neuropathy (DPNP) ($n = 60$).

	OADs ($n = 29$)	Insulin ($n = 11$)	OADs plus insulin ($n = 20$)	p
Laboratory				
HgbA1c (%)	7 (6.2–7.8)	9 (8.3–10.5)	9.6 (8.3–11.4)	**< 0.001**
Vitamin D (ng/mL)	13 (9–23.5)	14 (9–35)	9 (6–14.5)	**0.029**
Vitamin B12 (pg/mL)	345 (213.5–509.5)	403 (332–1209)	449 (300.7–724)	0.099
NSE (ng/mL)	13.8 ± 7.7	13.6 ± 6.0	12.7 ± 5.1	0.839
Neuropathic pain scale				
DN4 score	8 (6.5–9)	7 (6–8)	8 (6–9)	0.285
LANSS score	15 (13.5–18.5)	13 (13–14)	13.5 (13–16)	**0.020**

Note: Values are expressed as mean ± SD or median (interquartile range). HgbA1c: hemoglobin A1c, DN4: Douleur Neuropathique 4 questionnaire. The bold p values indicate statistical significance ($p < 0.05$).
Abbreviations: LANSS, Leeds Assessment of Neuropathic Symptoms and Signs; OADs, oral antidiabetic drugs; NSE, neuron-specific enolase.

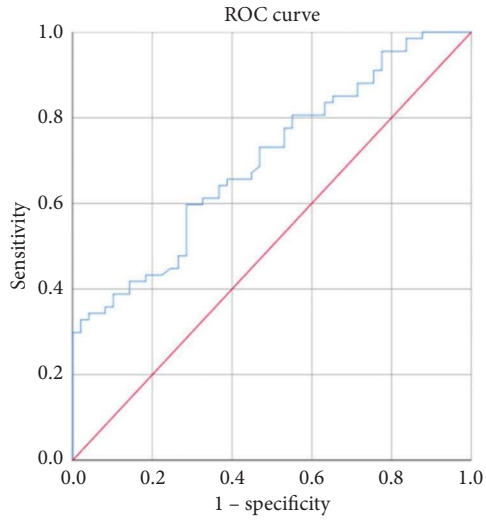

Figure 1: ROC analysis demonstrating the predictive value of serum NSE levels for DPNP in diabetic patients.

patients with diabetic neuropathy [12, 25] but decreased with treatment [24]. NSE assay may aid the diagnosis and monitoring of diabetic neuropathy. Li et al. showed that the NSE level was higher in patients with than without diabetic neuropathy [12]. In an Indian study, biophotomodulation therapy decreased the NSE level, which was associated with clinical improvement [24]. Another study from India found a correlation between the NSE level and neuropathic pain [17]. In the present study, the NSE level was somewhat higher in diabetics with neuropathic pain than in those without, but statistical significance was not attained, perhaps because of the relatively small sample size or the fact that the diabetes duration differed significantly between these groups. Furthermore, unlike previous reports [12, 16], our control group was composed of diabetics.

Diabetes primarily affects thinly myelinated Aδ and unmyelinated C-fibers. The resulting (early) neuropathic pain is termed small-fiber neuropathy [26, 27]. Normal-to-high HbA1c levels (5.8%–6.4%) reduced the pain threshold in the absence of a diabetes diagnosis or an impaired fasting glucose uptake [28]. Thus, neuropathic complaints may develop even when glucose metabolism is only minimally (but pathologically) impaired, although the electrophysiological findings are generally normal. Later in disease, the large fibers become affected, and DPNP is detected via evaluation of neuronal conduction. We lacked quantitative sensory/sudomotor testing to explore the small-fiber neuropathy in diabetic patients. In the present study, the diagnosis of DPNP was established based on the results of ENMG showing the large fiber involvement. In line with the literature [12, 17, 29], we found a significantly higher NSE level in diabetic patients with than without DPNP. Thus, we speculate that NSE may serve as a marker of large-

fiber involvement in diabetic patients. DPNP initially destroys axons, with demyelination occurring subsequently, especially in severe cases [30]. NSE contributes to both axonal regeneration and the repair of myelin sheaths. Because larger fibers have thicker myelin, extensive Schwann cell damage in severe DPNP may lead to increased NSE release [12]. Consistent with this, our diabetic patients with severe neuropathy exhibited significantly higher NSE levels (data not shown). Li et al. reported an optimal cutoff point of 10.10 μg/L for serum NSE level to distinguish patients with diabetic neuropathy from those without. In the present study, we found a cutoff of 9.51 ng/mL to independently predict DPNP development. The reason for this difference may be that our control group consisted of diabetic patients, whereas Li et al.'s control group was nondiabetic [12]. In fact, an AUC value of 0.70 for NSE was obtained, indicating a moderate diagnostic accuracy, which may limit clinical applicability without further validation.

Twenty five-OH Vitamin D deficiency predisposes to DPNP development [31, 32]. Elevated glucose levels decrease that of 25-OH Vitamin D, which protects peripheral nerves by stimulating the production of neurotrophins such as nerve growth factor and by ensuring neuronal calcium homeostasis [32]. In the present study, the high HgbA1c level of patients on OADs plus insulin reflected their relatively advanced stage of diabetes. In this treatment group, we found low 25-OH Vitamin D level as expected. But, the serum NSE levels did not increase, and the LANSS score was not high, perhaps due to the effects of the antidiabetic drugs. The detailed mechanisms by which antidiabetic drugs affect diabetic neuropathy were poorly understood. Previous data have shown that a decrease in the cobalamin level and an increase in the homocysteine level exacerbated DPNP in metformin-treated diabetic patients [33]. In contrast, recent studies have reported that metformin may be neuroprotective against DPNP [34, 35]. Also, sodium-glucose Cotransporter 2 inhibitors (SGLTis) may protect against DPNP [36–38]. SGLTis improved NC and reduced neuropathic pain in diabetic rats [36], and SGLTis lowered sympathetic nervous system activity [38]. EL-Haggar et al. found that, compared to controls, significant electrophysiological improvement was apparent in diabetics with DPNP who had taken SGLTis for 3 months [39]. In the present study, approximately two-fold more diabetic patients with than without DPNP used SGLTis (26.8% and 15.0%, respectively), which may have suppressed the development of neuropathy, and this may explain the lack of increases in both LANSS score and NSE in patients receiving OADs plus insulin.

Our work had certain limitations. First, it was cross-sectional in design, that we cannot determine a causal link. Second, it was a single-center study with a relatively small sample size that may limit the generalizability of the findings. Future multicenter studies are essential. Third, diabetic patients without neuropathic pain did not undergo ENMG; some patients may have had painless neuropathy. Fourth, we lacked a control group of nondiabetic healthy individuals; such a group would possibly contribute to our understanding of NSE's specificity for diabetic neuropathy. Fifth, it was impossible to environmentally homogenize all subjects in terms of, e.g., smoking status, nutritional habits, and physical exercise status, all of which may impact the NSE level.

5. Conclusion

Elevated serum NSE levels were significantly associated with DPNP in diabetic patients, indicating that NSE may serve as a potential biomarker for large-fiber involvement. However, this finding warrants confirmation in larger, multicenter studies.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Concept/design: S.D. and A.C.; data collection and/or processing: S.D., A.C., A.Y., A.K., and H.D.; data analysis and interpretation: S.D., A.C., A.Y., B.E.S., B.I., A.K., and H.D.; literature search: S.D., B.E.S., B.I., A.K., and H.D.; drafting the manuscript: S.D., A.C., A.Y., B.E.S., and B.I.; critical revision of the manuscript: S.D. and A.C.; supervision: A.C., S.D., and A.C. are contributed equally.

Funding

The authors received no specific funding for this work.

Acknowledgments

The authors have nothing to report.

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