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SERUM BIOMARKER PROFILING FOR EARLY DETECTION OF DIABETIC NEUROPATHY: A PROSPECTIVE OBSERVATIONAL STUDY

Summaya Fatima	Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad, India
Noorain Mohammed*	Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad, India *Corresponding Author
Salla Shruthika	Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad, India
Chennuri Arun	Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad, India
Sabita Sahoo	Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad, India
Mohammed Zoheb	Department of Neurology, Owaisi Group of Hospitals and Research Centre, Hyderabad, India

ABSTRACT **Background:** Diabetic neuropathy is among the most prevalent and debilitating microvascular complications of diabetes mellitus, characterized by progressive nerve damage that leads to sensory impairment, chronic pain, and increased risk of foot ulcers and amputations. Conventional diagnostic approaches depend on clinical symptom scoring and electrophysiological testing, both of which typically identify nerve damage only after significant injury has occurred. Serum biomarker profiling presents a non-invasive, potentially earlier alternative. **Objective:** To investigate serum levels of inflammatory markers (TNF-α, IL-6), neuronal injury markers (neuron-specific enolase [NSE]), and endothelial dysfunction markers (CD144/VE-cadherin, CD62E/E-selectin) across different stages of diabetic peripheral neuropathy (DPN), and to correlate these biomarkers with clinical and biochemical parameters. **Methods:** A single-center prospective observational study was conducted at Owaisi Group of Hospitals and Research Centre, Hyderabad, over five months (October 2024 to February 2025). One hundred diabetic patients aged 18-85 years were enrolled and classified by neuropathy severity using the Toronto Clinical Neuropathy Scoring System (TCNS). Biomarker mRNA expression levels were quantified via RT-qPCR. Statistical analysis employed one-way ANOVA with post-hoc testing. **Results:** All five biomarkers demonstrated statistically significant variation across DPN stages (NSE: $F=57.94$, $p=3.53\times10^{-17}$; IL-6: $F=264.02$, $p=1.29\times10^{-30}$; TNF-α: $F=120.28$, $p=2.03\times10^{-23}$; CD62E: $F=249.22$, $p=4.46\times10^{-30}$; CD144: $F=103.90$, $p=3.99\times10^{-22}$). IL-6, TNF-α, NSE, and CD62E increased progressively with worsening neuropathy severity. Serum albumin correlated inversely with inflammatory markers and positively with NSE. HbA1c showed a positive association with all biomarkers. **Conclusion:** Serum biomarker profiling of NSE, IL-6, TNF-α, CD62E, and CD144 shows significant promise for early, non-invasive detection of diabetic neuropathy. These markers reflect underlying neuroinflammatory and vascular pathology and exhibit detectable changes even in subclinical stages. Integration of biomarker screening into routine diabetic care could facilitate earlier intervention and improved patient outcomes.

KEYWORDS : Diabetic Peripheral Neuropathy; Serum Biomarkers; TNF-α; IL-6; Neuron-Specific Enolase; VE-cadherin; E-selectin; Early Detection; RT-qPCR

1. INTRODUCTION

Diabetes mellitus (DM) is a global public health emergency affecting an estimated 537 million adults, a figure projected to rise to 783 million by 2045. Diabetic peripheral neuropathy (DPN) represents the most common microvascular complication, affecting approximately 50-60% of individuals with long-standing diabetes. DPN is characterized by progressive, length-dependent axonal degeneration that initially manifests as distal sensory loss in a "glove and stocking" distribution, with subsequent involvement of motor and autonomic fibers.

The clinical and economic burden of DPN is considerable. It is the leading cause of non-traumatic lower limb amputation globally, and neuropathic pain substantially impairs quality of life. Despite its prevalence, early detection remains a major unmet clinical need. Current screening tools, including the 10-g monofilament test, 128-Hz tuning fork assessment, and the Toronto ClinicalNeuropathy Scoring System (TCNS), detect neuropathy only after appreciable nerve fiber loss has occurred. Nerve conduction studies, while more sensitive, are resource-intensive and impractical for routine population-level screening.

The pathogenesis of DPN involves multiple converging mechanisms. Chronic hyperglycemia activates the polyol pathway, advanced glycation end-product (AGE) accumulation, protein kinase C (PKC) signaling, hexosamine pathway dysregulation, and mitochondrial superoxide overproduction. These processes collectively drive oxidative stress, neuroinflammation, and endothelial dysfunction, culminating in axonal degeneration and demyelination.

Circulating serum biomarkers reflecting these pathophysiological processes offer an attractive, non-invasive approach to earlier detection. Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) are pro-inflammatory cytokines elevated in diabetic neuropathy. Neuron-specific enolase (NSE) is released from damaged neurons and correlates with neuronal injury severity. CD144 (VE-cadherin) and CD62E (E-selectin) are endothelial adhesion molecules whose altered expression reflects vascular inflammation and blood-nerve barrier disruption.

The present study aimed to prospectively profile these five serum biomarkers across TCNS-defined stages of DPN in a cohort of diabetic patients, and to explore their correlations with metabolic parameters including HbA1c and serum albumin, with the goal of validating their diagnostic and prognostic potential.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This was a single-center, prospective observational study conducted at the Department of Neurology and Department of General Medicine, Owaisi Group of Hospitals and Research Centre, Hyderabad, India. The study was conducted over five months from October 2024 to February 2025. Ethical approval was obtained from the institutional committee, and written informed consent was obtained from all participants prior to enrollment.

2.2 Study Population

One hundred patients with confirmed diabetes mellitus (type 2) were enrolled consecutively from the neurology and general medicine outpatient departments. Inclusion criteria required age 18-85years and a diagnosis of diabetes mellitus with or without clinical symptoms of neuropathy. Patients with chronic kidney disease, congestive heart failure, liver disease, autoimmune disorders, known genetic disorders, or pregnancy were excluded to minimize confounding effects on biomarker levels. A cohort of 50 healthy volunteers without diabetes or known neurological conditions served as healthy controls (HC) for comparative biomarker analysis.

2.3 Clinical Assessment and Neuropathy Classification

All participants underwent a standardized clinical assessment including neurological examination and neuropathy screening using the 10-g Semmes-Weinstein monofilament test and the 128-Hz vibration tuning fork. DPN severity was classified using the Toronto Clinical Neuropathy Scoring System (TCNS), which incorporates symptom scores (pain, numbness, tingling, weakness, ataxia, upper-limb symptoms), reflex scores (knee and ankle), and sensory test scores (pin prick, temperature, light touch, vibration, position sense). TCNS categories were: no neuropathy (0-5), mild (6-8), moderate (9-11), and severe (≥12). For analytical purposes, diabetic patients without clinical neuropathy on TCNS were designated the DNN (Diabetic Non-Neuropathy) group.

2.4 Blood Sample Collection and Processing

Venous blood samples were collected in EDTA tubes following an 8-12 hour fast. Samples were centrifuged, and plasma was stored at -80°C pending analysis. Routine biochemical parameters including fasting blood glucose, HbA1c, complete blood picture, lipid profile (cholesterol, HDL, LDL, triglycerides), and serum albumin were determined using standard automated laboratory analyzers.

2.5 RNA Extraction and RT-qPCR

Total RNA was extracted from peripheral blood mononuclear cells using a standardized isolation protocol. RNA purity and concentration were assessed by NanoDrop spectrophotometry; integrity was confirmed by gel electrophoresis. Complementary DNA (cDNA) was synthesized using 100 ng total RNA in a 12 µL reaction with reverse transcriptase and random hexamer primers at 37-55°C for 30-60 minutes.

RT-qPCR was performed using SYBR Green master mix with gene-specific forward and reverse primers for TNF-α, IL-6, NSE, CD144 (VE-cadherin), and CD62E (E-selectin). GAPDH and β-actin were used as endogenous reference genes. Thermal cycling conditions included initialdenaturation at 95°C for 2-3 minutes, followed by 40 cycles of 95°C for 15-30 seconds and 55-65°C for 15-30 seconds. Relative quantification was calculated using the $2^{-}\Delta\Delta Ct$ method.

2.6 Statistical Analysis

Descriptive statistics were expressed as mean ± standard deviation (SD). Differences in biomarker expression across DPN severity groups were analyzed by one-way ANOVA, followed by post-hoc multiple comparisons using Tukey's HSD test. Pearson or Spearman correlation analyses were performed to examine associations between biomarker levels and clinical/biochemical parameters (HbA1c, serum albumin). Statistical significance was set at $p < 0.05$. Analyses were performed using SPSS version 25.0.

3. RESULTS

3.1 Study Population Characteristics

A total of 100 diabetic patients were enrolled alongside 50 healthy controls. The study population comprised 57% male and 43% female patients. The age distribution approximated a normal curve, with most patients falling in the 40-70 year range and peak frequency in the 50-60 year age group, consistent with known epidemiological patterns of DPN risk increasing with age and diabetes duration. Among patients, 20 reported smoking, 35 alcohol use, and 12 tobacco use; patients with multiple concurrent substance use showed the highest indices of oxidative stress.

3.2 Neuropathy Severity Classification

Using TCNS classification, 38 patients were asymptomatic (DNN), 36 had mild neuropathy, 23 had moderate neuropathy, and 3 had severe neuropathy. The preponderance of asymptomatic and mild cases is consistent with early-stage detection in a prospective study and may reflect the benefit of active screening.

3.3 One-Way ANOVA Results for Biomarker Expression

All five biomarkers demonstrated highly statistically significant differences across DPN severity groups (Table 1).

Table 1. One-Way ANOVA Results for Serum Biomarker Expression Across DPN Severity Stages (HC, DNN, Mild, Moderate, Severe).

Biomarker	F-Statistic	p-Value	Significance
NSE	57.94	3.53×10^{-27}	Significant
IL-6	264.02	1.29×10^{-50}	Highly Significant
TNF-α	120.28	2.03×10^{-29}	Highly Significant
CD62E	249.22	4.46×10^{-50}	Highly Significant
CD144	103.90	3.99×10^{-24}	Highly Significant

3.4 Biomarker Trends Across DPN Stages

IL-6, TNF-α, NSE, and CD62E demonstrated consistent progressive increases from the HC group through DNN, mild, moderate, and severe DPN stages, with statistically significant pairwise differences on post-hoc testing ($p < 0.01$ to $p < 0.001$). CD144 showed a divergent pattern: expression was markedly elevated in the DNN group relative to HC ($p < 0.001$), but declined progressively in moderate and severe neuropathy, reflecting evolving endothelial dysfunction. Biomarker elevation was detectable even in DNN patients who had no clinical symptoms of neuropathy, suggesting that molecular changes precede clinical manifestations.

3.5 Correlation with Serum Albumin

Serum albumin exhibited an inverse relationship with inflammatory markers TNF-α and IL-6 and with CD62E, consistent with the known role of albumin as a negative acute-phase protein. A weak positive correlation was observed between serum albumin and NSE, suggesting that in patients with better nutritional and metabolic status, neuronal stress markers remained relatively elevated, potentially reflecting subclinical nerve injury preceding overt clinical decline. The relationship between albumin and CD144 was weakly positive, with notable variability, possibly reflecting heterogeneity in endothelial repair capacity.

3.6 Correlation with HbA1c

A positive correlation was observed between HbA1c and all five biomarkers across the patient cohort (HbA1c range 5-11%). Patients with higher HbA1c levels exhibited elevated inflammatory and neuronal markers, supporting the critical role of chronic glycemic dysregulation in driving neuroinflammation, oxidative stress, and microvascular damage. However, a subset of patients with very high HbA1c (≥10%) demonstrated relatively lower biomarker levels, potentially indicative of genetic resilience, early disease stage, or compensatory mechanisms warranting further investigation.

3.7 Age-Wise Biomarker Distribution

The 55-65 year age group exhibited the highest frequency and peak biomarker values across all five markers. Individuals over 65 showed the greatest variability in biomarker expression, consistent with heterogeneous disease progression influenced by glycemic control, comorbidities, and individual susceptibility. Younger patients (<50 years) generally had lower biomarker levels, reflecting shorter diabetes duration and relatively preserved nerve function.

4. DISCUSSION

This prospective study provides evidence supporting the clinical utility of a multi-biomarker serum panel for the early detection and severity stratification of diabetic peripheral neuropathy. The highly significant ANOVA results across all five biomarkers underscore their potential as objective, quantifiable indicators of neuropathy progression.

The progressive elevation of IL-6 across DPN stages aligns with established literature demonstrating IL-6 as a key mediator of neuroinflammation. IL-6 sustains the pro-inflammatory milieu through activation of the JAK/STAT3 signaling cascade, sensitizes peripheral nociceptors, and amplifies the effects of other neuropathic pain mediators. Notably, the significant elevation of IL-6 in the DNN group indicates that inflammatory dysregulation precedes clinical neuropathy, corroborating findings by Chanda et al. (2022) showing higher IL-6 in painful versus painless DPN.

Similarly, the progressive rise in TNF-α with worsening DPN is consistent with its pathogenic roles: inducing Schwann cell apoptosis, promoting demyelination, activating NF-κB-mediated neuro-inflammatory cascades, and reducing nitric oxide availability in the endoneurial vasculature. The approximately 2.6-fold greater risk of DPN among diabetic patients with elevated TNF-α reported in prior meta-analyses is consistent with the trend observed here. The therapeutic implication is significant; inhibition of TNF-α in animal models has improved nerve conduction velocity and structural integrity.

NSE elevation across DPN stages reflects the extent of neuronal injury and provides a direct correlate of axonal degeneration. Its significant elevation even in the mild neuropathy group, and the statistically significant difference from HC, supports the potential for NSE to serve as an earlyneuronal biomarker. This is consistent with Morgenstern et al. (2022), who identified NSE as predictive of both hypo- and hyperalgesia in DPN patients.

The divergent CD144 pattern, with peak expression in DNN patients and subsequent decline in advanced neuropathy, is physiologically interpretable. In early diabetic endothelial stress, VE-cadherin upregulation may represent a compensatory response to maintain junctional integrity. As disease advances, endothelial cell loss and progressive blood-nerve barrier disruption lead to falling CD144 expression, consistent with the endothelial failure seen in advanced diabetic microvascular disease. This non-linear pattern may complicate its use as a simple linear severity biomarker but underscores its importance as an indicator of early vascular change.

CD62E (E-selectin), by contrast, showed consistent progressive increases with neuropathy severity, reflecting escalating endothelial inflammation and leukocyte recruitment to the nerve microvasculature. Its highly significant F-statistic (249.22, $p = 4.46\times10^{-30}$) suggests it may be among the most robust biomarkers for tracking DPN progression.

The inverse associations between serum albumin and inflammatory markers (TNF-α, IL-6, CD62E) corroborate prior research demonstrating that hypoalbuminemia reflects systemic inflammation and is independently associated with increased DPN prevalence. ROC analysis in prior studies has identified a serum albumin cut-off of 39.95 g/L for predicting DPN in T2DM patients. Our data reinforce the concept that nutritional optimization may attenuate inflammatory biomarker burden and reduce neuropathy risk.

The positive HbA1c-biomarker correlations confirm the primacy of chronic glycemic control in DPN pathogenesis. The mechanistic basis involves AGE accumulation, PKC activation, and mitochondrial superoxide overproduction, all downstream of persistent hyperglycemia. These findings align with the DCCT/EDIC and UKPDS trial data demonstrating that tight glycemic control reduces DPN incidence and progression.

Male predominance (57% vs. 43%) in this cohort is consistent with epidemiological data suggesting that estrogen may exert neuroprotective effects, attenuating oxidative stress and inflammatory signaling in peripheral nerves. Additionally, higher rates of metabolic syndrome, cardiovascular risk factors, and substance use in male patients likely contribute to the differential.Alcohol use was the most prevalent substance use pattern in this cohort, with implications for neuropathy risk that are independent of diabetes. Alcohol-related neuropathy and diabetic neuropathy share overlapping pathomechanisms including oxidative stress and mitochondrial dysfunction, potentially accelerating neuropathy progression in co-affected patients.

5. Limitations

This study has several limitations. The sample size of 100 patients, while adequate for demonstrating statistical significance, limits subgroup analyses, particularly for the severe neuropathy group (n=3). The single-center design and five-month recruitment window may limit generalizability. The study did not include longitudinal follow-up to assess predictive validity of biomarker changes over time. The RT-qPCR approach measures mRNA expression in peripheral blood cells, which is a surrogate for, rather than direct measurement of, serum protein levels; future studies should validate these findings with ELISA-based protein quantification. Variability in individual metabolic parameters, treatment regimens, and disease duration was not fully controlled.

6. CONCLUSION

This prospective study demonstrates that serum biomarkers NSE, IL-6, TNF-α, CD62E, and CD144 exhibit highly significant variation across DPN severity stages, with detectable changes even in clinically asymptomatic diabetic patients. These findings support the potential of a multi-biomarker panel as a non-invasive, early diagnostic tool for diabetic peripheral neuropathy that complements existing clinical scoring systems. Incorporation of biomarker screening into routine diabetic follow-up could facilitate earlier identification of at-risk patients, enabling timely pharmacological, nutritional, and lifestyle interventions to slow neuropathy progression. Future large-scale, longitudinal, multi-center studies are needed to establish standardized reference ranges, define optimal diagnostic cut-offs, and validate predictive models incorporating these biomarkers alongside conventional risk factors.

Declarations*: Ethics Approval and Consent to Participate*

The study was conducted in accordance with the Declaration of Helsinki. Institutional ethical clearance was obtained prior to study commencement. Written informed consent was obtained from all participants.

Competing Interests

The authors declare no competing interests.

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Authors' Contributions

CA, NM, SS, and SF conducted data collection, sample processing, and RT-qPCR analysis. SSahoo provided institutional supervision and manuscript guidance. MZ provided clinical supervision and neurological assessment. All authors reviewed and approved the final manuscript.

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