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Early Neuroinflammatory Signatures and Glucagon-Like Peptide 1 Levels in Neuropathic Symptoms: Evidence from Newly Diagnosed Type 2 Diabetes Mellitus

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Abstract

Aim

Neuropathic symptoms are among the earliest neurological manifestations of type 2 diabetes mellitus (T2DM) and may precede electrophysiologically confirmed diabetic sensorimotor polyneuropathy (DSPN). The underlying mechanisms in treatment-naïve individuals remain incompletely understood. This study investigated the associations of serum glucagon-like peptide-1 (GLP-1), tumor necrosis factor-alpha (TNF-α), and intercellular adhesion molecule-1 (ICAM-1) with neuropathic symptoms in newly diagnosed, treatment-naïve patients with T2DM.

Methods

This case-control study included 150 participants: 50 newly diagnosed T2DM patients with neuropathic symptoms (defined by DN4 ≥ 4 and compatible clinical findings), 50 newly diagnosed T2DM patients without neuropathic symptoms, and 50 healthy controls. Nerve conduction studies were performed in all participants and were predominantly within normal limits, indicating a symptom-defined neuropathic phenotype rather than electrophysiologically confirmed DSPN. Fasting serum GLP-1, TNF-α, and ICAM-1 concentrations were measured using ELISA. Multinomial and binary logistic regression models were adjusted for age, sex, BMI, HbA1c, blood pressure, and lipid profile.

Results

Higher GLP-1 concentrations were independently associated with neuropathic symptoms (adjusted OR 1.09; 95% CI 1.01–1.18; p = 0.037). TNF-α levels were inversely associated with neuropathic symptoms (adjusted OR 0.94; 95% CI 0.89–0.99; p = 0.048). ICAM-1 levels were higher in diabetic participants compared with controls (adjusted OR 1.03; 95% CI 1.00–1.06; p = 0.029).

Conclusion

In newly diagnosed, treatment-naïve T2DM, GLP-1, TNF-α, and ICAM-1 levels were associated with neuropathic symptom status. These cross-sectional findings do not establish causality and warrant confirmation in longitudinal studies incorporating objective measures of peripheral nerve structure and function.

INTRODUCTION

Neuropathic manifestations are among the most common and disabling neurological complications of type 2 diabetes mellitus (T2DM), affecting up to half of patients during the course of the disease. These manifestations reflect structural and functional alterations of peripheral nerves and may involve both small- and large-fiber dysfunction, and leads to symptoms such as burning, numbness, and pain in a length-dependent manner, typically beginning in the distal lower limbs [1]. As diabetic neuropathic involvement progresses, serious consequences such as foot ulceration, gait disturb-ances, and lower-limb amputation may occur, leading to significant impairment in quality of life and increased mortality risk.

Although hyperglycemia has long been considered the primary driver of diabetic neuropathy, it is now well established that neuropathic symptoms may develop even in the early stages of T2DM or in individuals with prediabetes before the onset of sustained hyperglycemia [2, 3]. This observation suggests that additional mechanisms contribute to neural injury independently of glucose toxicity. Indeed, recent studies have highlighted the roles of oxi-dative stress, mitochondrial dysfunction, impaired insulin signaling, lipid-induced toxici-ty, and low-grade inflammation in the pathogenesis of neuropathy [1, 4].

Emerging consensus statements emphasize that confirmed diabetic sensorimotor polyneuropathy (DSPN) requires a combination of neuropathic symptoms, clinical signs, and objective abnormalities such as nerve conduction studies (NCS) to increase diagnostic certainty. For research grading, at least two abnormalities among neuropathic symptoms, clinical signs, nerve conduction studies (NCS), quantitative sensory testing (QST), or quantitative autonomic reflex testing are recommended to increase diagnostic certainty [5]. Although NCS remains one of the technical gold-standard tools, it primarily reflects large-fiber function and is limited by accessibility, examiner dependence, and low sensitivity in early disease. In diabetes and prediabetes, small-fiber involvement frequently precedes large-fiber abnormalities; therefore, quantitative small-fiber assessments—such as QST, intraepidermal nerve fiber density from skin biopsy, or corneal confocal microscopy (CCM)—have been increasingly emphasized as objective metrics of early neural injury [6].

Among these, neuroinflammation has emerged as a pivotal early event in the development of diabetic neuropathy. Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and endothelial adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) have been shown to mediate microvascular injury, endothelial dysfunction, and leukocyte infiltration into neural tissue [7, 8]. ICAM-1 in particular is upregulated in re-sponse to inflammatory stimuli and facilitates immune cell adhesion to vascular endothe-lium, contributing to perineural inflammation and neural ischemia [9]. Moreover, TNF-α can upregulate ICAM-1 expression, perpetuating a vicious cycle of neurovascular inflammation [8].

In parallel, endogenous counter-regulatory molecules have also been explored. Glucagon-like peptide-1 (GLP-1), an incretin hormone primarily known for its glucose-lowering effects, has demonstrated anti-inflammatory and neuroprotective actions in experimental models of diabetes [4, 10]. GLP-1 receptor agonists have been shown to attenuate inflammatory cytokine production, reduce oxidative stress, and improve nerve conduction velocity and structural integrity via modulation of the p38 MAPK and NF-κB signaling pathways [4, 10]. However, whether GLP-1 also plays a compensatory or pathological role in the earli-est stages of diabetic neuropathic involvement in humans particularly before treatment initiation remains unclear.

Although several studies have independently investigated TNF-α, ICAM-1, or GLP-1 in diabetic neuropathy, limited data are available evaluating these markers concurrently in newly diagnosed and treatment-naïve patients. Furthermore, the potential associations between endogenous GLP-1 levels and inflammatory pathways have not been clarified in the early neuroinflammatory phase of diabetic neuropathic involvement.

In this study, we aimed to investigate the relationship between fasting GLP-1 levels and the neuroinflammatory markers TNF-α and ICAM-1 in patients with newly diagnosed T2DM, both with and without neuropathic symptoms defined by clinical evaluation. Our goal was to better characterize non-glycemic contributors to early nerve injury and to explore the relationship between circulating GLP-1 levels and neuropathic symptom status in early T2DM. We hypothesized that fasting GLP-1, TNF-α, and ICAM-1 concentrations would differ according to neuropathic symptom status in newly diagnosed and treatment-naïve patients with T2DM.

MATERIALS AND METHODS

Study Population and Design

This study was designed as a case-control study. Newly diagnosed T2DM was defined as a first diagnosis of diabetes made either at our center or at another healthcare facility within the past 6 months, provided that no glucose-lowering therapy (oral antidiabetic agents or insulin) had been initiated. Patients were considered treatment-naïve only if no structured lifestyle modification programme (dietary prescription or exercise programme) had been prescribed or initiated by the patient. The study included 50 newly diagnosed T2DM patients presenting with neuropathic symptoms at diagnosis, 50 newly diagnosed T2DM patients without neuropathic symptoms, and 50 age- and sex-matched healthy controls who presented to the Internal Medicine outpatient clinic of Balıkesir University Faculty of Medicine between October 2023 and September 2024. A power analysis was conducted using G*Power 3.1 with an effect size of Cohen's d = 0.6, power = 80%, and α = 0.05. Based on this, a minimum of 45 patients per group was required; however, to enhance statistical reliability and account for potential data loss, 50 patients were included in each group.

Inclusion and Exclusion Criteria

Inclusion criteria consisted of newly diagnosed, treatment-naïve T2DM patients fulfilling ADA 2023 diagnostic criteria, and age-and sex-matched healthy controls.

Exclusion criteria were grouped into predefined categories to improve clarity:

- Gastrointestinal conditions affecting GLP-1 physiology: prior gastrointestinal surgery, active ulcerative colitis with bleeding, enteritis.
- Metabolic / endocrine disorders other than T2DM: severe diabetic crises (diabetic ketoacidosis, hyperosmolar state), hypothyroidism, liver disease, end-stage renal disease.
- Hematological disorders: hemolytic anemia, aplastic anemia, moderate–severe anemia, bone marrow disorders.
- Autoimmune / connective tissue disease: systemic lupus erythematosus, scleroderma, Sjögren's disease… etc.
- Infectious etiologies associated with neuropathy: HIV infection, tuberculosis, other severe systemic infections.
- Neoplastic / hereditary causes of neuropathy: malignancies, hereditary neuropathies.
- Vitamin B12 deficiency
- Alcohol and recreational substance use

Any patient who had previously received glucose-lowering treatment (oral antidiabetic agents or insulin) or had begun a structured lifestyle modification programme (prescribed diet or exercise plan) prior to enrollment was excluded.

Clinical and Biochemical Assessments

General patient information was collected using a standardized form (age, sex, comorbidities, surgical history, habits, and medications). Anthropometric measurements (height, weight, waist circumference, blood pressure, and pulse) were recorded. Detailed neuropathy-focused history was obtained from all participants, and a comprehensive neurological examination was performed by a neuromuscular-specialized neurologist. The sensory examination included assessment of light touch perception, pinprick, temperature discrimination, vibratory sensation using a 128-Hz tuning fork at the hallux, and joint position sense. Motor examination included detailed grading of upper and lower extremity muscle strength and evaluation for muscle atrophy. Deep tendon reflexes and autonomic findings were also assessed. In addition, all participants completed the DN4 neuropathic pain questionnaire. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg on at least two visits, with 2–3 measurements per visit. Diabetic retinopathy was diagnosed by an ophthalmologist based on fundoscopic examination for proliferative or pre-proliferative retinopathy.

MASLD was diagnosed based on ultrasonographic evidence of hepatic steatosis together with at least one cardiometabolic risk factor, in accordance with the 2023 international consensus. Cardiometabolic risk factors included overweight or obesity (BMI ≥25 kg/m²), type 2 diabetes mellitus, prediabetes, dyslipidemia (elevated triglycerides or low HDL-C), hypertension, or evidence of insulin resistance. Individuals with significant alcohol intake or alternative causes of chronic liver disease were excluded to avoid misclassification. This definition reflects the contemporary shift from purely biochemical thresholds to a broader cardiometabolic framework for characterizing hepatic steatosis [11].

Metabolic syndrome was defined according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP–ATP III) criteria. Accordingly, the diagnosis required the presence of at least three of the following components: (1) central obesity, defined as a waist circumference ≥102 cm in men or ≥88 cm in women; (2) elevated triglycerides (≥150 mg/dL) or current treatment for hypertriglyceridemia; (3) reduced HDL-cholesterol (<40 mg/dL in men or <50 mg/dL in women); (4) elevated blood pressure (systolic ≥130 mmHg, diastolic ≥85 mmHg, or use of antihypertensive medication); and (5) impaired fasting glucose (≥100 mg/dL) or antidiabetic therapy. These widely used diagnostic thresholds allow for standardized identification of cardiometabolic risk clustering in clinical research [12].

Dyslipidemia was defined as LDL-C ≥130 mg/dL, triglycerides ≥150 mg/dL, HDL-C <40 mg/dL in men or <50 mg/dL in women, or the use of lipid-lowering therapy. This definition reflects standard clinical criteria and ensures consistent classification across participants [13].

Fasting blood samples were collected between 08:00 and 09:00 AM from an antecubital vein after an 8-hour fast. Complete blood count and subgroup analyses were performed using the DXH 800 (Beckman Coulter, USA) autoanalyzer. Serum glucose, creatinine, ALT, AST, triglyceride (Tg), total cholesterol, and HDL-cholesterol (HDL-C) levels were measured using a spectrophotometric method (AU680, Beckman Coulter, USA). LDL-cholesterol (LDL-C) levels were calculated using the Friedewald formula; for patients with Tg levels >400 mg/dL, LDL-C was directly measured with the AU680 system. Hemoglobin A1c levels were measured using high-performance liquid chromatography (HPLC) (Variant II Turbo, Bio-Rad Laboratories, CA). Microalbuminuria was evaluated using the urine albumin-to-creatinine ratio (ACR) expressed in mg/g.

After clot formation, venous blood samples were centrifuged at 2000 g for 10 minutes within 15 minutes of collection. Hemolyzed specimens were excluded from analysis. Serum was aliquoted into cryovials and stored at −40°C until the day of measurement. All ELISA measurements were performed in a single batch using the same lot-numbered kits. Serum GLP-1 (Cat. No: 201-12-0023), TNF-α (Cat. No: 201-12-0083), and ICAM-1 (Cat. No: 201-12-0213) concentrations were determined by ELISA (SunRed Biotechnology Company, China). The measurement ranges were 0.6–180 pmol/L for GLP-1, 3–900 ng/L for TNF-α, and 0.4–60 ng/mL and 22–6000 ng/L for ICAM-1 (according to kit specifications). According to manufacturer information, intra-assay and inter-assay CV values are <10% and <12% for the TNF-α kit, <4.1% and <7.7% for the ICAM-1 kit, respectively; CV data for the GLP-1 kit are not provided For ICAM-1, serum samples were diluted 1:100 with saline prior to analysis. No pre-treatment was applied for GLP-1 or TNF-α measurements.

Assessment of Neuropathic Symptoms

Detailed neuropathy-focused history was obtained from all participants, and a comprehensive neurological examination was performed by a neuromuscular-specialized neurologist. The sensory examination included assessment of light touch perception, pinprick, temperature discrimination, vibratory sensation using a 128-Hz tuning fork at the hallux, and joint position sense. Motor examination included detailed grading of upper and lower extremity muscle strength and evaluation for muscle atrophy. Deep tendon reflexes and autonomic findings were also assessed. In addition, all participants completed the DN4 neuropathic pain questionnaire. The Douleur Neuropathique-4 (DN4) questionnaire — consisting of 10 items (7 symptom descriptors and 3 clinical findings) — was used to assess neuropathic pain characteristics [14]. Sensory and motor nerve conduction studies (NCS) were performed using the Medelec® CareFusion Synergy system (6-channel configuration) to evaluate large-fiber function and to exclude alternative neuropathic patterns. Sensory and motor conduction studies of the median and ulnar nerves in the upper limbs and sensory (sural) and motor (tibial and peroneal) nerve conduction studies in the lower limbs were performed antidromically using standardized protocols. F-wave latencies were also evaluated [15,16].

Because NCS findings were predominantly within normative limits, group classification was based on neuropathic symptoms and clinical examination rather than electrophysiological confirmation of polyneuropathy. Patients were grouped according to the presence or absence of neuropathic symptoms (D+N vs D), as determined by clinical evaluation and DN4 assessment. In the neuropathic symptom group, all participants reported neuropathic pain. Five patients additionally exhibited hypoesthesia, reduced deep tendon reflexes, and impaired vibration and position sense. Electrophysiological abnormalities were identified in a small subset of patients: two showed sensorimotor involvement, two demonstrated sensory involvement, and one was diagnosed with carpal tunnel syndrome. The remaining participants had normal nerve conduction studies.

Deep sensory loss and reduced deep tendon reflexes in the lower extremities were observed in 32 patients. Allodynia was detected in three patients. In ten patients, pain and temperature sensation loss accompanied decreased lower-extremity reflexes. Patients presenting with neuropathic pain, together with abnormalities detected in at least two parameters on detailed neuromuscular examination and electrophysiological testing, were classified as having diabetic neuropathy.

In the group without neuropathic symptoms, no neurological symptoms or abnormal findings were detected. Electrophysiological studies were within normal limits.

Ethical Statement

All patients were provided with written informed consent, and they agreed to partic-ipate in the study. Ethical approval was obtained from the Balıkesir University Faculty of Medicine Ethics Committee with decision number 2023/139 on October 25, 2023. The study was conducted in accordance with the principles of the Helsinki Declaration and followed good clinical practice.

Statistical Analyses

Data were analyzed using IBM SPSS Statistics for Windows, version 30.0 (IBM Corp., Armonk, NY). The Kolmogorov-Smirnov test and Q-Q plots were used to check the as-sumption of normality. Independent samples t-tests and ANOVA were used to compare means for normally distributed data, and the Mann-Whitney U test and Kruskal-Wallis was used for non-normally distributed data. Pairwise post-hoc group comparisons were adjusted using the Bonferroni correction to control for multiple testing. Categorical variables were analyzed using the chi-squared test with Yates' correction.

Multinomial logistic regression analysis was used to examine the efficacy of variables in predicting predicting neuropathic symptom status (D vs D+N) of patients in comparison to the healthy control group. For the multinomial logistic regression model, the overall model fit was statistically significant (−2 Log Likelihood = 164.43, $\chi^2 = 165.15$, df = 40, $p < 0.001$), and the Nagelkerke pseudo R^2 value indicated high explanatory power ($R^2 = 0.75$). Odds ratios were reported with 95% confidence intervals, and all multivariable models were adjusted for age, sex, BMI, HbA1c, lipid profile (LDL-C, HDL-C, Tg), and blood pressure (systolic/diastolic). Covariates included in the multivariable model were selected a priori based on biological plausibility and on previously published associations with diabetic neuropathic complications.

GLP-1 concentrations were entered into the regression model as a continuous variable in their original ELISA unit (pmol/L); therefore, the odds ratios represent the change in odds per 1 pmol/L increase in GLP-1.

Binary logistic regression was used to compare D and D+N groups. For the binary logistic regression model, model fit was evaluated using the Hosmer and Lemeshow goodness-of-fit test, which indicated an acceptable fit ($\chi^2 = 5.46$, df = 8, $p = 0.71$). The model's explanatory power was assessed using the Nagelkerke pseudo R^2 statistic ($R^2 = 0.33$).

The associations between DN4 and GLP-1, TNF-α, and ICAM-1 variables were examined via Spearman correlation analysis. Mean, standard deviation, median, and range statistics were used to describe quantitative data. Frequencies and percentages were used to describe categorical data. A significance level of $p = 0.050$ was used for all statistical tests.

RESULTS

50 newly diagnosed treatment-naïve patients with T2DM without neuropathic symptoms (D group), 50 newly diagnosed treatment-naïve patients with T2DM with neuropathic symptoms (D+N group), and 50 age- and sex-matched healthy controls (C group). Sex distribution was similar across groups **(Table 1).**

Anthropometric and Clinical Characteristics

Mean age differed significantly across groups: participants in the D+N group were older (55.5 ± 8.3 years) than both controls (48.2 ± 10.1 years, p = 0.001) and the D group (50.6 ± 8.9 years, $p = 0.033$). BMI increased progressively from controls to diabetic groups (C: 26.4 ± 2.9 kg/m²; D: 29.4 ± 3.6; D+N: 29.5 ± 2.9), and waist circumference demonstrated a similar gradient (C: 88 ± 10 cm; D: 97 ± 14; D+N: 98 ± 13), with both diabetic groups significantly exceeding controls (p = 0.001 for both comparisons).

Systolic blood pressure was higher in the D (131 ± 11 mmHg) and D+N groups (131 ± 10 mmHg) relative to controls (125 ± 11 mmHg), while diastolic blood pressure followed the same pattern (C: 72 ± 9 mmHg; D: 80 ± 11; D+N: 80 ± 9). Fasting glucose was markedly elevated in diabetes (C: 97 ± 14 mg/dL; D: 156 ± 77; D+N: 146 ± 58), and HbA1c was significantly higher in the D (8.0 ± 2.1%) and D+N groups (7.4 ± 1.6%) compared to controls (5.1 ± 0.7%), though no difference was observed between D and D+N.

Biochemical Findings

Urea levels were significantly higher in the D+N group (37 ± 26 mg/dL) compared with both the D group (28 ± 9 mg/dL) and controls (27 ± 10 mg/dL), whereas creatinine and eGFR values did not differ between groups.

Lipid parameters showed clear cardiometabolic divergence: LDL-C increased stepwise from controls (89 ± 20 mg/dL) to D (123 ± 43 mg/dL) and D+N (131 ± 41 mg/dL), and triglyceride levels were similarly higher in diabetes (C: 146 ± 52 mg/dL; D: 181 ± 89; D+N: 193 ± 98). HDL-C levels did not differ significantly.

Vitamin D levels were lower in the D (18.1 ± 7.3 ng/mL) and D+N groups (20.2 ± 5.9 ng/mL) compared with controls (24.2 ± 11.4 ng/mL). Biochemical data are presented in **Table 1.**

MASLD prevalence was markedly higher in both diabetic groups (D: 82% (n=41); D+N: 86% (n=43)) compared with controls (14% (n=7), $p < 0.001$). Hypertension and obesity were also more common among individuals with diabetes, without significant differences between D and D+N. Retinopathy occurred in 8% of participants in both diabetic groups, and microalbuminuria showed no significant difference between D and D+N.

Dyslipidemia was present in 54.8% (n = 27) of controls, 70% (n = 35) of diabetic participants without neuropathic symptoms, and 92% (n = 46) of those with neuropathic symptoms ($p < 0.05$). Direct comparison of the diabetic subgroups confirmed a significantly higher prevalence of dyslipidemia among individuals with neuropathic symptoms (90.5% vs. 64.3%, $p < 0.05$).

Inflammatory and Endothelial Biomarkers

GLP-1 concentrations were similar across groups (C: 27.7 ± 13.8 pg/mL; D: 24.8 ± 7.8; D+N: 26.8 ± 6.8). ICAM-1 levels were modestly higher in both diabetic groups (D: 172.9 ± 33.9 ng/mL; D+N: 168.8 ± 31.6) compared with controls (165.3 ± 36.9), although differences did not reach statistical significance. TNF-α showed a downward trend in the D+N group (153.8 ± 52.2 pg/mL) relative to controls (174.1 ± 135.2 pg/mL), but univariate comparisons were nonsignificant (Table 2).

Multivariable Regression Analysis

Results of the multinomial and binary logistic regression models are summarized in **Table 3.** After adjustment for metabolic, inflammatory, and clinical variables, several independent predictors of group classification emerged.

Compared with controls, the likelihood of belonging to the D group was higher with increasing BMI (OR = 1.83; 95% CI: 1.20–2.79) and LDL-C (OR = 1.06; 95% CI: 1.02–1.10), whereas higher vitamin D levels were inversely associated (OR = 0.87; 95% CI: 0.77–0.99).

In adjusted models, ICAM-1 remained an independent discriminator of the D group compared with controls (OR = 1.03, 95% CI: 1.00–1.07), although this association did not persist for the D+N group versus controls (OR = 1.01, 95% CI: 0.98–1.05). ICAM-1 did not differentiate the D+N and D groups in the final model (OR = 0.99, 95% CI: 0.94–1.04).

In the comparison of D+N versus controls, higher BMI (OR = 1.94; 95% CI: 1.27–2.98), LDL-C (OR = 1.07; 95% CI: 1.03–1.11), systolic blood pressure (OR = 1.05; 95% CI: 1.00–1.10), and fasting glucose (OR = 1.15; 95% CI: 1.07–1.23) were significant predictors. Lower TNF-α levels also contributed modestly to this model (OR = 0.985; 95% CI: 0.97–1.00).

When directly comparing D+N with D, neuropathic symptom status was independently associated with higher GLP-1 concentrations (OR = 1.09; 95% CI: 1.01–1.18) and lower TNF-α levels (OR = 0.94; 95% CI: 0.89–0.99), as well as older age

(OR = 1.07; 95% CI: 1.00–1.14).

Overall, type 2 diabetes was associated with greater adiposity and adverse cardiometabolic parameters. Neuropathic symptom status was associated with older age, higher urea levels, and higher GLP-1 concentrations after multivariable adjustment.

DISCUSSION

In this study, we investigated the relationship between inflammatory and endothelial biomarkers—GLP-1, TNF-α, and ICAM-1—and neuropathic symptom status in newly diagnosed, treatment-naïve patients with T2DM. Our findings provide insight into biomarker patterns associated with early neuropathic manifestations in T2DM. Although GLP-1 levels did not differ significantly between groups in univariate analyses, the association became significant in the adjusted regression model. This apparent discrepancy likely reflects confounding by age, BMI, glycemic measures, lipid profile, and blood pressure variables that differed across groups and are biologically linked to endogenous GLP-1 secretion. After adjusting for these covariates (age, sex, BMI, HbA1c, LDL-C, HDL-C, triglycerides, systolic and diastolic blood pressure), the independent relationship between higher GLP-1 levels and neuropathic symptom status emerged. Previous experimental and clinical studies provide context for GLP-1 biology [17–19]; however, our findings represent an association between circulating GLP-1 levels and neuropathic symptom status and do not imply a causal or mechanistic relationship. Elevated GLP-1 levels may alternatively reflect altered incretin secretion, metabolic dysregulation, insulin resistance, or changes in hormone clearance rather than a direct effect on peripheral nerve biology. Accordingly, this observation should be considered hypothesis-generating. ICAM-1 levels were significantly elevated in both diabetic groups compared to controls but did not differ between patients with and without neuropathic symptoms. This suggests that ICAM-1 elevation is an early vascular response associated with the diabetic state itself, rather than a marker specific to neuropathic symptom status. ICAM-1 plays a key role in leukocyte adhesion and endothelial dysfunction, and its elevation has been previously associated with sub-clinical atherosclerosis and microvascular complications in T2DM [20,21].

In the adjusted regression model, TNF-α levels were lower in the D+N group, even after adjusting for age, sex, BMI, HbA1c, LDL-C, HDL-C, triglycerides, and systolic and diastolic blood pressure. Although previous meta-analyses and cohort studies have described higher TNF-α concentrations in diabetic neuropathic conditions, our finding diverges from that pattern [22,23]. Several non-mechanistic explanations are possible, including biological variability in cytokine expression, early-stage disease heterogeneity, and limited statistical power. In addition, reliance on a single fasting cytokine measurement may not capture short-term inflammatory fluctuations. Because longitudinal cytokine profiling and direct assessments of inflammatory signaling pathways were not included, the finding should be interpreted cautiously. Rather than suggesting a mechanistic effect, the observed pattern may simply reflect heterogeneity in inflammatory activity among newly diagnosed, treatment-naïve individuals with T2DM.

Serum urea was significantly higher in the D+N group despite normal creatinine and eGFR levels. Recent studies indicate that urea may act as a neurotoxic molecule by increasing oxidative stress and promoting endothelial dysfunction even in the absence of overt renal impairment [24,25]. Therefore, elevated urea may reflect systemic metabolic stress potentially associated with neuropathic symptom status.

While microalbuminuria values did not significantly differ between groups, the frequency of individuals with microalbuminuria (≥30 mg/day) was slightly higher in the D group. Similarly, diabetic retinopathy was observed equally in D and D+N groups (8%), suggesting that neuropathic symptom presentation may not parallel the timeline of other microvascular complications. MASLD and hypertension were both highly prevalent in diabetic patients regardless of neuropathy status, indicating that these metabolic comorbidities may not serve as distinguishing features for neuropathy symptoms in early diabetes.

Electrophysiological studies were performed in all participants with neuropathic symptoms. Although a small subset demonstrated sensory or sensorimotor abnormalities, most had normal large-fiber conduction parameters. Because

objective small-fiber assessments were not performed, no conclusions can be drawn regarding fiber-specific involvement or structural nerve pathology in this cohort.

Accordingly, the observed differences in GLP-1, TNF-α, and ICAM-1 levels should be interpreted as associations with neuropathic symptom status rather than evidence of underlying neural injury. ICAM-1 elevation appears to reflect the diabetic state overall rather than symptom status specifically, consistent with prior heterogeneous findings in the literature [26]. A key strength of this study is the inclusion of treatment-naïve patients with newly diagnosed T2DM, minimizing confounding by prior therapies. However, several limitations should be acknowledged. Although age- and sex-matching was attempted, the D+N group was older than the D and control groups despite our best efforts. We deliberately did not increase the age of the control group to force age equivalence, because doing so would have increased the risk of including individuals with latent dysglycemia or prior glucose-lowering exposure—thereby compromising the “treatment-naïve” definition and reducing phenotype purity. In our study, quantitative small-fiber assessments (quantitative sensory testing, corneal confocal microscopy, or skin biopsy) were not performed. Therefore, electrophysiologically confirmed polyneuropathy could not be systematically characterized within the cohort. In addition, DPP-4 inhibitors were not added to prevent GLP-1 degradation; however, samples were centrifuged within 15 minutes, which is an accepted pre-analytical condition for GLP-1 stability. Finally, electrophysiological evaluation focused on large-fiber function, which may underestimate neuropathic changes not detectable by standard large-fiber testing. In conclusion, our findings underscore the contribution of non-glycemic mechanisms to neuropathic symptom expression in early T2DM. Elevated GLP-1 levels were associated with neuropathic symptom status; however, the underlying biological mechanisms cannot be determined from this cross-sectional analysis, while increased ICAM-1 and urea are consistent with broader vascular and metabolic alterations observed in early diabetes. These results support the exploration of novel diagnostic and therapeutic strategies targeting inflammatory and endothelial pathways in diabetic neuropathic conditions.

CONCLUSION

This study demonstrated that higher GLP-1 levels are independently associated with the presence of neuropathic symptom status in newly diagnosed, treatment-naïve individuals with type 2 diabetes mellitus. The elevation of ICAM-1 across diabetic groups suggests early endothelial activation, while increased serum urea may reflect additional metabolic stress in patients with neuropathy. These findings emphasize the relevance of non-glycemic mechanisms in the neuropathic manifestations in early T2DM and support further investigation into inflam-matory and vascular markers as potential contributors to its pathophysiology.

Declarations

Author Contributions: Conceptualization, Ö.K.B. and U.S.S.; methodology, Ö.K.B., U.S.S., S.U.; software, M.U.Y; validation, Ö.K.B., U.S.S., S.U.; formal analysis, Ö.K.B.; investigation Ö.K.B., U.S.S., S.U., M.U.Y. ; resources, , Ö.K.B. and U.S.S.; data curation, Ö.K.B and M.U.Y.; writ-ing—original draft preparation, Ö.K.B., U.S.S., S.U., M.U.Y.; writing—review and editing, Ö.K.B.; visualization, Ö.K.B.; supervision, U.S.S; project administration, Ö.K.B.; funding acquisition, Ö.K.B., U.S.S., S.U.. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper

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Data Availability Statement: The datasets generated and/or analysed during the current study are publicly available in the Zenodo repository at https://doi.org/10.5281/zenodo.19065807. All data have been de-identified in accordance with ethical standards.

Consent for Publication: Not Applicable.

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Tables

Table 1. Descriptive Statistics for Continuous Study Variables

	Groups								
	Control n=50			D n=50			D+N n=50		
	Mean	Median	Standard Deviation and Range	Mean	Median	Standard Deviation and Range	Mean	Median	Standard Deviation and Range
Age	50_a	49	14 (51)	50_a	49.5	10 (43)	56_b	57	12 (43)
Waist circumference	88_a	87	10 (40)	97_b	94	14 (66)	98_b	96	13 (46)
Microalbuminuria	12.5_a	12.8	4.0 (21)	19.5_a	8.5	42.9 (294)	14.5_a	15	7.7 (35.1)
BMI	26.43_a	26.55	2.93 (13.5)	29.35_b	29.15	3.58 (23)	29.47_b	29.4	2.87 (11.5)
FPG	97_a	96	14 (82)	156_b	129.5	77 (376)	146_b	128	58 (330)
25(OH)D	24.24_a	22.55	11.45 (64.4)	18.05_b	18.05	7.33 (30.4)	$20.19_{a,b}$	20	5.87 (28.4)
HbA1c	5.15_a	5.35	.73 (2.6)	8.01_b	7	2.14 (8.9)	7.41_b	6.8	1.65 (8.7)
Urea	27_a	25.5	10 (45)	28_a	27.5	9 (39)	37_b	32	26 (149)
Creatinine	$.87_a$	0.85	.24 (1.21)	$.83_a$	0.84	.18 (.75)	$.87_a$	.89	.21 (1.07)
eGFR	89_a	91	21 (86)	93_a	94.58	17 (63)	87_a	87	18 (69)
CRP	3.41_a	3	.93 (4)	3.39_a	3	.81 (4)	3.43_a	3	.66 (2.1)
Vitamin B12	326_a	284	144 (707)	289_a	269.5	99 (458)	316_a	272	226 (1414)
LDL-C	89_a	86.5	20 (99)	123_b	114	43 (210)	131_b	133	41 (202)
HDL-C	49_a	48	11 (39)	52_a	48	15 (60)	46_a	47	13 (63)
TG	146_a	150	52 (218)	$181_{a,b}$	163.5	89 (464)	193_b	179	98 (422)
Systolic BP	125_a	126.5	11 (49)	131_b	130	11 (55)	131_b	130	10 (41)
Diastolic BP	72_a	70	9 (33)	80_b	83	11 (40)	80_b	81	9 (35)
GLP-1	27.7_a	24.9	13.8 (82.1)	24.8_a	24.35	7.8 (46.6)	26.8_a	25.8	6.8 (37.1)
TNF-a	174.1_a	141	135.2 (699)	171.5_a	136.46	150.0 (857.7)	153.8_a	139.8	52.2 (238.7)
ICAM-1	165.3_a	162	36.9 (195)	172.9_a	171	33.9 (141)	168.8_a	166.8	31.6 (162.4)

Note: Values in the same row and subtable not sharing the same subscript are significantly different at p< .05 in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances.[1]

*Tests are adjusted for all pairwise comparisons within a row of each innermost subtable using the Bonferroni correction. Superscript letters (a, b) within the same row indicate post hoc pairwise comparisons. Values sharing the same letter are not significantly different.

¶Abbreviations: 25(OH)D, 25-hypdroxyvitamin D; BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GLP-1, glucagon-like peptide-1; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; ICAM-1, intercellular adhesion molecule-1; LDL-C, low-density lipoprotein cholesterol; TIBC, total iron-binding capacity; TNF-α, tumor necrosis factor-alpha.

Table 2. Descriptive Statistics for Categorical Study Variables

Variables		Groups					
		Control		D		D+N	
		Count	Row N %	Count	Row N %	Count	Row N %
Sex	0	21_a	29.6%	22_a	31.0%	28_a	39.4%
	1	29_a	36.7%	28_a	35.4%	22_a	27.8%
NAFLD	0	49_a	75.4%	9_b	13.8%	7_b	10.8%
	1	1_a	1.2%	41_b	48.2%	43_b	50.6%
Retinopathy	0	50^1	35.2%	46_a	32.4%	46_a	32.4%
	1	0^1	0.0%	4_a	50.0%	4_a	50.0%
Hypertension	0	43_a	51.8%	18_b	21.7%	22_b	26.5%
	1	7_a	10.4%	32_b	47.8%	28_b	41.8%
Dyslipidemia	0	23_a	54.8%	15_a	35.7%	4_b	9.5%
	1	27_a	25.0%	35_a	32.4%	46_b	42.6%
Metabolic Syndrome	0	50^1	98.0%	1_a	2.0%	0^1	0.0%
	1	0^1	0.0%	49_a	49.5%	50^1	50.5%
Obesity	0	50^1	46.7%	30_a	28.0%	27_a	25.2%
	1	0^1	0.0%	20_a	46.5%	23_a	53.5%
Gestational Diabetes	0	50^1	34.2%	49_a	33.6%	47_a	32.2%
	1	0^1	0.0%	1_a	25.0%	3_a	75.0%

Note: Values in the same row and subtable not sharing the same subscript are significantly different at p< .05 in the two-sided test of equality for column proportions. Cells with no subscript are not included in the test. Tests assume equal variances.[2]

1. This category is not used in comparisons because its column proportion is equal to zero or one.

2. Tests are adjusted for all pairwise comparisons within a row of each innermost subtable using the Bonferroni correction.

Table 3. Multivariable logistic regression analysis for predictors of diabetes and neuropathy status

Predictor	D vs Control OR (95% CI)	p-value	D+N vs Control OR (95% CI)	p-value	D+N vs D OR (95% CI)	p-value
BMI (kg/m²)	1.83 (1.20–2.79)	0.008	1.94 (1.27–2.98)	0.003	–	–
LDL-C (mg/dL)	1.06 (1.02–1.10)	0.012	1.07 (1.03–1.11)	0.010	–	–
25(OH)D (ng/mL)	0.87 (0.77–0.99)	0.042	–	–	–	–
ICAM-1 (ng/mL)	1.03 (1.00–1.07)	0.044	–	–	–	–
Systolic BP (mmHg)	–	–	1.05 (1.00–1.10)	0.030	–	–
Fasting Glucose (mg/dL)	–	–	1.15 (1.07–1.23)	<0.001	–	–
TNF-α (ng/L)	–	–	0.985 (0.97–1.00)	0.050	0.94 (0.89–0.99)	0.048
GLP-1 (pmol/L)	–	–	–	–	1.09 (1.01–1.18)	0.037
Age (years)	–	–	–	–	1.07 (1.00–1.14)	0.036

Notes: Values represent adjusted odds ratios (OR) with 95% confidence intervals (CI). Multivariable multinomial logistic regression was used to compare D and D+N vs controls. Binary logistic regression was used for D+N vs D. Only predictors with statistically significant associations are shown ($p < 0.05$).

Abbreviations: BMI: body mass index; LDL-C: low-density lipoprotein cholesterol; 25(OH)D: 25-hydroxyvitamin D; ICAM-1: intercellular adhesion molecule-1; BP: blood pressure; GLP-1: glucagon-like peptide-1; TNF-α: tumor necrosis factor-alpha; OR: odds ratio; CI: confidence interval.