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Endocan expression and correlation with other endothelial determinants in developing a score for early identification of diabetic peripheral neuropathy

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Diabetic peripheral neuropathy is a common complication of diabetes mellitus that has dire outcomes, affecting the economic profile of a country. Despite the multifactorial background of the pathogenesis of this disease, the mechanism underlying peripheral neuropathy is still unclear. Endothelial damage is a new determinant of pathogenesis, with endocan as a potential biomarker for endothelial dysfunction in diabetic peripheral neuropathy patients. In this cross-sectional study, with 49 patients with diabetes with peripheral neuropathy and 48 patients with diabetes without neuropathy, endothelial biomarkers such as endocan, hs-CRP, vitamin D, and lipid profiles were measured and analyzed in both groups. The standardized scores for dyslipidemia, inflammation, vitamin D and endocan were calculated. All of these biomarkers were significantly altered in peripheral neuropathy patients. A strong correlation between endocan levels and lipid profiles and between hs-CRP and vitamin D levels was detected. The inflammatory score and a combined score including all the above biomarkers might help in the early stratification of diabetic patients who are at greater risk of developing peripheral neuropathy.

Keywords Blood-nerve barrier, Cytokines, DIVE score, Dyslipidemia, Endothelial, Inflammation

Abbreviations

IDF	International Diabetes Federation
DPN	Diabetic peripheral neuropathy
BNB	Blood-nerve-barrier
VEGF	Vascular endothelial growth factor
ABPI	Ankle Brachial Pressure Index
PAD	Peripheral artery disease
OPD	Out patient department
CLIA	Chemiluminescence immunoassay
BMI	Body mass index
LDL-C	Low-density lipoprotein cholesterol
HDL-C	High-density lipoprotein cholesterol
TG	Triglycerides
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
IQR	Interquartile range
ROC	Receiver operating characteristics curve

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The worldwide prevalence of diabetes mellitus has risen dramatically, with a diabetes incidence estimate in India of 125 million in 2045[1,2]. The most common complication is diabetic peripheral neuropathy(DPN), with a prevalence ranging from 18.84 to 45.6%[3–8]. DPN mainly involves small nerve fibers and is diagnosed after excluding peripheral neuropathies due to other causes[9]. With an insidious onset, symptoms in the early stages are rare, so symptoms eventually progress to the late stage, causing serious complications such as diabetic foot ulcers resulting in amputations affecting the quality of life[10].

DPN with a multifactorial pathophysiology has interactions between hyperglycemic vascular factors and metabolic cascade pathophysiological factors[11,12]. The vascular influence of altered function of the blood-nerve-barrier(BNB) with endoneurial microangiopathy seems to be the first marker for the development of DPN[13]. Histopathologically the endoneurial microvessels show as thickening of capillary basement membrane, loss of capillary pericyte coverage along with hyperplasia of endothelial cells and increased vascular permeability[11]. Furthermore, there is a continued electrolyte imbalance and thickening of the basal laminae, leading to progressive edema and ischemia of the neurons[13,14]. The above changes result in microcirculatory disturbances leading to tissue hypoxia and the promoting activity of vascular endothelial growth factor(VEGF) pathway to increase BNB permeability and thus BNB breakdown[15].

Endocan, secreted by endothelial cells and is expressed by tissues, including capillary endothelial cells and neurons[16]. Proinflammatory cytokines and VEGF, upregulate the expression of endocan releasing molecules that increase the adhesion of leukocytes to endothelial cells, promoting the migration of inflammatory cells and damage[16]. Thus, an increase in endocan may be a potential immune-inflammatory marker. A positive association between endocan and diabetes and microvascular complications such as diabetic retinopathy and diabetic neuropathy has been observed, indicating that endocan is a new biomarker of angiogenesis in patients with complications of diabetes[17,18].

The diagnosis of DPN is based on nerve conduction studies, wherein a positive finding is associated with late manifestation of DPN[10]. The screening methods proposed for the early detection of DPN are still not widely used due to their disadvantages. Therefore, it is necessary to develop a grading or scoring system to assess the severity of DPN, perform early intervention and improve the quality of life. Endocan is involved in endothelial dysfunction and a similar status has been suggested for the pathogenesis of DPN. However, studies conducted in this area are grossly inadequate, and therefore, this study could be of diagnostic and therapeutic importance for characterizing alterations in endocan in patients with diabetes and DPN.

Therefore, the aim of this study was to measure and analyze the angiogenic biomarker endocan between individuals with diabetes with and without neuropathy and to compare the inflammatory state among the groups.

Materials and methods

Study design

This was a hospital-based cross-sectional study conducted in the Department of Biochemistry and Endocrinology of our institute. The study was granted ethical approval by the Institute Ethics Committee vide Proposal No. AIIMSRPR/IEC/2022/1227 and Approval Ref No 2492/IEC-AIIMSRPR/2022, dated 26th September 2022. All procedures of the study followed the guidelines of the Declaration of Helsinki and Tokyo. The recruitment of subjects and analysis of the samples were performed between April 2023 and December 2023. The study population comprised 97 adult patients with diabetes who visited the OPD of the Department of Endocrinology for more than five years. The patients were categorized into two groups: those with diabetes with peripheral neuropathy (DPN, n = 49) and those with diabetes without peripheral neuropathy (No DPN, n = 48).

DPN was defined as impairment in one or more of the following criteria: a vibration perception threshold ≥ 25 V, heat pain threshold > 48 °C, or cold pain threshold < 10 °C. Conversely, the absence of peripheral neuropathy was determined by the presence of all specified conditions: a vibration perception threshold ≤ 15 V, heat pain threshold < 42 °C, and cold pain threshold > 20°C[19–21].

The exclusion criteria included diabetes with clinical evidence of cardiovascular disease like coronary, peripheral or carotid artery disease, major disease(hepatic or renal failure, malignancy, autoimmune disease and any acute or chronic infections), recent trauma or surgery within one month, thyroid disease, megaloblastic anemia, chronic alcoholism, and pregnancy.

Study procedure

Patients who satisfied the inclusion criteria were enrolled after providing informed written consent. The detailed clinical history of the patient and any recent biochemical data (within three months) were obtained from patient records. The demographic profiles of age and sex were recorded, and biophysical parameters such as height, weight, and systolic and diastolic blood pressure were measured. The ankle brachial pressure index (ABPI) was assessed to rule out peripheral arterial disease (PAD). Diabetic peripheral neuropathy assessment was performed on areas of both feet(great toe, first meta-tarsal, fifth metatarsal, medial arch, lateral arch and heel) of an individual by quantitative sensory testing (QST) using a Vibrotherm-Dx neuropathy analyzer.

Blood sampling

Blood samples for investigations, such as glucose (fasting and postprandial), urea, creatinine, lipid profile, hs-CRP, and vitamin D, were collected and processed the same day in an AU5800 autoanalyzer (Beckman Coulter, Inc.). HbA1c was measured by ion exchange high-performance liquid chromatography (HPLC) using D-10 equipment from Bio-Rad Laboratories, Inc. Serum vitamin D was estimated by chemiluminescence immunoassay (CLIA) in a Siemens Advia Centaur XP autoanalyzer. The plasma was collected in EDTA vials, centrifuged at − 4 °C and stored at − 80 °C for Endocan analysis.

Endocan assay

The quantitative determination of human endothelial cell-specific molecule-1/Endocan (ESM-1/Endocan) activity was performed based on the principle of a double-antibody sandwich enzyme immunoassay (ELISA) kit. (Coon Koon Biotech China, Cat No. CK-Bio11325, LOT No. 202304). The samples were processed within 2 months of collection and stored at − 80 °C to avoid loss of bioactivity. The intra-assay CV% was < 7%, with a sensitivity of 1 pg/mL.

Statistical analysis

All the statistical analyses were performed using Jamovi software version 2.3.26 (Sydney, Australia). The Shapiro–Wilk test was used to assess normality. Normally and nonnormally distributed data are represented as the mean and standard deviation (SD) and as the median and interquartile range (IQR), respectively. Comparisons were performed using Student's t test and the Mann−Whitney U test. Spearman's rho was used to determine the correlation of endocan with various biochemical parameters. Binomial logistic regression analysis was performed to identify the best predictive model, and based on this, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value of the ROC curve were measured. A p value < 0.05 was considered to indicate statistical significance.

Results

This study was performed to assess the difference in endocan levels between two groups of diabetic patients without peripheral neuropathy and those with peripheral neuropathy. According to the operational definitions, 49 diabetic patients suffered from peripheral neuropathy, whereas 48 diabetic patients did not have neuropathy.

The clinico-anthropometric measurements of the study population are summarized in Table 1. The two groups were similar in age and sex distribution. A comparable height, weight, BMI, and systolic and diastolic blood pressure were observed in both groups.

Table 2 compares the biochemical variables between the study groups. Fasting plasma glucose (FPG) did not significantly differ between the groups ($p = 0.198$), while postprandial plasma glucose (PPPG) was significantly greater in the DPN group ($p = 0.015$). Notably, the DPN group exhibited elevated HbA1c levels compared to those in the non-DPN group ($p = 0.006$). Additionally, significant differences were observed in total cholesterol ($p = 0.005$), TG ($p = 0.003$), HDL cholesterol ($p < 0.001$), urea ($p < 0.001$), creatinine ($p = 0.004$), eGFR ($p = 0.004$), hs-CRP ($p = 0.002$), vitamin D ($p < 0.001$), and Endocan ($p < 0.001$).

To calculate the dyslipidemia score, we standardized all the variables by calculating Z scores for LDL-C, TG and HDL-C using the mean and standard deviation. Thereafter, the dyslipidemia score was calculated according to the following equation: average of the standardized variables (LDL-C + TG)/2 minus standardized HDL-C[22]. The inflammation score was also determined in both groups after standardization of hs-CRP; similarly, the standardized vitamin D and standardized endocan levels were determined. Figure 1 shows high standardized dyslipidemia ($p = 0.007$*), high standardized inflammation markers such as hs-CRP ($p < 0.001$*), high standardized endocan ($p = 0.004$*) and low standardized vitamin D ($p < 0.001$*) scores between diabetic patients with and without neuropathy. These results highlight significant differences in these parameters between the two groups, providing insights into potential associations with diabetic neuropathy.

The results from Spearman's correlation analysis of the interrelationship of endocan with various biochemical parameters and standardized scores are presented in Table 3. Notably, endocan was significantly positively correlated with T-CSF, TG, LDL, urea, creatinine, standardized hs-CRP, and the standardized Dys score. Conversely, it exhibits a significant negative correlation with HDL, eGFR and standardized vitamin D. These findings suggest potential associations between endocan levels and the abovementioned variables, shedding light on its potential implications in various physiological processes.

With the above findings, the authors assessed the influence and extent of dyslipidemia, inflammation and endothelial factors as comprehensive DIVE scores in both groups. The DIVE score, as shown in Fig. 2, was significantly greater ($p < 0.021$) in patients with DPN than in those without DPN. This finding implies that the

Parameter	No DPN (n = 48)	DPN (n = 49)	95% Confidence interval	p value
Age (years)	53.81 ± 7.60	53.71 ± 6.43	−0.411–0.384	0.945
Males: Females	27:21	36:13	0.918–5.05	0.07
Duration of disease (years)	8 (9)	10 (6)	−2.00–2.00	0.733
Weight (kg)	65.43 ± 11.59	66.76 ± 9.02	−0.268–0.529	0.521
Height (metre)	1.55 ± 0.06	1.57 ± 0.08	−0.208–0.592	0.345
BMI (kg/m^2)	26.86 ± 4.00	27.66 ± 4.58	−0.214–0585	0.361
Systolic BP (mmHg)	128.45 ± 14.11	133.38 ± 16.79	−0.087–0.719	0.121
Diastolic BP (mmHg)	81.43 ± 6.32	81.36 ± 7.45	−0.408–0.388	0.960

Table 1. Clinico-anthropometric measurements in the study group. The data are presented as the number of subjects, mean ± SD or median (IQR). *BMI* body mass index; *BP* blood pressure. p was calculated by the chi-square test for categorical data and Student's t test or the Mann−Whitney U test for continuous data.

Parameter	No DPN (n = 48)	DPN (n = 49)	95% Confidence Interval	*p* value
FPG (mg/dL)	129 (67.5)	149 (80)	− 0.351–0.445	0.198*
PPPG (mg/dL)	218.5 (125.5)	280 (97)	− 0.028–0.782	0.015*
HbA1C (%)	7.9 (2.4)	9.3 (3.3)	0.102–0.925	0.006*
T. Chol (mg/dL)	161.18 ± 40.53	185.49 ± 42.60	0.166–0.996	0.005**
TG (mg/dL)	106.5 (45.5)	142 (64)	− 0.233–0.565	0.003*
HDL-C (mg/dL)	43.49 ± 8.0	37.24 ± 9.20	− 1.144 to − 0.297	< 0.001**
LDL-C (mg/dL)	87.18 ± 29.27	99.23 ± 31.52	− 0.011–0.800	0.054**
Urea (mg/dL)	25.56 ± 6.24	34.79 ± 12.58	0.483–1.361	< 0.001**
Creatinine (mg/dL)	0.94 (0.20)	1.0 (0.4)	0.185–1.017	0.004*
eGFR (ml/min/1.73 m^2)	83.05 (23.20)	68.58 (32.74)	− 1.012 to − 0.181	0.004*
hs-CRP (mg/L)	4.1 (7.02)	14 (15.51)	0.218–1.054	0.002*
Vitamin D (ng/mL)	23.8 (14.69)	17.0 (11.55)	− 1.168 to − 0.319	< 0.001*
Endocan (pg/mL)	1699.81 (372.47)	1945.41 (627.04)	0.539–1.428	< 0.001*

Table 2. Comparison of biochemical variables between the study groups. The data are presented as the means ± SDs or medians (IQRs). *FPG* fasting plasma glucose; *PPPG* postprandial plasma glucose; *HbA1C* glycated hemoglobin; *T* total cholesterol; *TG* triglycerides; *HDL* high-density cholesterol; *LDL* low-density lipoprotein; *eGFR* estimated glomerular filtration rate. The *p* value was calculated by the *Mann—Whitney U test and **Student's t test.

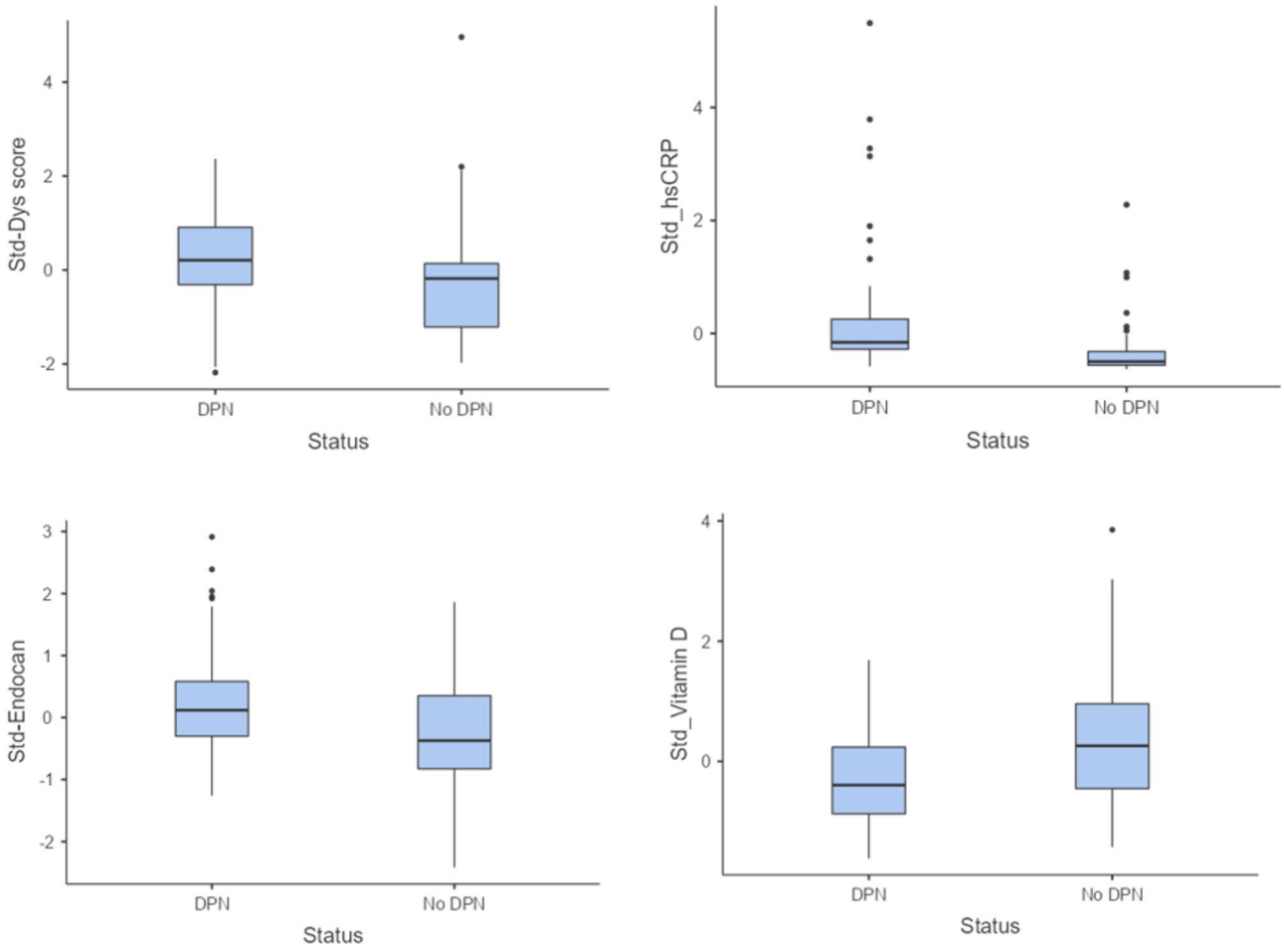

Figure 1. Comparison of standardized dyslipidemia*, standardized inflammation*, standardized vitamin D* and standardized endocan** scores between diabetic patients with and without peripheral neuropathy. * Mann—Whitney U test, ** Student's t test.

Parameter	Spearman's rho	95% CI	*p* value
T. Chol(mg/dL)	0.219	0.035–0.413	0.031
TG(mg/dL)	0.213	− 0.089–0.305	0.037
HDL-C(mg/dL)	− 0.287	− 0.448 to − 0.077	0.004
LDL-C(mg/dL)	0.200	− 0.042–0.347	0.049
Urea(mg/dL)	0.295	0.115–0.477	0.003
Creatinine(mg/dL)	0.203	0.036–0.414	0.046
eGFR(ml/min/1.73 m^2)	− 0.247	− 0.247 to − 0.323	0.015
Std_hsCRP	0.242	− 0.129–0.268	0.017
Std_Vitamin D	− 0.295	− 0.471 to − 0.107	0.003
Std_Dys Score	0.289	− 0.049–0.425	0.004

Table 3. Correlations of endocan with various biochemical parameters and various standardized scores. *Std* standardized.

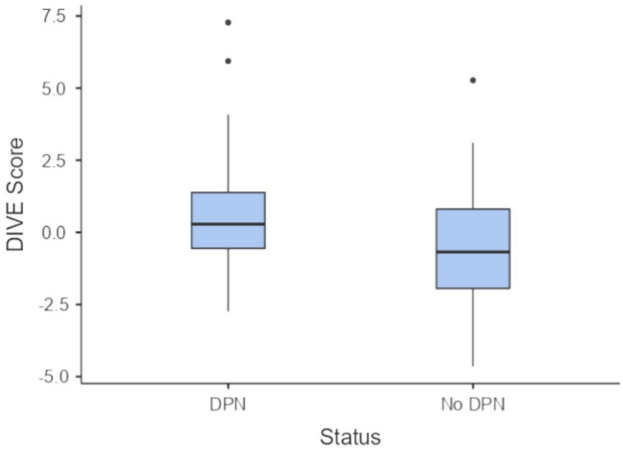

Figure 2. Box plot comparing DIVE scores between the DPN group and the non-DPN group (p = 0.021).

DIVE score could serve as a useful metric for distinguishing between these two groups in the context of diabetic peripheral neuropathy.

In-depth independent associations of clinical data were tested with binomial logistic regression analysis to determine the influence of endocan, Std hs-CRP, the Std Dys score, Std Vit D and the DIVE score on DPN. The best models that determined the variability in the distinct scores are shown in Table 4. Predictive models using multivariate logistic regression identified endocan and the other standardized scores as independent predictors for DPN (odds ratio of endocan: 1.002, $p = 0.002$; odds ratio of Std hsCRP: 2.672, $p = 0.017$; odds ratio of Std Dys score: 1.118, $p = 0.05$; odds ratio of Std Vit D: 0.480, $p = 0.019$; odds ratio of DIVE score: 1.28, $p = 0.022$). The adjusted R^2 indicated that approximately 28% of the variability in Std Dys score values, 45.3% of the variability in hsCRP, 34.8% of the variability in vitamin D, and 27.2% of the variability in endocan were related to DPN. The adjusted R^2 indicated that approximately 7.8% of the DIVE score was responsible for DPN.

The ROC curve (Fig. 3) and analysis in Table 5 reveal the diagnostic efficacy of various biomarkers in distinguishing diabetic peripheral neuropathy (DPN) patients from diabetic patients without DPN. Notably, hs-CRP had a robust AUC of 0.781, indicating high discriminative power. The standardized Dys score, with a cutoff of 0.07, exhibited moderate sensitivity (63.27%) and specificity (72.92%). Despite its high sensitivity (89.8%), vitamin D has a low specificity (6.25%) at a cutoff of 9.73. Endocan achieved a balanced performance

Predictor	Estimate	Z	95% Confidence Interval	Odds Ratio	Nagelkerke's R^2	*p* value
Std Dys score	0.11233	2.212	0.339–1.695	1.118	0.280	0.05
Std hsCRP	0.98287	2.393	1.195–5.977	2.672	0.453	0.017
Std Vit D	− 0.73292	− 2.353	0.261–0.885	0.480	0.348	0.019
Endocan	0.00293	3.147	1.001–1.005	1.002	0.272	0.002
DIVE score	0.2479	2.295	1.037–1.58	1.28	0.078	0.022

Table 4. Predictive models to identify independent predictors of diabetic peripaheral neuropathy.

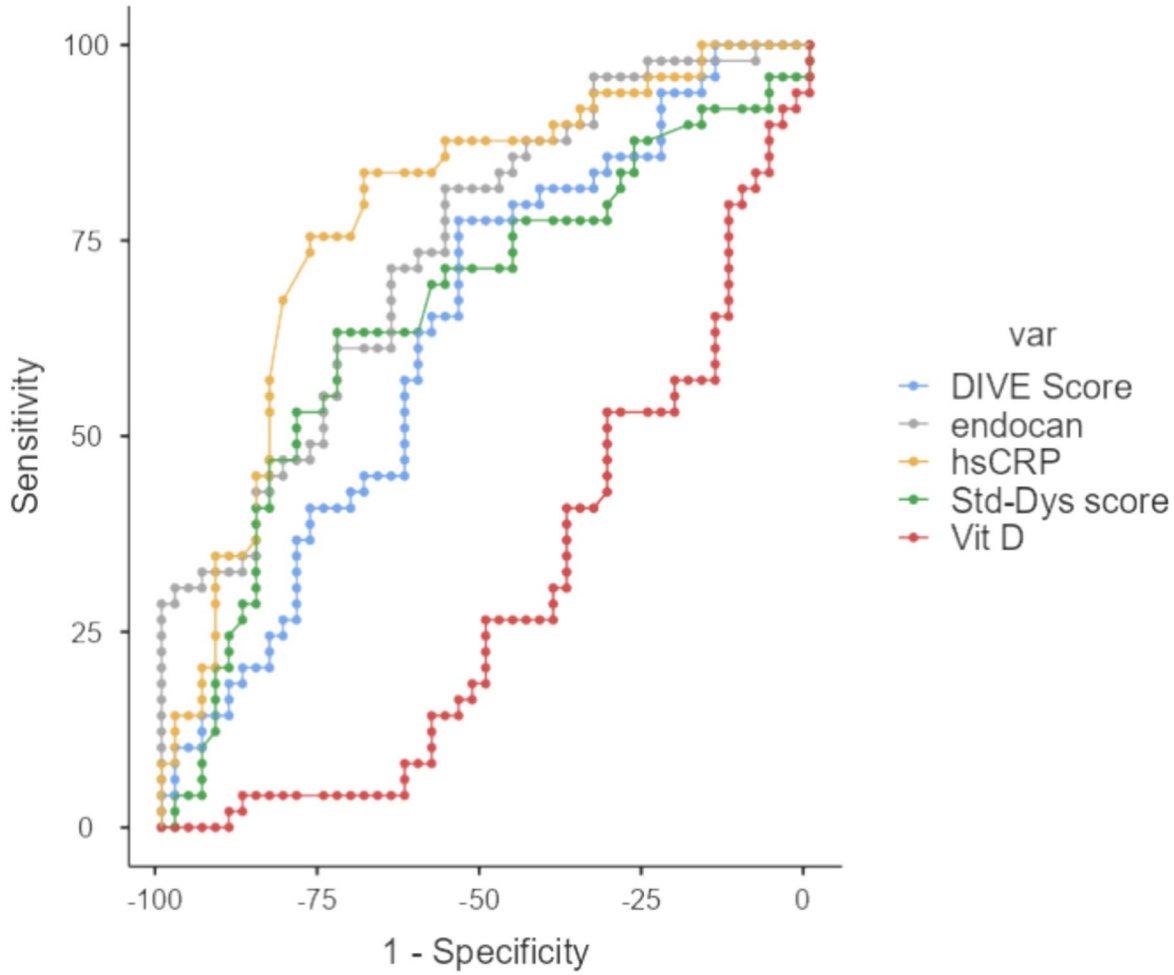

Figure 3. ROC curve of the significant biomarkers as predictors of diabetes with peripheral neuropathy.

Parameter	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	95% Confidence interval
Std Dys score	0.07	63.27	72.92	70.45	66.04	0.658	0.549–0.966
hsCRP	10.49	75.51	77.08	77.08	75.51	0.781	0.689–0.872
Vitamin D	9.73	89.8	6.25	49.44	37.5	0.30	0.195–0.404
Endocan	1727.95	81.63	56.25	65.57	75	0.739	0.640–0.837
DIVE score	− 0.58	77.55	54.17	63.33	70.27	0.636	0.525–0.846

Table 5. Cutoff, sensitivity (%), specificity (%), PPV (%), NPV (%), and AUC of the various biomarkers for differentiating diabetes patients with peripheral neuropathy from those without peripheral neuropathy.

with sensitivity (81.63%) and specificity (56.25%), resulting in an AUC of 0.739. At a cutoff of -0.58, the DIVE score had a moderate AUC of 0.636, reflecting satisfactory sensitivity (77.55%) and specificity (54.17%). These findings provide valuable insights into the ability of biomarkers to accurately identify individuals with DPN.

Discussion

To the best of our knowledge, this is the first study to explore the complex interplay between immune-inflammatory markers, dyslipidemia, vitamin D levels, and endocan in patients with diabetes and peripheral neuropathy. The increasing global prevalence of diabetes mellitus and its associated complications, including DPN, emphasizes the urgency for in-depth investigations to unveil potential biomarkers and pathways for early diagnosis that can aid early intervention.

First, the study revealed a significant association between dyslipidemia and DPN. The dyslipidemia score, which incorporates the standardized variables LDL-C, TG, and HDL-C, revealed distinct differences between diabetic patients with and without neuropathy. Dyslipidemia has long been a modifiable risk factor for the

incidence and progression of DPN[23].The interaction of dyslipidemia with the entry of long-chain fatty acids through the blood–neutrophil barrier triggers neurogenic inflammation and the release of a cascade of cytokines and chemokines, promoting tissue injury. The role of a high-fat diet in inducing DPN and subsequent dietary reversal, demonstrating the normalization of neuropathy, adds importance to the role of dyslipidemia in the pathogenesis of DPN[24]. Recent lipidomic studies revealed alterations in lipid classes related to mitochondrial dysfunction in patients who developed peripheral neuropathy[25].

Second, the study explored the status of inflammation in the two groups of patients with diabetes with and without peripheral neuropathy. The inflammation score, determined by standardized hs-CRP levels, demonstrated a pronounced difference between the two groups. In this study, both groups exhibited a cluster of metabolic factors characterized by mild chronic inflammation that shifted the immune microenvironment to a proinflammatory state. In one follow-up study, hs-CRP above 2.5 mg/L was shown to predict the development of diabetic neuropathy[26]. Inflammatory cells in diabetic nerves release excessive TNF-α or interleukins and activate the mitogen activated protein (MAP)-kinase pathway and NF-κB pathway, causing microangiopathy in diabetic neuropathy[27].

Moreover, this study examined the potential impact of vitamin D deficiency on DPN. Evidence from studies has shown that vitamin D deficiency might affect the development of DPN[28–30]. These studies suggest early monitoring and evaluation of vitamin D in the early stages of disease. Moreover, vitamin D increases the level of nerve growth factor, preventing neurotrophic deficits. The vitamin D receptor is highly expressed in the dorsal ganglion of small fibers that respond to pain, an explanation for painful neuropathy in diabetes patients[31] In this study, standardized vitamin D levels were significantly different between individuals with and without DPN, supporting the hypothesis that vitamin D status may play a role in the pathogenesis of painful DPN through elevated inflammation.

Although there are diverse underlying factors in the pathogenesis of DPN, endothelial dysfunction is a mainstay and crucial aspect. This leads to altered blood–nerve–barrier permeability, impacting nutrition and oxygen supply and subsequent demyelination of nerves in DPN patients[12]. A novel aspect of the study involves the investigation of endocan as a potential biomarker for DPN. Endocan levels were greater in patients with DPN than in those without DPN. Endocan, a secreted and expressed vascular endothelial cell, regulates endothelium activation, permeability, and proliferation. It is controlled by proinflammatory cytokines and is increased in inflammation and disorders such as atherosclerosis and hypertension[32]. Since it affects inflammatory and vasculoprotective signals, it may be a marker of endothelial dysfunction. A meta-analysis by Khalaji A et al. revealed that endocan levels are increased in diabetic patients and are greater in diabetic patients with complications; therefore, endocan may play a functional role in endothelium-dependent pathological disorders such as DPN[33]. In this study, correlation analysis revealed associations between endocan and various biochemical parameters, emphasizing the potential relevance of these parameters for endothelial dysfunction in DPN pathophysiology. Considering these findings, endocan and a combination of these biomarkers might be suitable candidates for early identification of the risk of developing complications such as peripheral neuropathy in diabetes patients.

The study's integrative approach culminated in the development of a comprehensive DIVE score, incorporating dyslipidemia, inflammation, vitamin D, and endocan scores. The DIVE score demonstrated a statistically significant difference between individuals with and without DPN, suggesting its potential utility as a metric for distinguishing between these two groups. The predictive models, derived from multivariate logistic regression analysis, highlighted the independent predictive value of endocan, standardized hs-CRP, standardized dyslipidemia score, standardized vitamin D, and the DIVE score for DPN. These findings underscore the multifactorial nature of DPN, involving dyslipidemia, inflammation, and vitamin D status, with endocan emerging as a potential key player. The ROC analysis evaluated the cutoff value with the highest Youden's index of various biomarkers. Individually, hs-CRP exhibited high discriminative power, and the DIVE score demonstrated moderate sensitivity and specificity, reinforcing the potential utility of this integrated approach in clinical settings. Future studies are needed to assess the predictive ability of the DIVE score for DPN onset. Some individuals in the cohort without DPN might be at risk, which could explain the poorer AUC for the DIVE score.

Strengths and limitations

To the best of our knowledge, this is the first study in the Indian subcontinent to comprehensively characterize direct and surrogate endothelial biomarkers that are related to diabetic neuropathy. Using these biomarkers, the authors have derived a novel summary score (DIVE score) for the first time that contributes to the potential development of risk prediction tools, aiding clinicians in identifying individuals at greater risk for DPN. Although QST for DPN, involves subjective assessments, since outcomes depend on patient responses, we have used clear cut-off points for severely impaired sensory thresholds.

However, to reach well-reasoned conclusions about our new findings, prospective studies are needed to measure the ability of these combined biomarkers and scores to mitigate the risk of DPN. Future studies should be done to assess the association of the DIVE score and endocan with objective assessments for early neurodegeneration, including corneal confocal microscopy, skin biopsies, and nerve conduction studies.

Conclusion

In conclusion, this study revealed the overexpression of endocan in patients with DPN. This research contributes valuable insights into the intricate web of factors influencing DPN in individuals with diabetes mellitus. This comprehensive approach, encompassing dyslipidemia, inflammation, vitamin D, and endocan, provides a holistic perspective on the pathogenesis of DPN. The findings pave the way for further research and potential clinical applications aiming to improve early diagnosis and intervention for DPN, ultimately enhancing the quality of life for individuals with diabetes.

Data availability

Data and materials supporting the results of this article are included within the article.

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Author contributions

P.P.J.: Sample collection, gathering clinical data, and initial draft of the paper. R.N.: Conception, design, and conduct of the study and edited and approved the final version of the manuscript. A.G.: Design of study, resources, investigation, edited the manuscript. S.P.: Conceptualization, analysis, writing—review and editing of manuscript S.S.: Supervision, investigation, data curation, manuscript editing. E.M.: Design of study, investigation, supervision, editing the final draft. All authors have read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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