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Neurofilament light chain and nerve growth factor as biomarkers of axonal injury and neurotrophic dysfunction in diabetic neuropathy

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ABSTRACT

Introduction and aim. Diabetic peripheral neuropathy (DPN) is the most common complication of diabetes and is often diagnosed at an advanced stage when nerve damage becomes irreversible. Neurofilament light chain (NfL), a neuroaxonal injury marker, and nerve growth factor (NGF), a neurotrophin, are potential biomarkers for evaluating axonal damage and neurotrophic support in DPN. This study aimed to evaluate the efficacy of serum NfL and NGF as concurrent biomarkers of axonal injury and impaired neurotrophic support in a single cohort of patients with DPN, exploring their relationship with clinical and electrophysiological parameters.

Material and methods. A case-control study was conducted, including 30 patients with type 2 diabetes mellitus (T2DM) and DPN, 30 patients with T2DM without neuropathy (diabetic controls), and 60 healthy control subjects. Serum NfL and NGF concentrations were measured by enzyme-linked immunosorbent assay (ELISA). The primary outcome was the receiver operating characteristic (ROC) performance of serum NfL for discriminating patients with DPN from healthy controls.

Results. Serum NfL levels were significantly elevated in patients with DPN (13.9±1.20 ng/mL) compared with diabetic controls (9.83±2.08 ng/mL) and healthy controls (5.60±2.53 ng/mL) ($p<0.0001$). Conversely, NGF levels were significantly lower in both diabetic groups than in healthy controls ($p<0.0001$). In this case-control sample, ROC analysis showed that serum NfL discriminated against DPN from healthy controls with an area under the curve (AUC) of 0.994 (sensitivity 96.6%, specificity 100.0%), though this exceptionally high performance reflects discrimination against healthy individuals rather than diabetic controls.

Conclusion. Serum NfL and NGF represent promising candidate biomarkers of axonal injury and impaired neurotrophic support in DPN, respectively, and were correlated with glycemic control and disease duration

in this cohort. However, given the small, single-center, case-control design, further validation in larger, longitudinal, and duration-matched cohorts is required before clinical implementation can be considered.

Keywords. diabetic peripheral neuropathy, nerve growth factor, neurofilament light chain

Introduction

Diabetes mellitus is a growing global health problem. According to the International Diabetes Federation, 589 million adults were living with diabetes in 2024, and this burden is expected to increase further.[1] Diabetic peripheral neuropathy (DPN) is among the most common chronic complications of diabetes mellitus, causing pain, sensory loss, and substantial impairment in quality of life.[2] DPN arises from chronic hyperglycemia, oxidative stress, and neuroinflammation, which together drive progressive nerve injury, demyelination, and ultimately neuronal apoptosis.[3,4]

A critical issue in the clinical management of DPN is its often-delayed diagnosis, which typically occurs at an advanced stage when significant and irreversible nerve damage has already occurred.[5,6] This diagnostic latency underscores the urgent need for sensitive and specific biomarkers that can facilitate early detection and intervention in this patient population. In this context, two molecular candidates have garnered considerable attention: neurofilament light chain (NfL) and nerve growth factor (NGF). NfL is a 68-kDa structural protein that is an integral component of the neuronal cytoskeleton and is essential for maintaining axonal caliber and structural integrity.[7] Upon neuroaxonal injury, NfL is released from damaged neurons into the extracellular space and subsequently into the peripheral bloodstream, where its concentration reflects the magnitude of ongoing axonal damage. Elevated circulating NfL levels have been consistently demonstrated across a wide range of neurodegenerative and peripheral nerve disorders, and emerging evidence supports its role as a dynamic and sensitive biomarker for DPN.[8,9]

Conversely, nerve growth factor is a member of the neurotrophin family and is indispensable for the development, survival, differentiation, and long-term maintenance of peripheral sensory and sympathetic neurons. Its biological effects are mediated primarily through the high-affinity tropomyosin receptor kinase A (TrkA), which promotes neuronal survival and axonal regeneration. A growing body of evidence indicates that a neurotrophic deficit, characterized by reduced NGF synthesis and impaired TrkA signaling, contributes to DPN and renders neurons increasingly vulnerable to hyperglycemia-induced injury.[10,11] While previous studies have independently investigated NfL and NGF in diabetic neuropathy, research evaluating both markers in the same cohort remains limited. Their ability to distinguish diabetic participants with neuropathy from those without neuropathy, and their relationships with glycemic exposure and disease duration, therefore merit further investigation.

Aim

Accordingly, this study aimed to evaluate serum NfL and NGF as individual and concurrent biomarkers of axonal injury and neurotrophic dysfunction in DPN, and to explore their associations with clinical and electrophysiological parameters. The primary novelty of this study lies in the simultaneous assessment of both serum NfL and NGF within the same cohort of patients with DPN, which allows for a direct comparison of axonal damage and neurotrophic support. However, this novelty statement is made cautiously, as individual assessments of these biomarkers have been reported previously. The analysis of DPN severity was considered exploratory.

Material and methods

Study design and ethical considerations

This case-control study was conducted at Al-Imameen Al-Kadhimayn Medical City in Baghdad, Iraq, over a duration of 5 months, from September 2025 to January 2026. The study protocol was developed in accordance with ethical principles for medical research involving human subjects as outlined by the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board of the College of Medicine, Babylon University, Iraq (Approval Number: MB5-69, date: 18/9/2025). Participants were given a full explanation of the study purposes and procedures, and written informed consent was obtained prior to study enrollment.

Participant recruitment and group allocation

A total of 120 participants aged 41–77 years were recruited for this study. The sample size was determined based on previous literature evaluating biomarker variations in DPN, ensuring sufficient power (80%) to detect a significant difference at an alpha level of 0.05. The principal analytic focus was the discrimination of participants with DPN from healthy controls, whereas severity subgroup comparisons were planned as exploratory analyses. Potential confounding factors such as age, metabolic status (BMI), and renal function were considered during patient selection and data interpretation, as these variables may influence circulating NfL and NGF levels. Participants were allocated to one of three distinct cohorts:

- Group 1 (DPN patients, n=30): This group comprised patients with a pre-existing diagnosis of type 2 diabetes mellitus and a clinically and electrophysiologically confirmed diagnosis of diabetic peripheral neuropathy.
- Group 2 (diabetic controls, n=30): This group comprised patients with a diagnosis of type 2 diabetes mellitus but with no clinical or electrophysiological evidence of peripheral neuropathy.
- Group 3 (healthy controls, n=60): This group included apparently healthy individuals with no history of diabetes, neurological disorders, or other chronic illnesses that could potentially interfere with the study's endpoints.

The inclusion criteria for the diabetic groups were a confirmed diagnosis of type 2 diabetes mellitus. The exclusion criteria for all groups included a history of significant renal or hepatic disease, active malignancy, acute inflammatory conditions, or other known causes of peripheral neuropathy.

Clinical and demographic data collection

A standardized case report form was used to collect detailed information from all participants. This included demographic data (age and sex), comprehensive medical history, and duration of diabetes for participants in Groups 1 and 2. Anthropometric measurements, including height and weight, were used to calculate the Body Mass Index (BMI) for each participant, quantified as kg/m^2.[12]

Assessment of peripheral neuropathy

All participants in the diabetic cohorts (groups 1 and 2) underwent comprehensive nerve conduction studies (NCS) to objectively assess the functional status of their peripheral nerves. The examinations were performed by an experienced neurophysiologist using a Nihon Kohden electrophysiology system (Nihon Kohden, Japan). All studies were conducted under standardized, temperature-controlled conditions to ensure the reliability and consistency of the results. The NCS protocol included the assessment of both sensory and motor nerves to confirm the diagnosis of DPN and grade its severity. The severity of DPN was stratified into mild, moderate, or severe categories based on a standardized composite score derived from NCS parameters. Mild DPN was defined as a reduction in sensory nerve action potential (SNAP) amplitude (<50% of the lower limit of normal, LLN) in at least two sensory nerves (sural or superficial peroneal) with normal motor conduction. Moderate DPN was defined as abnormal sensory conduction combined with mild-to-moderate reductions in motor conduction velocity (<10% below LLN) or compound muscle action potential (CMAP) amplitudes in the common peroneal or tibial nerves. Severe DPN was characterized by the complete absence of recordable SNAPs in the lower extremities, accompanied by severe motor conduction velocity slowing (>20% below LLN) or a marked reduction in CMAP amplitudes (<50% of LLN).[13]

Biochemical analyses

Sample collection and processing

Fasting venous blood samples were collected from all participants in the morning. Samples were harvested in serum separator tubes and allowed to clot at room temperature. After that, the samples were centrifuged at 3000 rpm for 15 min to obtain the serum. Further, the serum obtained was aliquoted into cryovials and stored at −80°C until analysis to maintain stability of all biomarkers.

Immunoassays

Serum levels of NfL and NGF were measured using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, Shanghai, China; lot no of NfL: E4467Hu and NGF E2102Hu), strictly adhering to the manufacturer's instructions. The assay procedure was as follows: 1) Standards and samples were prepared and diluted as recommended by manufacturer; 2) 50 μL of standard or sample was added to the appropriate wells; 3) 50 μL of biotinylated antibody was added to each well and incubated; 4) Wells were washed, and streptavidin-HRP was added; 5) After further washing, substrate solution was added for color development; 6) The reaction was stopped, and optical density was measured at 450 nm. All samples were measured in duplicate to ensure accuracy. The analytical sensitivity for the NFL assay was 0.054 ng/mL with a standard curve range of 0.1–35 ng/mL. The analytical sensitivity for the NGF assay was 3.48 pg/mL with a detection range 7–1500 pg/mL. For both assays, the intra-assay coefficient of variation (CV) was <8%, and the inter-assay CV was <10%.

Statistical analysis

Statistical analysis of the data obtained was performed using the SPSS software package (version 26.0, IBM, Armonk, NY, USA). Summary statistics of quantitative variables are expressed as mean±standard deviation (SD). Differences between the three study groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. For categorical variables, numbers and percentages are shown, and comparisons between groups were made with the chi-square (χ^2) test. The prespecified primary outcome was the area under the receiver operating characteristic (ROC) curve for serum NfL in discriminating participants with DPN from healthy controls. Secondary analyses included between-group comparisons for NfL and NGF, bivariate correlations, and exploratory comparisons according to DPN severity. ROC curve analysis was used to evaluate diagnostic accuracy, and the AUC, optimal cut-off values, sensitivity, and specificity were calculated from the ROC curves. No multivariable regression model or combined biomarker ROC model was generated in the present dataset. Statistical significance was defined as $p<0.05$.

Results

Demographic and clinical data of the 120 participants are described in Table 1. The mean glycated hemoglobin (HbA1c) level was significantly higher in patients with DPN compared to those without neuropathy (8.33±2.36% vs. 6.71±1.13%; $p<0.0001$). The duration of diabetes was significantly longer in the DPN group compared to the non-DPN group (16.9±6.77 vs. 2.5±1.35 years; $p=0.003$). A significant difference in glycemic control (HbA1c) was observed among the three groups ($p<0.0001$), with the highest HbA1c level found in patients with DPN (8.33±2.36%). Diabetic cohorts demonstrated significantly elevated levels of NfL concentrations compared to healthy controls ($p<0.001$), and patients with DPN had

the highest mean level at 13.9±1.20 ng/mL, which was significantly higher than in diabetic controls (9.83±2.08 ng/mL, p<0.001). On the other hand, NGF concentrations were markedly decreased in both diabetic groups compared to healthy controls (p<0.0001) and especially low in diabetic patients with neuropathy (202.6±21.3 pg/mL), although the difference between DPN patients and diabetic controls did not reach statistical significance (p>0.05).

Table 1. Demographic characteristics of the population under study*

Characteristic	Diabetes patients with neuropathy (n=30)	Diabetes patients without neuropathy (n=30)	Healthy control (n=60)	p
Age (years)				
Mean±SD	56.66±6.67	53.26±8.63	54.69±8.78	0.086
Range	(42.0–70.0)	(43.0–73.0)	(41.0–77.0)	
Sex				
Male, n (%)	20 (66.7%)	18 (60.0%)	30 (50.0%)	0.1128
Female, n (%)	10 (33.3%)	12 (40.0%)	30 (50.0%)	
Duration of disease (years)				
Mean±SD	16.9±6.77	2.5±1.35	—	0.003
Severity of disease				
Mild, n (%)	10 (33.3%)	—	—	N/A
Moderate, n (%)	11 (36.7%)	—	—	
Severe, n (%)	9 (30.0%)	—	—	
HbA1c (%)				
Mean±SD	8.33 ± 2.36^{A}	6.71 ± 1.13^{B}	5.14 ± 0.53^{B}	<0.0001
NfL (ng/mL)				
Mean±SD	13.9 ± 1.20^{A}	9.83 ± 2.08^{B}	5.60 ± 2.53^{C}	<0.0001
NGF (pg/mL)				
Mean±SD	202.6 ± 21.3^{A}	212.3 ± 26.2^{A}	311.2 ± 32.2^{B}	<0.0001

***** n – number of cases, capital letters A, B and C indicate the level of significance following Tukey's multiple comparisons test; similar letters indicate no significant difference, whereas different letters indicate significant difference

A statistically significant positive correlation was observed between HbA1c and serum NfL levels (r=0.574, p<0.001) and a significant negative correlation was found between HbA1c and serum NGF levels

(r=−0.570, p<0.001). Serum NfL was positively correlated with diabetes duration (r=0.733, p<0.001). NGF demonstrated a strong inverse correlation with NfL (r=−0.613, p<0.001). The correlation of NGF with diabetes duration was not statistically significant (r=0.059, p=0.667).

Table 2. Correlation matrix of investigated biomarkers and clinical variables

Variables		AGE	BMI	Duration	HbA1c	NfL	NGF
NfL	R	0.221	-0.15	0.733	0.574	1	-0.613
	p	0.046	0.16	<0.001	<0.001		<0.001
	n	60	60	60	60	60	60
NGF	R	-0.071	0.10	0.059	-0.570	-0.613	1
	p	0.524	0.37	0.667	<0.001	<0.001	
	n	60	60	60	60	60	60

The ROC curve analysis results are presented in Table 3. Both biomarkers showed good discrimination between diabetic groups and healthy controls. NfL demonstrated an AUC of 0.886, with a sensitivity of 73.1% and specificity of 99.1%, at a cut-off value of >5.93 ng/mL in distinguishing diabetic patients without neuropathy from control subjects (p<0.001). For differentiating DPN patients from healthy controls, NfL demonstrated an AUC of 0.994 with 96.6% sensitivity and 100% specificity at a cut-off value of >11.9 ng/mL. NGF also showed good discriminative power, with an AUC of 0.85 (p<0.001) for differentiating diabetic subjects without neuropathy from healthy controls (cut-off: ≤226.2 pg/mL, sensitivity: 92.3%, specificity: 88.5%) and an AUC of 0.87 (p<0.001) for differentiating DPN patients from healthy controls (cut-off: ≤227.8 pg/mL, sensitivity: 96.7%, specificity: 88.0%). Importantly, to address the more clinically relevant distinction, we performed an exploratory ROC analysis comparing patients with DPN directly against diabetic controls (T2DM without neuropathy). For this comparison, serum NfL demonstrated a high discriminative capacity with an AUC of 0.892 (95% CI: 0.79–0.99, p<0.001) at an optimal cut-off of >11.8 ng/mL, yielding a sensitivity of 90.0% and a specificity of 83.3%. In contrast, serum NGF exhibited poor discriminative power between the two diabetic cohorts, with an AUC of 0.584 (95% CI: 0.43–0.74, p=0.26) at a cut-off of ≤208.5 pg/mL (sensitivity: 63.3%, specificity: 56.7%), which reflects the substantial overlap in NGF levels between diabetic patients with and without neuropathy.

Table 3. Diagnostic performance of NfL and NGF

Marker	Comparison Groups	Cut-off	Sensitivity	Specificity	AUC (95% CI)	p

NfL (ng/mL)	DM without neuropathy vs healthy control	>5.93	73.1 %	99.1%	0.886 (0.78–0.98)	<0.001
	DM with neuropathy vs healthy control	>11.9	96.6%	100%	0.994 (0.96–1.00)	<0.001
	DM with neuropathy vs DM without neuropathy	>11.8	90.0%	83.3%	0.892 (0.79–0.99)	<0.001
NGF (pg/mL)	DM without neuropathy vs healthy control	≤226.2	92.3%	88.5%	0.85 (0.73–0.96)	<0.001
	DM with neuropathy vs healthy control	≤227.8	96.7%	88%	0.87 (0.76–0.97)	<0.001
	DM with neuropathy vs DM without neuropathy	≤208.5	63.3%	56.7%	0.584 (0.43–0.74)	0.260

An analysis was conducted to compare the levels of biochemical parameters according to the severity of DPN (Table 4). The mean serum NfL concentrations were 13.9±1.10 ng/mL in the mild group, 13.52±1.3 ng/mL in the moderate group, and 13.61±2.3 ng/mL in the severe group. The mean serum NGF concentrations were 206.2±14.1 pg/mL, 196.1±13.2 pg/mL, and 211.3±24.4 pg/mL in the mild, moderate, and severe groups, respectively. Despite numerical variations, there were no statistically significant differences in the mean levels of either NfL (p=0.87) or NGF (p=0.31) across the three DPN severity stages.

Table 4. Comparison of biochemical parameters according to the severity of disease

Characteristic	**Mild n=10**	**Moderate n=11**	**Severe n=9**	**p**
NfL (ng/mL, mean±SD)	13.9±1.10	13.52±1.3	13.61±2.3	0.87
NGF (pg/mL, mean±SD)	206.2±14.1	196.1±13.2	211.3±24.4	0.31

Discussion

The present study evaluated serum NfL and NGF levels in patients with type 2 diabetes mellitus (T2DM) with and without peripheral neuropathy. We observed significantly higher serum NfL and lower serum

NGF levels in patients with DPN compared to healthy controls, reflecting progressive neuroaxonal injury and impaired neurotrophic support.[14,15]

Our observation that serum NfL levels were highest in patients with DPN (13.9±1.20 ng/mL), intermediate in diabetic controls (9.83±2.08 ng/mL), and lowest in healthy controls (5.60±2.53 ng/mL) supports the role of NfL as a sensitive marker of axonal damage. This graded elevation suggests that neuroaxonal injury is a continuous process in diabetes, potentially starting subclinically before the clinical onset of neuropathy. This is consistent with findings by Nasr-Eldin et al., who reported elevated serum NfL in diabetic patients regardless of neuropathy status, with the highest levels in those with confirmed DPN.[16] This indicates that diabetes may induce a state of subclinical neural injury that can be detected by NfL.

The strong positive correlation between serum NfL and diabetes duration ($r=0.733$, $p<0.001$) as well as HbA1c ($r=0.574$, $p<0.001$) suggests that cumulative glycemic exposure and metabolic stress drive progressive axonal degeneration. This is in agreement with prior longitudinal analyses,[14,17] although our cross-sectional design prevents causal inferences.

In terms of diagnostic performance, the exceptionally high AUC of 0.994 for NfL in distinguishing patients with DPN from healthy controls must be interpreted with caution, as case-control studies comparing diseased cohorts with healthy individuals typically overstate diagnostic accuracy. Indeed, when we performed the more clinically relevant comparison between DPN patients and diabetic controls, the AUC for NfL decreased to 0.892. While still reflecting good diagnostic performance, this indicates that the biomarker's utility is more modest in clinical settings where the goal is to differentiate neuropathy from diabetes-related changes. This is consistent with Chen et al., who reported an AUC of 0.829 for NfL alone in distinguishing DPN from non-DPN diabetic patients.[15] Therefore, serum NfL should be regarded as a promising candidate biomarker requiring further validation, rather than a tool ready for routine clinical use.

Interestingly, our study did not find a significant association between NfL levels and clinical severity of DPN (mild, moderate, severe). This finding should be interpreted cautiously because the severity subgroups were small, making the analysis exploratory and underpowered. Nevertheless, the absence of a clear gradient is consistent with recent reports suggesting that serum NfL may reflect axonal injury more directly than symptom-based severity phenotypes.[18,19]

Our study identified significantly lower serum NGF levels in patients with DM, with the most pronounced reduction in the DPN group. This supports the neurotrophic deficit hypothesis, which posits that a deficiency in essential growth factors, such as NGF, contributes to neuronal vulnerability and degeneration in DPN.[3,4] The significant negative correlation between NGF and HbA1c ($r=-0.570$, $p<0.001$) in our cohort reinforces the link between poor glycemic control and impaired neurotrophic support.

A comprehensive review by Massimino et al. explains that disruption of the NGF/proNGF axis is a key element in DPN pathophysiology.[20] Our findings are also directionally consistent with prior clinical work by Sun et al., who reported lower serum NGF levels in patients with DPN and suggested diagnostic utility

for this marker.[10] These external data should be interpreted as contextual support rather than confirmation of our single-center cross-sectional observations.

The structural and functional alterations observed in DPN, reflected by altered NfL and NGF levels, are fundamentally driven by chronic hyperglycemia-induced oxidative stress and neuroinflammation. Recent experimental evidence highlights the potential of targeting these pathways to mitigate nerve injury. For instance, a recent study by Arı et al. investigated the protective effects of dichloroacetic acid (DCA) in a rat model of diabetic neuropathy. The authors demonstrated that DCA administration significantly alleviated neuropathic alterations by reducing lipid peroxidation and inflammation, leading to improved nerve conduction and motor function.[21] Such mechanistic insights underscore the critical role of oxidative stress and inflammation in the pathogenesis of DPN and provide a vital context for our biomarker findings, suggesting that therapeutic interventions targeting these pathways may help preserve neuroaxonal integrity and neurotrophic support.

The diagnostic performance of NGF in our study (AUC=0.87) was good, although not as high as that of NfL. This is consistent with the general understanding that structural damage markers NfL often exhibit higher diagnostic accuracy than markers of regulatory pathways or trophic factors (NGF).[22] NGF may be more reflective of the underlying state of neurotrophic dysregulation than a direct measure of the extent of axonal breakdown.

The inverse correlation between NfL and NGF ($r=-0.613$, $p<0.001$) suggests that increasing neuroaxonal injury may coexist with declining neurotrophic support. This conceptual framework is biologically appealing; however, we did not construct a combined biomarker model, compare a combined ROC curve with NfL alone, or test incremental discrimination. Therefore, the potential added value of using both markers together should be considered hypothesis-generating rather than established.

It is important to acknowledge that the statistical analyses employed in this study, namely one-way ANOVA, bivariate Pearson correlation, and ROC curve analysis, do not account for the potential confounding effects of variables such as age, BMI, and renal function on biomarker levels. Although age and sex were not significantly different between groups, multivariable regression analysis would have provided a more rigorous assessment of the independent association of NfL and NGF with DPN status. In addition, renal function was addressed through exclusion criteria rather than direct adjustment with creatinine or eGFR values, which warrants caution because circulating NfL may be influenced by kidney clearance

Study limitations

Several limitations of this study should be acknowledged. First, the relatively small sample from a single center may limit generalizability. Second, the cross-sectional design precludes causal inference. Third, although major comorbid conditions were excluded, renal function was not incorporated as a measured

covariate, and unmeasured subclinical renal impairment or systemic inflammation may have affected biomarker levels. Fourth, no multivariable regression, confidence intervals, standardized effect sizes, or combined biomarker models were generated, limiting inference about independent or additive diagnostic value. Fifth, the severity analysis was underpowered because the DPN subgroup sizes were small (10/11/9) and should therefore be regarded as exploratory. Finally, the ROC analyses were performed against healthy controls, and the exceptionally high diagnostic accuracy observed for NfL requires validation in larger cohorts that include clinically relevant diabetic comparison groups. Additionally, there was a significant difference in the duration of diabetes between the DPN group and the non-neuropathy diabetic group. Because both NfL and NGF levels may be influenced by cumulative glycemic exposure over time, this disparity makes it difficult to definitively determine whether the observed changes in biomarker levels are directly attributable to the presence of neuropathy or simply reflect the longer duration of diabetes. Future studies with duration-matched cohorts are required to clarify this.

Conclusion

In conclusion, serum NfL levels were significantly elevated and serum NGF levels were significantly reduced in patients with DPN compared to healthy controls. These findings support the potential of serum NfL and NGF as promising candidate biomarkers of axonal injury and impaired neurotrophic support in DPN, respectively. However, given the small, single-center, cross-sectional case-control design, lack of multivariable adjustment, and the reduced diagnostic performance of NfL in distinguishing DPN from diabetic controls, these results must be considered preliminary. Further validation in larger, longitudinal, and duration-matched cohorts is strictly required before these biomarkers can be considered for clinical implementation or routine diagnostic monitoring.

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Declarations

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Author contributions

Conceptualization: R.A.A.K. and T.M.J.; Methodology: R.A.A.K. and T.M.J.; Validation R.A.A.K. and T.M.J.; Formal Analysis: R.A.A.K; Investigation: R.A.A.K. and T.M.J.; Resources: R.A.A.K.; Data Curation: R.A.A.K.; Writing – Original Draft Preparation: R.A.A.K. and T.M.J.; Writing – Review & Editing: R.A.A.K. and T.M.J.; Visualization: R.A.A.K.; Supervision: R.A.A.K.; Project Administration: TMJ; Funding Acquisition: R.A.A.K. and T.M.J..

Conflicts of interest

The authors declare that they have no competing interests.

Data availability

The datasets produced and examined in the present study can be obtained from the corresponding author upon a reasonable request, adhering to ethical and privacy considerations.

Ethics approval

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of the College of Medicine, Babylon University (Approval Number: MB5-69, date: 18/9/2025).

Use of AI and AI-assisted technologies in the writing process

AI tools (PaperPal) were used only for language editing and grammar improvement. All scientific content, data analyses, and conclusions are the authors' own.

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