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ORIGINAL ARTICLE

Investigation of Plasma Spexin, Visfatin and Leptin Levels in Patients with Diabetic Neuropathy

Adeviye OZKAN IBIS[1], Huriye Eda OZTURAN OZER[2], Gülhan SARICAM[3], Orkun SARICAM[4], Ahmet ERGUN[5]

[1] Ankara Yildirim Beyazit University, Department of Health Services Vocational School, Ankara, Türkiye
[2] Baskent University, Faculty of Medicine, Department of Biochemistry, Ankara, Türkiye
[3] Ankara Pursaklar State Hospital, Department of Neurology Clinic, Ankara, Türkiye
[4] Ankara Pursaklar State Hospital, Department of Internal Medicine, Ankara, Türkiye
[5] Baskent University, Faculty of Medicine, Department of Physiology, Ankara, Türkiye

ABSTRACT

Aim: Diabetic neuropathy (DN) is one of the most common chronic complications of diabetes mellitus (DM). The aim of this study was to investigate plasma spexin, visfatin and leptin levels of patients with Type 2 DM who developed DN as a complication and patients who did not develop DN and to determine whether any significant differences existed between the groups. **Method:** The study included 93 patients diagnosed with type 2 DM. Electromyography (EMG) was performed as an electrophysiologic nerve conduction study. According to EMG results, patients were divided into two groups: those with DN (n=55) and those without (n=38). Two tubes of venous blood samples (5 ml each) were collected from each patient. HbA1c levels of patients were checked in the first blood sample. According to HbA1c levels, the groups were divided into two subgroups as HbA1c between 6.5-8.4 and ≥8.5, and a total of four study groups were formed. The second blood sample was separated into plasma, spexin, visfatin and leptin levels were determined by ELISA method. The results were compared between the groups. **Results:** Of the participants with DN, 31 were female and 24 were male. Of those without DN, 29 were female and 9 were male. DN patients had significantly higher glucose and HbA1c values than those without ($p<0.05$). When lipid profiles were compared, although total cholesterol and triglyceride levels were high in both groups, the difference was not statistically significant ($p>0.05$). There was no significant difference between groups in terms of High Density Lipoprotein (HDL) levels. Low Density Lipoprotein (LDL) levels were higher in the non-DN group, but not significantly ($p>0.05$). Body mass index (BMI), glucose, triglycerides, total cholesterol, HDL, LDL, spexin, visfatin and leptin values of patients with and without DN with HbA1c levels between 6.5-8.4 did not show statistically significant difference according to the diagnosis of neuropathy ($p>0.05$). Among patients with HbA1c level ≥8.5, there was no statistically significant difference between BMI, glucose, triglyceride, HDL, spexin, visfatin and leptin levels of patients with and without DN ($p>0.05$), whereas there was a statistically significant difference between total cholesterol and LDL levels of the same group ($p<0.05$). LDL levels were higher in patients with DN. When the correlation between spexin, visfatin, and leptin levels of patients with and without DN was examined, a strong and statistically significant positive correlation was found between them ($p<0.01$).**Conclusion:** Plasma levels of spexin, visfatin and leptin did not differ significantly between the groups of patients with and without DN. However, a significant positive correlation was found between plasma spexin, visfatin and leptin levels of the patients. This result suggests that plasma levels of spexin, visfatin and leptin act together and may be clinically important.

Keywords: Diabetes Mellitus, Diabetic Neuropathy, Leptin, Spexin, Visfatin.

ÖZET

Amaç: Diyabetik nöropati (DN), Diabetes Mellitus'un (DM) en sık görülen kronik komplikasyonlarından biridir. Bu çalışmada, Tip 2 DM'li olup komplikasyon olarak DN gelişen hastalar ile DN gelişmeyen hastaların plazma speksin, visfatin ve leptin değerlerinin incelenmesi ve gruplar arasında farklılık olup olmadığının araştırılması amaçlanmıştır. **Yöntem:** Çalışmaya Tip 2 DM tanısı almış olan 93 yetişkin hasta dahil edildi. Elektrofizyolojik sinir ileti çalışması olarak elektromiyografi (EMG) yapıldı. EMG bulgularına göre hastalar DN olan (n = 55) ve DN olmayanlar (n = 38) olarak iki gruba ayrıldı. Her hastadan, biri 5 ml olacak şekilde iki tüp venöz kan örneği alındı. İlk kan örneğinde hastaların HbA1c düzeyleri kontrol edildi. HbA1c düzeylerine göre gruplar kendi aralarında, HbA1c 6,5-8,4 arası ve ≥8,5 olmak üzere iki alt gruba ayrılarak toplam dört çalışma grubu oluşturuldu. Diğer kan örneği plazmaya ayrıldıktan sonra ELISA yöntemi ile speksin, visfatin ve leptin düzeyleri belirlendi. Sonuçlar gruplar arasında karşılaştırıldı.

Corresponding Author: Adeviye ÖZKAN İBİŞ **Correspondence Adress:** Ankara Yildirim Beyazit University, Department of Health Services Vocational School, Yıldırım Beyazıt Mahallesi Ankara Bulvarı No:35 - 06760 Çubuk, Ankara, Türkiye **Mail:** adevye.ozkan@gmail.com **Received:** 29.01.2025**; Accepted:** 15.05.2025

Bulgular: Katılımcılardan DN olanların 31'i kadın, 24'ü erkektir. DN olmayanların ise 29 kadın, 9'u erkektir. DN olan hastaların, olmayan hastalara göre glikoz ve HbA1c değerlerinin anlamlı derecede daha yüksek değerlere sahip olduğu görüldü ($p<0,05$). Lipid profilleri karşılaştırıldığında, her iki grupta da total kolesterol ve trigliserid seviyeleri yüksek olmasına rağmen, aralarındaki fark istatistiksel olarak anlamlı değildi ($p>0,05$). Yüksek Yoğunluklu Lipoprotein (HDL) düzeyleri açısından gruplar arasında net bir farklılık tespit edilemedi. Düşük Yoğunluklu Lipoprotein (LDL) seviyeleri genel olarak DN olmayan grupta daha yüksek bulundu ancak anlamlı değildi ($p>0,05$). HbA1c düzeyleri 6,5-8,4 arasında yer alan DN olan ve olmayan hastaların beden kitle indeksi (BKİ), glikoz, trigliserid, total kolesterol, HDL, LDL, speksin, visfatin ve leptin değerleri, nöropati tanısı alma durumuna göre istatistiksel olarak anlamlı bir farklılık göstermedi ($p>0,05$). HbA1c düzeyi ≥8,5 olan hastalardan, DN olan ve olmayanların BKİ, glikoz, trigliserid, HDL, speksin, visfatin ve leptin düzeyleri arasında istatistiksel olarak anlamlı bir fark bulunmazken ($p>0.05$), aynı gruptaki hastaların total kolesterol ve LDL düzeyleri arasında istatistiksel olarak anlamlı bir fark bulundu ($p<0.05$). LDL düzeyleri DN olan hastalarda daha yüksekti. DN olan ve olmayan hastaların speksin, visfatin ve leptin düzeyleri arasındaki korelasyon incelendiğinde ise, aralarında yüksek pozitif ve anlamlı bir ilişki tespit edildi ($p<0,01$). **Sonuç:** Speksin, visfatin ve leptinin plazma düzeyleri, DN olan ve olmayan hasta grupları arasında anlamlı farklılık göstermemiştir. Ancak, hastaların plazma speksin, visfatin ve leptin düzeyleri arasında anlamlı düzeyde pozitif korelasyon tespit edilmiştir. Bu sonuç, speksin, visfatin ve leptinin plazma düzeylerinin birlikte hareket ettiğini ve klinik olarak önemli olabileceğini düşündürmektedir.

Anahtar Kelimeler: Diabetes Mellitus, Diyabetik Nöropati, Leptin, Speksin, Visfatin.

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INTRODUCTION

Diabetic neuropathy (DN) is one of the most common chronic microvascular complications of diabetes mellitus (DM). It has a reported prevalence of approximately 50% among individuals with DM. It primarily targets unmyelinated and thinly myelinated nerve fibers. In the early stages of the disease, there are usually no symptoms, but there may be nerve conduction slowing that can be detected by electrophysiologic testing. In the early stages (<5 years), it is possible to evaluate the signs of small fiber neuropathy electrophysiologically and detect some nerve conduction abnormalities (1). The primary risk factor for the development of diabetic neuropathy is chronic hyperglycemia. However, this risk can be significantly reduced through tight glycemic and metabolic control. In the early stages of hyperglycemia, functional disorders such as decreased sensory nerve conduction velocity, motor nerve conduction velocity and increased sensitivity to pain (hyperalgesia), and painful response to normal stimuli (allodynia) are observed in the first months (2). It is known that with diabetic nerve damage; axonopathy increases, myelination decreases, nerve degeneration and hypoalgesia increase. Factors such as increased polyol pathway activity due to hyperglycemia, increased advanced glycation end products (AGEs), activation of the protein kinase C (PKC) pathway, increased flux through the hexosamine pathway, oxidative stress and decreased nerve growth factor levels are

among the main mechanisms thought to contribute to DN development (3).

Spexin is a new neuropeptide hormone 14 amino acids long and affects many physiological and pathophysiological processes, identified by the Markov modeling method in 2007 (4). Although the mechanism of action of spexin is not fully known, it is directly involved in the regulation of adrenocortical cell proliferation, and it has been proven that it is also secreted from the pancreas (5). Spexin, which has a prepropeptide structure, is encoded by the C12ORF39 gene. Although no specific receptor for spexin has been identified to date, it has been proven to be a natural ligand for galanin receptor 2/3 (GALR2/3) (6). Galanin, a classical neuropeptide, has effects on numerous physiological processes, such as regulating glucose homeostasis, accelerating the alteration of GLUT4 to the membrane of various insulin-sensitive cells and reducing insulin resistance in peripheral tissues, osmotic regulation and water intake, regeneration, feeding, and energy homeostasis, pain, and neuron renewal (7).

Visfatin is a 52 kDa cytokine identified by Fukuhara et al. in 2005 (8). Visfatin, known as pre-B cell colony enhancing factor (PBEF) and identified as a growth factor for B lymphocytes in previous years, is known to be primarily produced by human visceral adipose tissue and has insulin-mimicking effects. Visfatin binds to insulin receptors without competing with insulin and activates distinct signaling pathways downstream of the insulin receptor. In cell culture studies, visfatin treatment has increased glucose uptake in adipocytes and myocytes and inhibited hepatic glucose production. Visfatin is found in low concentrations in plasma and plays a physiological role in lowering plasma glucose (9).

Leptin is a polypeptide hormone that is mainly synthesized in adipose tissue. This hormone plays a key role in the regulation of on body fat mass, food intake, energy metabolism and glucose homeostasis. Exerts its metabolic functions through specific receptors. There are 6 different receptor types (LEPRa, LEPRb, LEPRc, LEPRd, LEPRe, and LEPRf) consisting of short and long forms and having different functions (10).

When the literature is examined, several studies have examined in which spexin, visfatin and leptin were examined separately in both types of diabetes (Type 1 DM and Type 2 DM) and various complications, however, no studies to date have simultaneously investigated all three parameters. Therefore, this study primarily aimed to examine the plasma levels of spexin, leptin and visfatin in patients with and without DN and to investigate how changes in HbA1c levels cause differences in the plasma values of these parameters.

MATERIAL AND METHODS

This study received approval from Başkent University Medical and Health Sciences Research Board and Non-Interventional Clinical Research Ethics Committee with the decision dated 20/04/2022

and numbered 22/90. All patients read and signed informed consent forms before inclusion in the study.

Between May 2022 and July 2023, 93 adult patients who applied to the internal medicine outpatient clinics of Ankara Pursaklar State Hospital, who were previously diagnosed with Type 2 DM according to the American Diabetes Association criteria and who agreed to participate in the study were included in this study. The inclusion criteria were as follows: confirmed diagnosis of Type 2 DM, with a minimum of two years since DN diagnosis, an HbA1C value of ≥6.5, and the absence of other complications such as retinopathy/nephropathy.

Personal information (height, weight, gender, body mass index, etc.) and medical history (history of diabetes, oral or insulin treatment, etc.) of the patients who agreed to participate in the study were recorded. Two tubes of venous blood, 5 ml each, were collected from the patients in the morning on an empty stomach. HbA1c values of the patients were determined with one of the blood samples. According to HbA1c values, patients were grouped as 6.5-8.4 and ≥8.5. The blood samples in the other tube were separated into plasma and stored at -20 oC until the analyses were performed. After all samples were collected, spexin, visfatin and leptin levels were determined by ELISA method. Other laboratory findings (glucose, LDL, HDL, triglycerides, total cholesterol,) of each patient were obtained from medical records. Body mass index (BMI) was calculated according to weight/height squared (kg/m2) ratio.

Electrophysiological evaluation was performed in each patient to confirm the diagnosis of DN. In order to better interpret abnormalities in nerve conduction velocities, patients with a diagnosis of DN of less than 2 years were excluded. Electromyography (EMG) was performed according to the protocol recommended by the American Society of Electrodiagnostic Medicine. In sensory nerve examination, conduction velocities of the median nerve in the upper extremity and the sural nerve in the lower extremity were analysed. In motor nerve examination, conduction velocities of the median nerve in the upper extremity and tibial nerve in the lower extremity were examined. After electrophysiological evaluation, patients with DN were divided into two groups as those with HbA1C values between 6.5-8.4 and those with ≥8.5. Grouping was done by taking into account patients with long duration of diabetes and those who could not maintain good glycemic control (11). Patients without DN were grouped in the same way and a total of four study groups were formed.

This study was approved by Baskent University Institutional Review Board and ethics Committee (Project no: KA22/202) and supported by Baskent University Research Fund.

Exclusion Criteria

Patients diagnosed with type 1 DM, those diagnosed with DN less than 2 years ago, those with HbA1c levels below 6.5%, and those diagnosed with nephropathy or retinopathy were excluded from the study.

Biochemical Analysis

Venous blood samples that were taken from patients on an empty stomach in the morning were centrifuged at 4.000 rpm for 10 minutes to separate plasma. The plasma samples obtained were transferred to Eppendorf tubes and stored at -20 °C until analysis. In plasma samples, Spexin, visfatin, and leptin levels were measured by ELISA using Sun-Red Bio brand commercial kits. The results were expressed as pg/ml for spexin and ng/ml for visfatin and leptin.

Statistical Analysis

The patient data collected for the study were analyzed using the IBM Statistical Package for the Social Sciences (SPSS) version 29.0 (IBM Corp., Armonk, NY) package application. Descriptive statistics were provided as mean and standard deviation for continuous data and frequency and percentage for categorical variables. For comparisons between groups, the “Independent Samples T-test” was used for two groups, and the “Pearson Chi-Square Test” was used to compare categorical variables. To compare more than two independent groups, a one-way analysis of variance (ANOVA) was applied. In groups that did not show normal distribution, in comparison to two independent groups, the Mann-Whitney U-test was used, and in comparison to two or more unrelated groups, the Kruskal Wallis test was used. The association between scale/sub-dimension scores was examined with the Spearman correlation coefficient. Statistical significance was established when the p-value was less than 0.05.

The power of the study was calculated in G*Power 3.1.9.7 package programme. The effect size for Student's t test was calculated as 70 people with d=0.6818217+, which will provide 80% test power at 95% confidence level, and the effect size for Pearson Correlation Test was calculated as 29 participants with ρ=0.5*, which will provide 80% test power at 95% confidence level. The minimum total sample size required for all test methods to be used in the study is 70 people. Accordingly, the power of the study completed with 93 participants was found to be 100% at 0.05 significance level.

RESULTS

Demographic and Clinical Features

A total of 93 patients with type 2 DM were included. While 55 (59.1%) participants had DN, 38 (40.9%) did not develop DN as a complication. Of the participants with DN, 31 were female, 24 were male. Of those without DN, 29 were female, and 9 were male. 41.82% of the patients with DN received oral medication, and 58.18% received insulin treatment. Among patients with DN, 68.42% received oral medications, while 31.58% received insulin treatment. It was observed that the rate of overweight and obese individuals in the groups with and without DN drew attention. Total cholesterol, triglyceride and plasma glucose levels were high in both groups. (Table 1).

Table 1. Findings Related to Some Categorical Characteristics According to the Diagnosis of Diabetic Neuropathy

Parameters		Diabetic Neuropathy			
		(+) (n:55)		(-) (n:38)	
		n	%	n	%
Gender	Female	31	56.36	29	76.32
	Male	24	43.64	9	23.68
Oral / insulin	Oral Medication	23	41.82	26	68.42
	İnsulin	32	58.18	12	31.58
BMI	Normal	4	7.27	1	2.63
	Overweight	20	36.36	13	34.21
	Obese	17	30.91	11	28.95
	Morbid obese	14	25.45	13	34.21
Glucose (70-100 mg/dL)	Normal	3	5.45	1	2.63
	High	52	94.55	37	97.37
Total cholesterol (0-200 mg/dL)	0-200	26	47.27	19	50.00
	over 200	29	52.73	19	50.00
Triglyceride (0-150 mg/dL),	Between 0-150	23	41.82	16	42.11
	Over 150	32	58.18	22	57.89
HDL (40-60 mg/dL),	Low	9	16.36	4	10.53
	Normal	33	60.00	23	60.53
	High	13	23.64	11	28.95
LDL (100-129 mg/dL),	Low	16	29.09	15	39.47
	Normal	22	40.00	7	18.42
	High	17	30.91	16	42.11

BMI: Body Mass İndex, **HDL:** High Density Lipoprotein, **LDL:** Low Density Lipoprotein

Comparison of Independent Variables

Patients with DN had significantly higher HbA1c and glucose values compared to those without DN ($p<0.05$). There was no statistically significant difference between DN diagnosis status and BMI, Triglycerides, HDL, LDL, Spexin, Visfatin and Leptin (Table 2).

Table 2. Comparison of BMI, HbA1c, Glucose, Triglyceride, HDL, LDL, Spexin, Visfatin, and Leptin Variables According to Diabetic Neuropathy Diagnosis Status

Parameters	DN (+) (n=55) Mean	DN (-) (n=38) Mean	Z statistics	p-value
BMI	32.34	32.25	-0.078	0.938
HbA1c	9.21	7.98	-2.780	**0.005***
Glucose (mg/dL)	192.13	151.76	-2.290	**0.022***
Triglyceride (mg/dL)	229.0	182.61	-0.274	0.784
HDL (mg/dL)	52.07	53.87	-0.618	0.537
LDL (mg/dL)	132.22	123.66	-0.043	0.966
Spexin (pg/ml)	799.97	730.41	-0.461	0.645
Visfatin (ng/ml)	27.11	25.61	-0.063	0.950
Leptin (ng/ml)	11.84	11.24	-0.332	0.740

DN: Diabetic Neuropathy, **BMI:** Body Mass İndex, **HDL:** High Density Lipoprotein, **LDL:** Low Density Lipoprotein *Significant at 0.05 level; Mann-Whitney U test

There was no statistically significant difference in plasma spexin, visfatin, and leptin values between patients with HbA1c levels between 6.5-8.4% and those with or without DN diagnosis ($p>0.05$). Similarly, BMI, glucose, total cholesterol, triglycerides, HDL and LDL values showed no significant difference between individuals with and without DN diagnosis (Table 3).

In patients with HbA1c levels ≥8.5%, there was a statistically significant difference in total cholesterol and LDL levels between the groups ($p<0.05$). On the other hand, when BMI, Glucose, Triglycerides, HDL, LDL, Spexin, Visfatin and Leptin variables of the patients in the same group were compared, there was no statistically significant difference with DN diagnosis status ($p>0.05$) (Table 4).

Table 3. Comparison of BMI, Glucose, Total Cholesterol, Triglyceride, HDL, LDL, Spexin, Visfatin and Leptin Values of Patients with HbA1c Levels Between 6.5-8.4% According to Diabetic Neuropathy Diagnosis Status

Parameters	DN (+) (n=23) Mean	DN (-) (n=26) Mean	Test statistics	p-value
BMI	30.83	32.10	-0.778	0.445[b]
Glucose (mg/dL)	135.61	129.19	-0.291	0.771[a]
Total Cholesterol (mg/dL)	191.83	218.92	-1.970	0.057[b]
Triglyceride (mg/dL)	160.52	177.85	-0.790	0.433[b]
HDL (mg/dL)	52.17	57.19	-1.490	0.143[b]
LDL (mg/dL)	108.13	126.73	-1.764	0.087[b]
Spexin (pg/ml)	934.07	746.35	-0.511	0.609[a]
Visfatin (ng/ml)	28.16	26.73	-0.461	0.645[a]
Leptin (ng/ml)	12.96	11.83	-0.220	0.826[a]

DN: Diabetic Neuropathy, BMI: Body Mass İndex, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein

[a]: Mann-Whitney U test; *significant at 0.05 level;

[b]: T-test for equality of means; *significant at 0.05 level.

BMI, glucose, triglyceride, total cholesterol, LDL, HDL, Spexin, Visfatin and Leptin levels in DN patients were compared according to HbA1c levels. Triglyceride and glucose levels were significantly higher in DN patients with HbA1c levels ≥8.5% ($p<0.05$). There was no statistically significant difference in BMI, total cholesterol, LDL, HDL, spexin, visfatin, and leptin levels between DN patients with HbA1c levels of 6.5-8.4% and those with HbA1c levels ≥8.5% ($p>0.05$).

When non-DN patients were grouped according to HbA1c levels, leptin and total cholesterol levels were higher in patients with HbA1c ≥8.5% compared to those with HbA1c levels of 6.5-8.4%, while HDL levels were lower ($p<0.05$). There was no statistically significant difference in BMI and triglyceride levels. In addition, patients with HbA1c levels ≥8.5% had higher plasma glucose levels ($p<0.05$), and patients with HbA1c levels between 6.5-8.4% had higher LDL and visfatin levels ($p<0.05$).

Table 4. Comparison of BMI, Glucose, Total Cholesterol, Triglyceride, HDL, LDL, Spexin, Visfatin and Leptin Variables of Patients with HbA1c Level ≥8.5% According to the Diagnosis of Diabetic Neuropathy

Parameters	DN (+) (n=32) Mean	DN (-) (n=12) Mean	Test statistics	p-value
BMI	33.43	32.60	0.443	0.661[b]
Glucose (mg/dL)	232.75	200.75	1.740	0.090[b]
Total Cholesterol (mg/dL)	212.75	172.17	2.430	**0.019[b]***
Triglyceride (mg/dL)	278.22	192.92	-1.146	0.252[a]
HDL (mg/dL)	52.00	46.67	-1.425	0.154[a]
LDL (mg/dL)	149.53	117.00	-2.150	**0.032[a]***
Spexin (pg/ml)	703.58	695.87	-0.105	0.916[a]
Visfatin (ng/ml)	26.36	23.18	-1.199	0.230[a]
Leptin (ng/ml)	11.04	9.96	-1.212	0.225[a]

DN: Diabetic Neuropathy, **BMI:** Body Mass İndex, **HDL:** High Density Lipoprotein, **LDL:** Low Density Lipoprotein
[a]: Mann-Whitney U test; *significant at 0.05 level;; [b]: T-test for equality of means; *significant at 0.05 level.

Correlation

Although there was a weak negative correlation between the plasma glucose values of the patients and spexin levels, this correlation was not significant. A high positive and significant correlation was found between spexin, visfatin, and leptin levels (p<0.01) (Table 5).

Table 5: Correlation Analysis Between Spexin, Visfatin and Leptin and Other Independent Variables

		Glucose	Total Cholesterol	Triglyceride	HDL	LDL	Spexin	Visfatin	Leptin
Spexin	r	-0,067	0,164	0,068	0,156	0,101	1	0,724	0.520
	p	0,646	0,261	0,642	0,285	0,490		**<0,001****	**<0.001****
Visfatin	r	0,034	0,163	0,075	0,056	0,125	0,724	1	0.699
	p	0,817	0,262	0,608	0,703	0,391	**<0,001****		**<0.001****
Leptin	r	0,026	0,149	0,177	0,049	0,048	0,520	0,699	1
	p	0,859	0,308	0,224	0,737	0,746	**<0,001****	**<0,001****	

HDL: High Density Lipoprotein, **LDL:** Low Density Lipoprotein **Significant at 0.01 level; Spearman correlation analysis

DISCUSSION

DN is one of the chronic complications of DM. Hyperglycemia plays a crucial role in the development of DN. A study conducted by Chang et al. in 2024 showed that hyperglycemia causes DN and blood glucose levels are high in DN patients (12). In another study, they showed that good glycemic control can significantly reduce the risk of developing DN and nerve conduction velocity abnormalities (13). In our study, it was striking that glucose levels were high in both groups (with and without DN). While this situation is considered a general feature of Type 2 DM, it was thought that adequate glycemic control could not be achieved in the patients.

HbA1c (glycosylated hemoglobin), among the diagnostic criteria for DM, shows the average plasma glucose level in the last 3 months. High HbA1c values indicate that patients have glycemic variability and inadequate glycemic control. Many studies have investigated the relationship between HbA1c and DM. A study of 1,632 patients with type 2 DM showed that high HbA1c levels were significantly associated with the presence of diabetic peripheral neuropathy (DPN) (14). Another study found similar results, showing that patients with DPN had higher HbA1c values than those without DPN (15). In our study, similar results were found in the literature, and it was observed that those diagnosed with DN had significantly higher values for HbA1c variable than those who did not. This finding suggests that high HbA1c levels may be related to the development of DN.

In our study, total cholesterol levels, one of the lipid parameters of the patients, were found to be high both in patients with and without a diagnosis of DN, but did not differ significantly based on the diagnosis of DN. These results suggest that total cholesterol levels do not play an important role in the development of DN or do not make a significant difference in this study sample. Triglyceride levels were also high in both groups, but no direct relationship with neuropathy was observed. The HDL levels were found to be similar between the groups. Although LDL levels were generally higher in the non-DN group, they were not associated with the development of DN. Similar results were found in a study by Su et al. and it was found that there was no difference between patients in terms of lipid profile and DN diagnosis (15). Again, the same results were obtained by Chang et al. showing that lipid profiles such as plasma LDL, HDL, and TG were similar between patients with and without DPN (12).

Spexin reduces insulin resistance and regulates glucose homeostasis. Studies examining the relationship between DM and spexin have shown that spexin levels are low in both types of diabetes (Type 1 DM and Type 2 DM) (16). In a study conducted by Karaca et al. the levels of spexin were lower in the type 1 DM group than in the healthy control group (16). Similarly, Gu et al.

discovered that spexin levels were significantly lower in patients with T2 DM compared to healthy controls and showed a negative correlation with glucose values (17). Although there are many studies in the literature examining the relationship between diabetes and spexin, studies examining the relationship between DN and spexin are quite limited. A study of 167 patients with type 2 DM found that serum spexin levels were low in patients with DPN and suggested that low serum spexin levels were independently associated with the presence of DPN and painful DPN (18). Again, the same study suggested that spexin may have a possible protective role in neuropathology and pain-related pathogenesis in diabetes (18). According to the results of another study with an in vitro DN model investigating the effects of spexin on the dorsal root ganglion, it was suggested that spexin has an effect to increase cell viability in dorsal root ganglion neurons and may have the potential to be a new therapeutic target in DN (19). In our study, contrary to previous literature, no significant difference was found between plasma spexin levels of patients with DN and patients without DN. When compared according to HbA1c levels, the same results were obtained and it was observed that there was no relationship between the patients' HbA1c levels and spexin levels. These results suggest that spexin has no role in the development of DN.

Various researches have been conducted investigating the relationship between DN and visfatin levels and ideas have been given about the possible relationships between them. Büyükaydın et al. investigated the relationship between serum visfatin and other metabolic parameters in DNP and showed that serum visfatin levels were significantly higher in patients with DNP compared to those without DNP and that there is a potential link between high visfatin levels and the presence of neuropathy in diabetic patients (20). In a study conducted by Mohammed et al. with a total of 120 participants, it was shown that the mean Visfatin values of men and women with DPN were higher than the control group and it was suggested that Visfatin levels are a strong indicator for early diagnosis of DN in both men and women (21). In a study investigating the effect of visfatin gene variations on late diabetic complications, it was reported that there was no difference between the variability in the genotype distribution of different variants of the visfatin gene and no statistically significant correlation was found in any of the late complications of T2 DM (22). In our study, although plasma visfatin values were found to be higher in patients with DN compared to those without DN, these results did not create a statistically significant difference. When the visfatin values of the patients were compared according to HbA1c levels, no significant correlation was found between HbA1c levels and visfatin values in patients with DN, whereas visfatin values decreased as HbA1c levels increased in patients without DN. While these findings indicate that visfatin levels may not play a role in the development of DN, contrary to previous studies, it was determined that further research

involving larger sample sizes is necessary due to the small group size in this study.

Studies examining the relationship between leptin and DN show varying results. In a case-control study conducted in recent years, leptin levels were measured in 205 diabetic patients with and without DPN and leptin levels were found to be higher in patients with DPN (23). In another study, similar results were obtained during the evaluation of leptin levels in microvascular complications of diabetes (diabetic nephropathy, retinopathy, neuropathy), and it was shown that leptin levels were significantly higher in patients with DN than in those without (22). However, there are also conflicting data suggesting no association between leptin and DN. In a study of 157 Type 2 DM patients, Sari et al. evaluated leptin levels in both macrovascular and microvascular complications of diabetes. As a result, they claimed that they found no significant difference in plasma leptin levels between diabetic patients with sensory or autonomic neuropathy and those without these complications (24). In our study, no difference was found between the plasma leptin levels of patients with and without DN. When the difference between the groups according to HbA1c levels was examined, a similar result was reached in patients diagnosed with DN, and it was seen that there was no difference in the plasma leptin levels of patients with DN as the HbA1c level changed. In patients without DN, it was seen that as the HbA1c level increased, the leptin level also increased. These findings could suggest that leptin levels are not a significant influence in the growth of DN.

Studies have examined plasma spexin, visfatin, and leptin levels separately in DN patients. However, no studies have reviewed these three parameters in the same patient group, and no correlation has been found between them. Our study looked at the association between spexin, visfatin, and leptin and discovered a positive and substantial relationship. The high positive and significant relationship between visfatin and leptin levels showed that leptin levels increased as visfatin increased. A strong positive correlation was found between spexin and visfatin levels, and it was determined that these two variables increased together. Leptin and spexin levels were found to be significantly positively correlated. This shows that spexin levels tend to increase as leptin levels increase. These results suggest that plasma spexin, visfatin, and leptin levels may act together and could be clinically important.

CONCLUSION

There was no significant difference between the plasma levels of spexin, visfatin, and leptin in patients with and without DN. Furthermore, these plasma values did not change significantly with fluctuations in HbA1c levels. These findings suggest that spexin, visfatin, and leptin do not play a significant role in the development of DN. However, it was observed that spexin, visfatin, and leptin levels in plasma appear to act together, which may have clinical significance.

Limitations of The Study

The most important limitations of this study were the small sample size and the lack of a healthy control group.

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Conflict of interest: The authors declare that they have no competing interests.

Ethics approval: This study was approved by Başkent University Non-Interventional Clinical Research Ethics Committee (Decision Number: 22/90, Date: 20.04.2022).

Data Availability: The data supporting this study's findings are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Patient Consent: All patients participating in the study read and signed an informed consent form.

AI Statement: No artificial intelligence (AI) assisted technology was used in the design of our study and in analyzing and writing up the results.

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