Association of serum adropin with risk and severity of diabetic peripheral neuropathy in patients with type 2 diabetes mellitus

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Background

Diabetic peripheral neuropathy (DPN) is the major microvascular complication of type 2 diabetes mellitus (T2DM). Adropin is a peptide hormone that has essential roles in metabolic homeostasis and the pathogenesis of T2DM and its complications. This study was designed to estimate serum adropin levels in patients with T2DM in correlation with risk factors of DPN. The authors also aimed to investigate the association between serum adropin level and clinical and electrophysiological tests of DPN.

Patients and methods

This case–control study enrolled 100 patients with T2DM (40 diabetic cases without DPN and 60 diabetic cases with DPN) and 50 controls. All participants were subjected to a complete neurological examination. The motor and sensory conduction velocities of the median nerve, ulnar nerve, and common peroneal nerve were measured. The severity of DPN was assessed by Toronto clinical scoring system (TCSS). Serum adropin levels were assessed using an enzyme-linked immunosorbent assay.

Results

Our results revealed decreased circulating serum adropin levels in patients with T2DM (3.5±1.2), especially diabetic patients with DPN (3.1±1.07), compared with controls (6.1±0.89). There is a negative correlation between serum adropin level and TCSS as well as electrophysiological tests: motor nerve conduction velocity of median and ulnar nerve, sensory nerve conduction velocity of median and ulnar nerve, compound muscle action potential amplitude (median and ulnar nerve), and sensory nerve action potential amplitude (median, ulnar, and perception threshold nerve) ($P < 0.001^*$).

Conclusion

Diabetic patients with DPN had lower values of serum adropin than diabetic patients without DPN, and serum adropin levels were negatively correlated with metabolic risk factors, TCSS, as well as electrophysiological tests of DPN.

Keywords:

adropin, diabetic polyneuropathy, nerve conduction studies, type 2 diabetes mellitus

Introduction

The prevalence of type 2 diabetes mellitus (T2DM) is rapidly increasing worldwide [1]. The disease burden of diabetes is mainly attributed to the morbidity and mortality associated with microvascular and macrovascular complications. Long-term hyperglycemia contributed to the main chronic metabolic disorders of DM. It leads to functional and structural deficits in both peripheral and central nervous systems in a progressive manner, resulting in diabetic peripheral neuropathy (DPN) [2].

DPN is the most common chronic diabetic complication and the major contributor to foot ulceration and lower limb amputation in persons with DM and has also a significant negative effect on patient's quality of life and associated with increased morbidity and mortality in diabetic patients [1]. DPN includes several distinct syndromes, of which symmetrical sensory polyneuropathy is the most common form of neuropathy [3]. Patients with diabetic neuropathy have symptoms that include sensory complaints of numbness and tingling, pain and loss of sensation, and motor complaints of weakness [4].

Past decades have witnessed a spurt in research activity that has shown a key role of inflammation in the pathogenesis of DPN. The etiology of DPN is still a mystery; however, there are evidence from various studies confirming that there are three main alterations involved in the pathologic changes of DPN:

inflammation, oxidative stress, and mitochondrial dysfunction. Inflammation induces activation of nuclear factor κB, activator protein 1, and mitogen-activated protein kinases [5].

Adropin is a secreted peptide hormone, encoded by the energy homeostasis-associated (Enho) gene [6]. Recent studies have suggested that adropin plays a pivotal role in metabolic homeostasis, including fatty acid metabolism, insulin resistance prevention, and development of dyslipidemia [7]

Adropin is a regulator in obesity-associated hyperinsulinemia, lipid/glucose homeostasis, and energy metabolism. Its secretion is mediated by dietary macronutrients [8]. In experimental studies, systemic injections of adropin have been shown to improve skeletal muscle insulin sensitivity and to promote weight loss [7]. Adropin has been shown in the literature to be decreased in many diseases, such as type 2 diabetes, diabetic nephropathies, coronary atherosclerosis, hypertension, and polycystic ovary disease [9,10].

DPN is the most common chronic diabetic complication and the major contributor to lower limb amputation and has also a significant negative effect on the patient's quality of life. Therefore, we intended early prediction of DPN. Substantial pieces of evidence implicate inflammation as a critical mediator in the pathophysiology of DPN. Interestingly, insulin resistance and chronic inflammation are the mainstays of this disease. Adropin significantly decreased the mRNA expression levels of tumor necrosis factor-α and interleukin-6 in the pancreas tissue of diabetic rats [4]. To best of our knowledge, no study has examined adropin level in DPN. Thus, this study was designed to estimate serum adropin levels in T2DM in correlation with risk factors of DPN. We also aimed to investigate the association between of serum adropin level and clinical and electrophysiological tests of DPN.

Patients and methods
Patients
This study was conducted in the Outpatient Clinic of Neurology Department at Zagazig University Hospital. This study included 150 participants: 100 patients with T2DM recruited from Diabetes and Endocrinology Outpatient Clinic of Internal Medicine Department of Zagazig University Hospitals and 50 healthy controls, who were matched to cases by age, sex, smoking habits, and ethnic origin.

The enrolled patients were included if they met all of the following criteria: aged greater than or equal to 18 years and current diagnosis of diabetes mellitus type 1 (duration ≥5 years) or type 2 (any duration). All participants were subjected to thorough history taking. Data were collected through a predesigned structured questionnaire to collect information about age, smoking habits, type of diabetes, duration of the disease, previous screening for diabetic complications, history of renal disease (dialysis or transplantation), and history of retinal laser photocoagulation, as well as symptoms of diabetic neuropathy.

The enrolled participants were subjected to full clinical assessment and neurological examination, and diabetic patients were subdivided into patients without DPN ($n=40$) and patients with DPN ($n=60$). There is no gold standard for the diagnosis of DPN. The expert panel of San Antonio conference recommends that it should be made based on neuropathic symptoms, signs, and nerve conduction studies [11].

Neurological examination was performed using the 10 g Semmes-Weinstein monofilament (Briggate Medical, Lakewood Blvd, Braeside VIC 3195, Australia), applying the test on nine different sites on the plantar surface of the foot and diagnosing sensory neuropathy when less than seven sites were felt by the patient. Vibration perception threshold (VPT) was also measured, using a biothesiometer, to define the presence of diabetic neuropathy with a cutoff VPT of more than 25 V for the diagnosis of loss of protective sensation.

Anthropometric variables, including BMI, calculated as weight in kg/height in m^2, and waist circumference (cm)/hip circumference (cm) ratio (WHR) were measured.

We excluded patients with any current psychiatric disorder that might affect the reliability of their response to the study questionnaire. Patients with T2DM with other types of neuropathic pain of nondiabetic origin including but not limited to lower back or neck pain (radiculopathy), postherpetic neuralgia, cancer-related pain, spinal cord injury pain, multiple sclerosis pain, carpal tunnel syndrome pain, phantom pain, trigeminal neuralgia, or fibromyalgia were excluded from the study. Moreover, we excluded patients with the following diseases: serious liver or renal insufficiency, thyroid dysfunction, refractory hypertension, recent history of infection, cerebral vascular disease, tumors, as well as pregnant patients. The Ethical Committee of Faculty of Medicine, Zagazig University, approved our study protocol, and all participants assigned written informed consent.

Severity of neuropathy

The severity of neuropathy was graded according to Toronto clinical scoring system (TCSS): 1–5 points for no neuropathy, 6–8 points for mild neuropathy, 9–11 points for moderate neuropathy, and 12–19 points for severe neuropathy. Symptom, reflex, and sensory tests, including pinprick, temperature, light touch, vibration, and position sensation, were performed as part of the TCSS [12].

Conventional nerve conduction detection

The motor conduction of median nerve, ulnar nerve, and common peroneal nerve (CPN) and the sensory conduction of median nerve, ulnar nerve, posterior tibial nerve (PTN), and sural nerve of all participants was measured by the Dantec Keypoint Workstation (Suite, California, USA). According to the revised criteria of Dyck *et al.* [13] in 2011, abnormal nerve conduction studies were defined as one of the following criteria: prolonged incubation period, slowing of conduction velocity, reducing of compound muscle action potential (CMAP) and sensory nerve action potential (SNAP) amplitude, or unsuccessful eliciting of certain arbitrary waveform.

Blood sampling

Blood samples were drawn from all participants after an overnight fast and divided into three portions: 1 ml of whole blood was collected into evacuated tubes containing EDTA, for HbA1c; 1 ml of whole blood was collected into evacuated tubes containing potassium oxalate and sodium fluoride (2 : 1) for fasting blood glucose; and serum was separated immediately from the remaining part of the sample and stored at –20°C until analysis.

Biochemical analysis

We determined fasting plasma glucose (FPG) using the glucose oxidase method (Roche Cobas 8000-e507; Roche Diagnostics, Mannheim, Germany) (Spinreact, Girona, Spain). Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured by routine enzymatic methods (Roche Cobas 8000-e507; Roche Diagnostics) (Spinreact). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [14].

Measurement of serum adropin levels

Serum adropin levels were determined by using a commercial enzyme-linked immunosorbent assay kit from Sun Red Biotechnology (catalogue no. 201-11-3361; China) based on the manufacturer's instructions [15].

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (version 21.0; SPSS Inc., Chicago, Illinois, USA). Data were expressed using descriptive statistic (mean±SD) and were analyzed using the 't' test. Pearson's correlation coefficient was used to assess the association between serum adropin and TCSS, nerve amplitude, and nerve conduction velocity. Receiver operating characteristic (ROC) curve analysis was performed to assess the potential accuracy of serum adropin levels, area under the curve (AUC), and the cutoff values for diagnosis of DPN among diabetic patients. We considered P to be significant at less than 0.05 with a 95% confidence interval (CI).

Results

Among studied participants, in the diabetic group, 69.6% were female and 30.4% were male, and their mean age was 43.83±10.17 years. In the control group, 64.3% were female and 35.7% were male, and their mean age was 41.25±9.45 years. Diabetic and control groups were matched for age, sex, and smoking.

Anthropometric and biochemical characteristics of the study participants are summarized in Table 1.

Anthropometric and biochemical characteristics of the studied groups

Diabetic patients had significantly higher values of BMI, waist/hip ratio, systolic and diastolic blood pressure, as well as lipid profile (triglycerides, total cholesterol, and LDL) compared with the control group. Furthermore, diabetic patients had significantly higher values of FPG, HbA1c, and TCSS compared with the control group. On the contrary, adropin and HDL levels were significantly lower in patients with T2DM compared with controls ($P<0.001$).

General characteristics of diabetic patients

In patients with DPN, there was a statistically significant long duration of diabetes as well as higher values of BMI, waist/hip, diastolic blood pressure, TG, FPG, HbA1c, and TCSS. Regarding diabetic treatment, the prevalence of patients with DPN treated with insulin was more common than diabetic patients without DPN. On the contrary, the prevalence of patients treated with oral hypoglycemic medications was more common in patients without PDN than patients with DPN. Regarding diabetic vascular complications, the prevalence of microalbuminuria and stroke was more common in patients with DPN than diabetic patients without

Table 1 Anthropometric and biochemical characteristics of the studied groups

Variables	Control group (*n*=50)	T2DM patients (*n*=100)	*P* value
BMI (kg/m^2)	22.18±1.189	37.03±4.96	<0.001*
Waist/hip ratio	0.86±0.011	1.05±0.21	<0.001*
Systolic blood pressure (mmHg)	117.8±9.40	145.8±19.44	<0.001*
Diastolic blood pressure (mmHg)	75.6±4.589	90.92±12.33	<0.001*
Total cholesterol (mg/dl)	185.3±19.90	220.88±29.1	<0.001*
Triglycerides (mg/dl)	179.26±33.019	265.16±68.6	<0.001*
LDL cholesterol (mg/dl)	101.08±23.067	128.91±33.4	<0.001*
HDL cholesterol (mg/dl)	47.48±6.87	35.25±5.63	<0.001*
Fasting plasma glucose (mg/dl)	89.72±6.304	196.97±30.04	<0.001*
HbA1c (%)	5.63±0.624	9.79±2.206	<0.001*
Adropin (ng/ml)	6.1±0.89	3.5±1.2	<0.001*
TCSS	0.565±0.011	8.54±3.12	<0.001*

HDL, high-density lipoprotein; LDL, low-density lipoprotein; T2DM, type 2 diabetes mellitus; TCSS, Toronto clinical scoring system.
*$P<0.05$ when compared with control group.

Table 2 Laboratory and anthropometric parameters of diabetic patients

Variables	Diabetic patients without DPN (*n*=40)	Diabetic patients with DPN (*n*=60)	*P* value
Duration of diabetes (years)	7.17±0.63	8.58±2.33	<0.001*
BMI (kg/m^2)	34.73±2.32	38.03±4.96	<0.001*
Waist/hip ratio	0.96±0.102	1.07±0.234	<0.001*
Systolic blood pressure (mmHg)	152.08±27.457	144.01±16.09	0.042
Diastolic blood pressure (mmHg)	84.52±11.061	92.84±12.12	<0.001*
Total cholesterol (mg/dl)	211.73±31.064	223.61±28.116	0.058
Triglycerides (mg/dl)	230.86±34.98	275.4±73.011	<0.001*
LDL cholesterol (mg/dl)	128.78±29.378	138.95±34.76	0.981
HDL cholesterol (mg/dl)	36.78±5.248	34.79±5.694	0.168
Fasting plasma glucose (mg/dl)	227.78±42.20	187.76±16.93	<0.001*
HbA1c (%)	7.2±0.265	9.79±2.206	<0.001*
TCSS	2.75±1.96	10.28±2.33	<0.001*
Medication			
Diet	20 (21.1)	23 (20.9)	0.557
Oral	30 (31.6)	15 (13.6)	<0.001*
Insulin	42 (44.2)	65 (59.1)	<0.001*
Complication of diabetes			
Retinopathy	20 (21.1)	51 (56.4)	<0.001*
Microalbuminuria	49 (51.6)	55 (50)	0.466
Stroke	22 (23.2)	29 (26.4)	<0.001*
CHD	34 (47.8)	14 (12.7)	0.254

CHD, coronary heart disease; DPN, diabetic peripheral neuropathy; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T2DM, type 2 diabetes mellitus; TCSS, Toronto clinical scoring system. *$P<0.05$ when compared with control group.

DPN. However, the prevalence of retinopathy and coronary heart disease (CHD) was more common in patients without DPN than patients with DPN (Table 2).
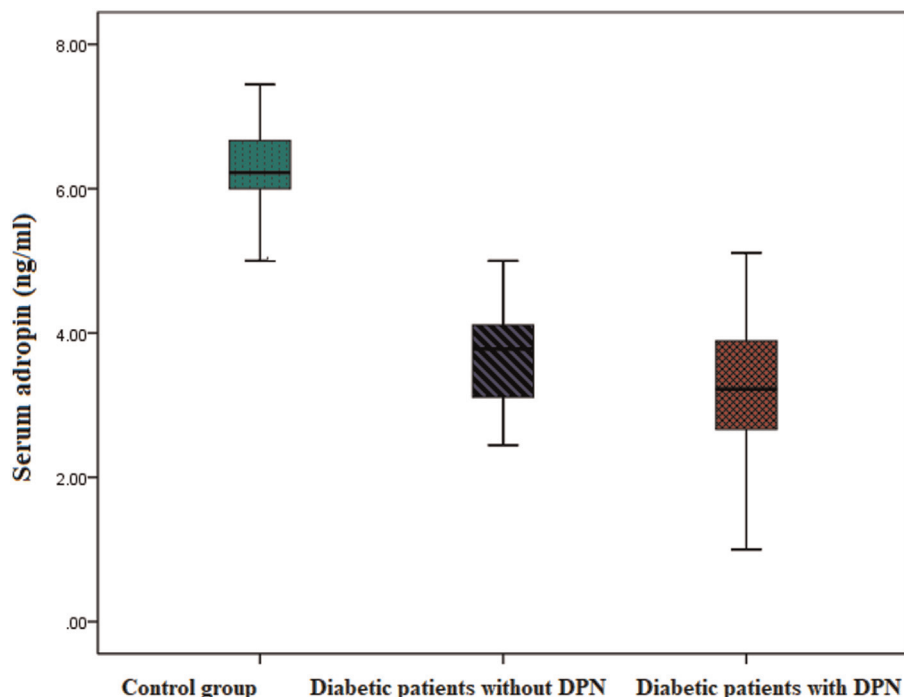
Serum adropin (ng/ml) levels in the studied groups
Our results show that diabetic patients had statistically significant lower values of serum adropin (3.5±1.26) compared with controls (6.1±0), (Table 1). Among diabetic patients, in patients with DPN, there were statistically significant lower values of serum adropin (3.1±1.07) compared with diabetic patients without DPN (3.7±1.3) (Fig. 1).

Correlations between serum adropin and Toronto clinical scoring system, nerve amplitude, and nerve conduction velocity in diabetic patients with diabetic peripheral neuropathy
Our results demonstrated a significant negative correlation between serum adropin and TCSS as well as electrophysiological tests: motor nerve conduction velocity (MNCV), sensory nerve conduction velocity (SNCV), CMAP amplitude (median and ulnar nerve) and SNAP amplitude (median, ulnar, and PT nerve). Interestingly, among the studied tests, the highest negative correlation was found between TCSS and serum adropin ($P<0.001$) (Tables 3 and 4).

Figure 1

Serum adropin (ng/ml) levels in the studied groups.

Table 3 Electrophysiological tests among studied groups

Electrophysiological tests	Control group (n=50) (mean ±SD)	Diabetic patients without DPN (n=40) (mean±SD)	Diabetic patients with DPN (n=60) (mean±SD)
MNCV (m/s)			
Median	53.27±9.8	52.8±10.92	45.66±4.11*
Ulnar	53.09±10.3	52.96±10.54	46.13±6.47*
CPN	52.17±9.8	52.96±10.76	56.8±8.93
SNCV (m/s)			
Median	51.29±9.8	52.12±11.04	42.66±4.11*
Ulnar	53.5±10.4	53.6±7.794	44.8±4.72*
PTN	51.2±9.8	50.4±4.4	52.78±8.4
Sural	50.33±9.8	51.41±4.317	51.01±5.6
CMAP amplitude (mV)			
Median	7.99±1.4	6.38±1.22	4.56±0.41*
Ulnar	8.97±1.484	7.36±1.22	5.54±0.411*
CPN	6.01±1.48	6.25±1.67	5.93±1.41
SNAP amplitude (µV)			
Median	9.94±1.97	8.18±1.67	6.69±1.38*
Ulnar	10.25±1.85	8.77±2.0	7.09±1.59*
PTN	7.44±1.97	5.68±1.67	4.19±1.380*
Sural	8.57±1.97	9.08±2.24	8.41±1.86

CMAP, compound muscle action potential; CPN, common peroneal nerve; DPN, diabetic peripheral neuropathy; MNCV, motor nerve conduction velocity; nerve; SNAP, sensory nerve action potential; PTN, posterior tibial; SNCV, sensory nerve conduction velocity; TCSS, Toronto Clinical Scoring System. *$P_1 < 0.001$ when comparing hypothyroid patients without PN with control group. *$P_2 < 0.001$ when comparing hypothyroid patients with PN with control group.

Pearson correlation between serum adropin with other parameters

There was a negative correlation between serum adropin and FPG (Fig. 2), TG (Fig. 3), as well as TCSS (Fig. 4) and BMI (Fig. 5) ($P < 0.001$*).

Linear regression analyses in patients with hypothyroidism

Linear regression analysis test was done to assess the main independent parameters associated with serum adropin. Our results showed that LDL and FPG, as well as TCSS, were independently correlated with serum adropin (Table 5).

Table 4 Pearson correlation coefficient between serum adropin (ng/ml) and nerve amplitude and nerve conduction velocity

Variables	Diabetic patients with DPN (*n*=60)	
	r	*P*
MNCV (m/s)		
Median	−0.282	<0.001*
Ulnar	−0.276	<0.001*
CPN	−0.134	0.103
SNCV		
Median	−0.308	<0.001*
Ulnar	−0.332	<0.05*
PTN	−0.159	0.051
Sural	−0.006	0.940
CMAP amplitude (mV)		
Median	−0.614	<0.001*
Ulnar	−0.614	<0.01*
CPN	−0.022	0.791
SNAP amplitude (μV)		
Median	−0.501	<0.001*
Ulnar	−0.504	<0.001*
PTN	−0.501	<0.001*
Sural	−0.007	0.932

CMAP, compound muscle action potential; CPN, common peroneal nerve; DPN, diabetic peripheral neuropathy; MNCV, motor nerve conduction velocity; nerve; SNAP, sensory nerve action potential; PTN, posterior tibial; SNCV, sensory nerve conduction velocity.

Electrophysiological tests of the studied groups

Nerve conduction velocities in the studied group showed that MNCV in the median and ulnar nerve were significantly decreased in diabetic patients with DPN compared with the control group. Moreover, SNCV in the median and ulnar nerve were significantly decreased ($P<0.001$), whereas all other nerve velocities difference was not significant.

Regarding amplitudes, CMAP amplitude in the median and ulnar nerve was significantly decreased in diabetic patients with DPN compared with the control group. SNAP amplitude in the median, ulnar, and PT nerve was significantly decreased in diabetic patients with DPN compared with the control group, whereas all other nerve amplitude differences were not significant.

The accuracy of serum adropin for discriminating diabetic patients from the control group by Receiver operating characteristic curve analysis

We investigated the potential diagnostic value of serum adropin by ROC test (Fig. 6). For discriminating diabetic patients from the control group, the cutoff value of serum adropin was 4.16 ng/ml and the AUC was 0.809 (95% CI=0.731–0.887); additionally, the sensitivity and the specificity were 86.7 and 55%, respectively.

The accuracy of serum adropin levels for discriminating diabetic peripheral neuropathy among diabetic patients by receiver operating characteristic curve analysis

We further investigated the potential diagnostic value of serum adropin by ROC test (Fig. 7). In diabetic patients, for discriminating patients with DPN from patients without DPN, the cutoff value of serum adropin was 3.72 ng/ml and the AUC was 0.655 (95% CI=0.537–0.773). Additionally, the sensitivity and the specificity were 60 and 54%, respectively.

Discussion

Omics studies have indeed demonstrated that neuropathy is the most common microvascular complications among patient with T2DM that can involve peripheral, central, and/or autonomic nervous systems [16–18].

DPN is defined as the presence of symptoms or signs of peripheral nerve dysfunction in people with diabetes after exclusion of other causes. Additionally, this definition reflects that the causality of neuropathy in diabetic patients is related to various factors in addition to hyperglycemia [19].

Intriguing reports are suggesting that adropin is a peptide marker correlated with energy homeostasis and obesity. Moreover, adropin also plays an important role in glucose metabolism, as experimental studies confirmed the improvement of glycemic profile and control after treatment with adropin [20].

Despite the growing evidence that the symptoms of DPN are not a reliable indicator for the presence of neuropathy in the disease course, as ∼50% of patients with neuropathy are asymptomatic, they are prone to insensate foot complications [21,22]. Thereby, early recognition of the high-risk population is enormously important. The current research is therefore increasingly focused on the discovery of novel biomarker profiles to further elucidate the complex pathophysiology of DPN.

To our knowledge, no report has been published evaluating the association between adropin and DPN. To addresses this need, we explored the possible association between serum adropin level and risk factors as well as clinical and electrophysiological tests of DPN.
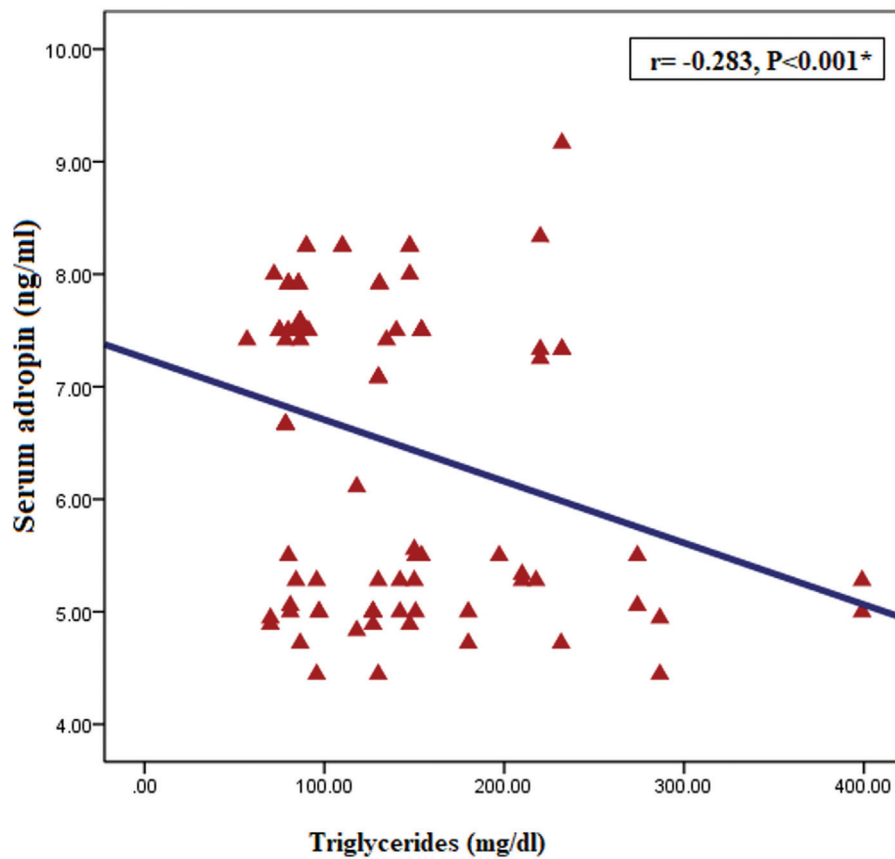
Our study revealed clear evidence that in patients with DPN, there was a longer duration of diabetes as well as
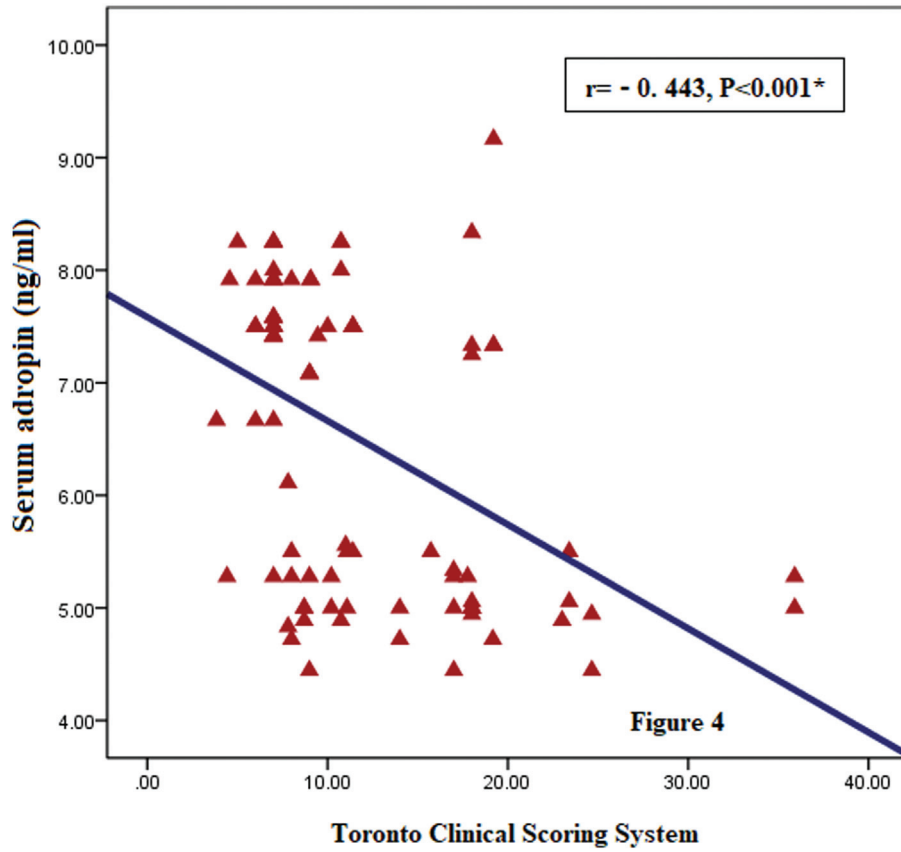
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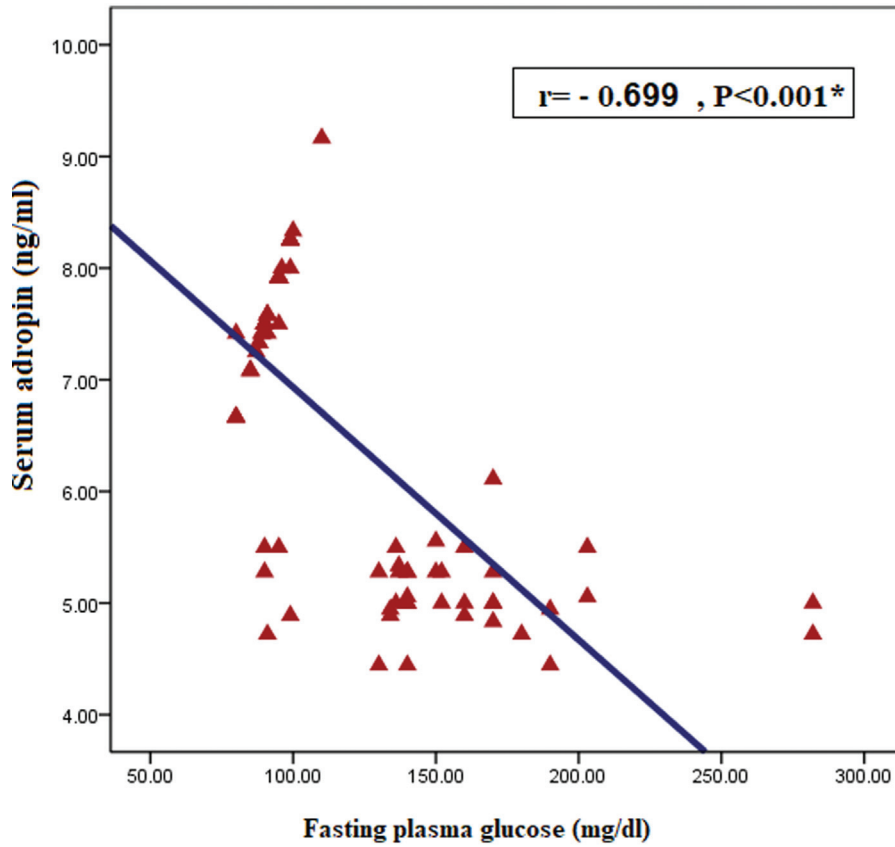
Correlation between serum adropin (ng/ml) levels and fasting plasma glucose in the type 2 diabetes mellitus groups.
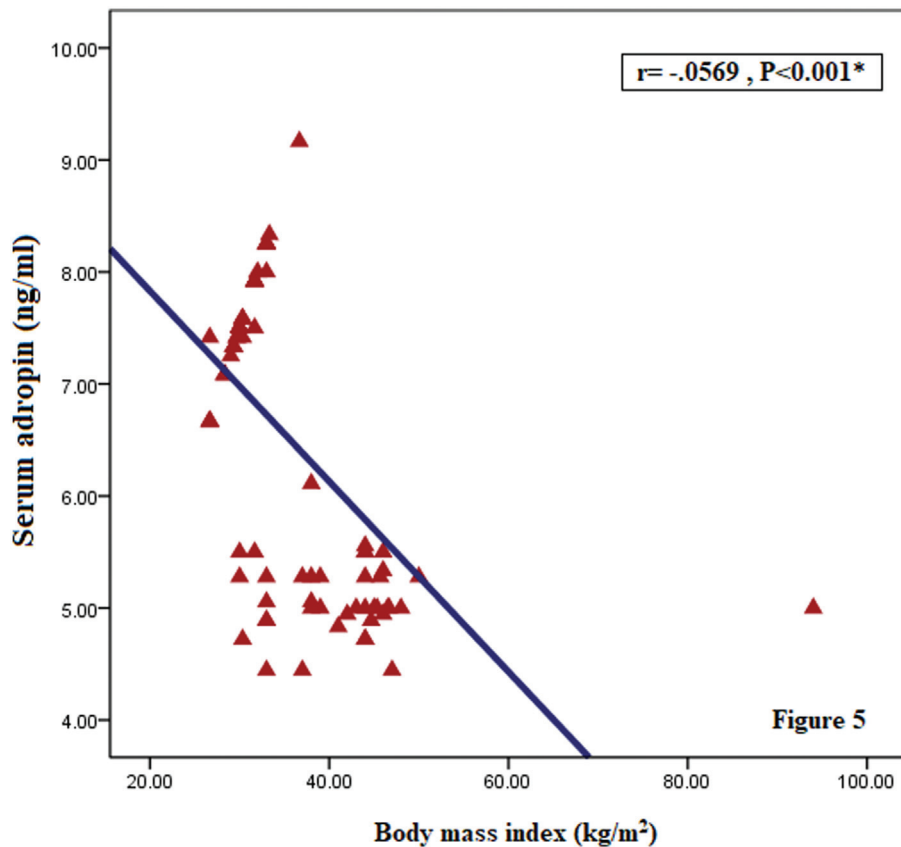
Figure 3

Correlation between serum adropin (ng/ml) levels and triglycerides serum level in the type 2 diabetes mellitus groups.

Figure 4

Correlation between serum adropin (ng/ml) levels and Toronto clinical scoring system in the type 2 diabetes mellitus groups.

Figure 5

Correlation between serum adropin (ng/ml) levels and BMI in the type 2 diabetes mellitus groups.

Table 5 Linear regression analyses to test the influence of the main independent variables against serum adropin (ng/ml) level (dependent variable) in obese patients

Model	Unstandardized coefficients	SE	Standardized coefficients	t	P value	95.0% confidence interval for B	
	B		β			Lower bound	Upper bound
(Constant)	7.089	2.963		2.393	0.018	12.940	1.239
DBP	0.001	0.002	0.048	0.747	0.456	0.002	0.004
SBP	0.001	0.001	0.104	1.659	0.099	0.000	0.003
FPG	−0.017	0.005	−0.635	−3.699	<0.001*	−0.026	−0.008
TCSS	0.012	0.002	0.466	4.815	<0.001*	0.007	0.016
LDL	0.007	0.002	0.318	3.296	<0.001*	0.003	0.010
HDL	0.083	0.051	0.211	1.634	0.104	0.017	0.184

DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL, high-density lipoprotein; SBP, systolic blood pressure; TCSS, Toronto clinical scoring system.

Figure 6

AUC=0. 809 , P<0.001*
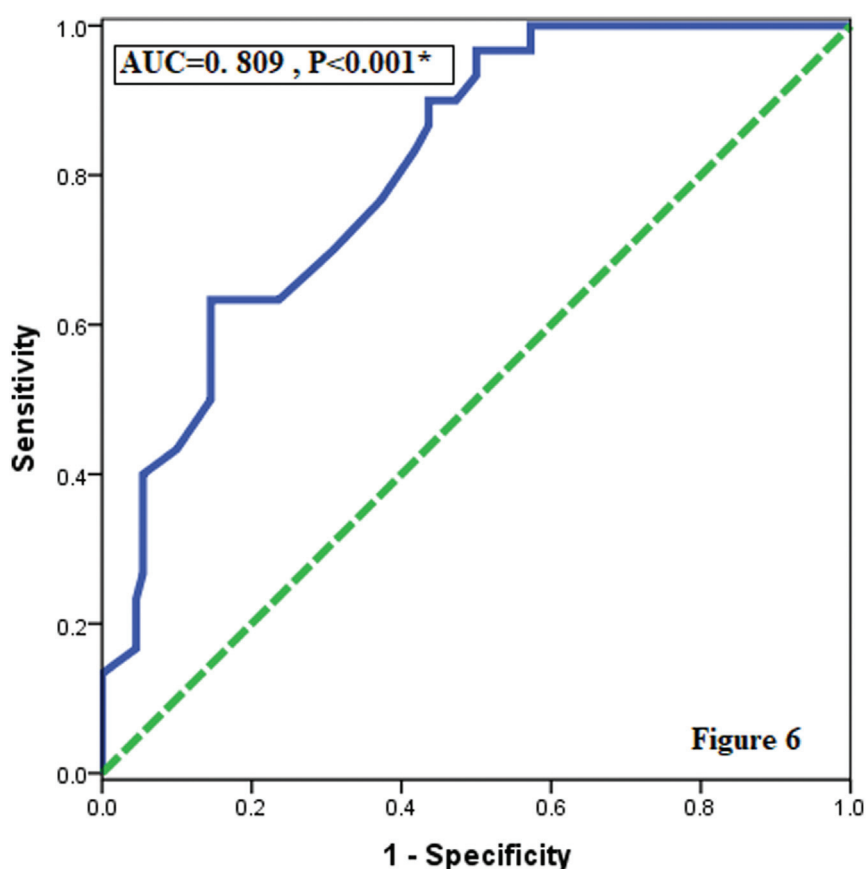
Accuracy of serum adropin for discriminating diabetic patients from control group by receiver operating characteristic curve analysis.

higher values of BMI, waist/hip ratio, diastolic blood pressure, TG, FPG, HbA1c, and TCSS compared with patients without PDN.

Similar to our results, the study by Khawaja et al. [23] revealed that dyslipidemia and long-standing DM (diabetes of ≥5 years) were significantly higher in patients with DPN.

In agreement with our results, the study by Dyck et al. [24] according to the results showed higher levels of

HbA1c were observed in mice with diabetic microvascular complications.

Similar results were described in previous studies that explored the association between abdominal obesity and DPN [25,26]. In contrast, a study by Tapp et al. [27] found a nonsignificant association between waist circumference and DPN in diabetic populations.

According to our results, the prevalence of patients with DPN treated with insulin was more common than

Figure 7
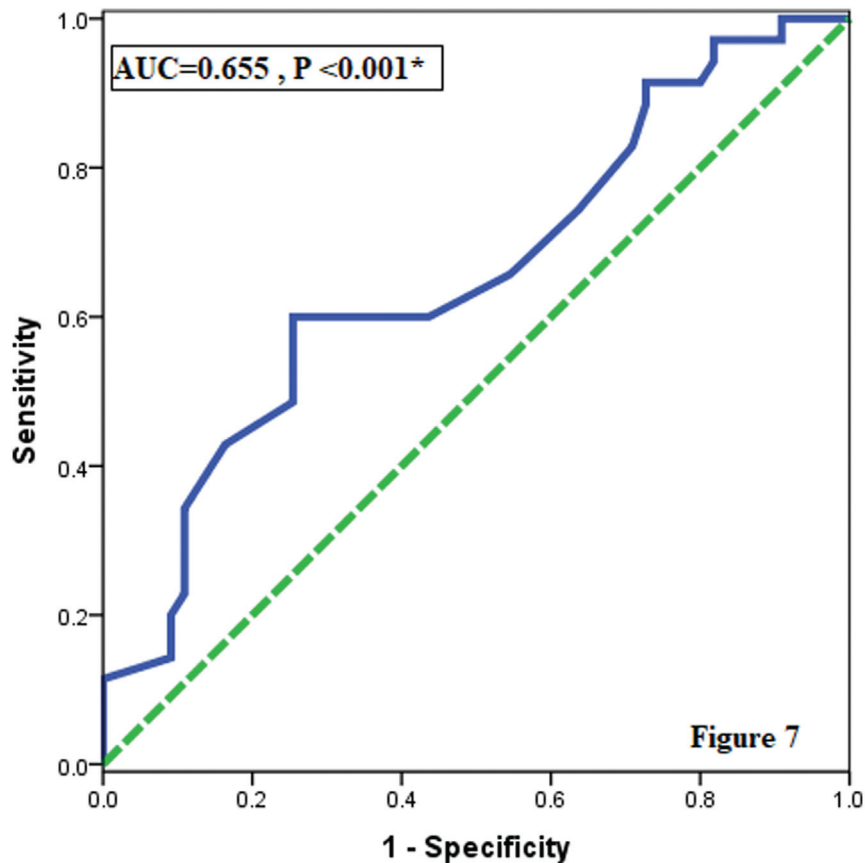
Accuracy of serum adropin levels for discriminating diabetic peripheral neuropathy among diabetic patients by receiver operating characteristic curve analysis.

diabetic patients without DPN. On the contrary, the prevalence of patients treated with oral hypoglycemic medications was more common in patients without PDN than patients with DPN. Regarding diabetic vascular complications, the prevalence of retinopathy and coronary heart disease was more common in patients with DPN than diabetic patients without DPN.

Similar results were confirmed by Morkrid *et al.* [28], they observed that a lower level of fasting blood glucose and oral hypoglycemic use was associated with lower odds of DPN, which emphasizes the role of intensive glycemic control in DPN prevention and treatment.

In agreement with our results, Sone *et al.* [29] found that insulin use was associated with DPN. In this study context, insulin use tends to be an indicator for longer duration and greater severity of diabetes. Similarly, reports of Adler *et al.* [30] detected a positive association between insulin use and DPN.

Similar to our results, a study by Khawaja *et al.* [23] revealed that diabetic retinopathy was significantly higher in patients with DPN.

Against our results, Maser *et al.* [31] demonstrated that nephropathy is the most common diabetes complication and is often concomitant with DPN.

The results presented herein are innovative, as this study performs a robust estimation of serum adropin levels as a biomarker of inflammation. Our results show that diabetic patients had statistically significant lower values of serum adropin compared with controls. Interestingly, in patients with DPN, there were statistically significant lower values of serum adropin compared with diabetic patients without DPN.

In agreement with our results, Gao *et al.* [32] observed lower serum level of adropin in a patient with T2DM compared with controls. Moreover, Beigi *et al.* [33] found lower serum adropin levels in gestational diabetes mellitus.

To the best of our knowledge, this study is the first study to explore the correlation of serum adropin with metabolic risk factors, clinical scoring (TCSS), and electrophysiological tests. In this report, we have demonstrated a negative correlation between serum adropin and FPG, TG, TCSS, and BMI. Intriguing

reports have suggested that serum adropin was correlated with decreased risk of developing T2DM after the logistic regression analysis [34].

Kumar *et al.* [8] found decreased level of serum adropin in obesity and insulin resistance in obese mice, and after injection of adropin, there was a significant improvement of these metabolic deregulations.An interesting study conducted by Hu and Chen [34] evaluated the correlation of adropin with metabolic risk factors. They detected that serum adropin was negatively correlated with BMI. However, they did not find any significant correlation between serum adropin and other metabolic risk factors.

Our results confirmed that serum adropin level had a significant negative correlation with TCSS as well as electrophysiological tests: MNCV (median and ulnar nerve), SNCV (median and ulnar nerve), CMAP amplitude (median and ulnar nerve), and SNAP amplitude (median, ulnar, and PT nerve).

To our knowledge, the role of adropin in the pathogenesis of DPN has not been addressed in previous studies. However, we found an interesting study conducted by Hu and Chen [34] that evaluated the role of serum adropin in diabetic nephropathy. They observed a lower level of serum adropin in diabetic nephropathy. Moreover, the level of adropin was negatively correlated with the progression of DN. Another experimental study detected the role of adropin in diabetic nephropathy. Adropin expression was detected in the kidney tissue of rats, including the glomerulus, peritubular interstitial cells, and peritubular capillary endothelial cells [35].

Accordingly, we analyzed our data by ROC to estimate the cutoff, AUC, sensitivity, and specificity of serum adropin as peptide biomarker. Our results detected that the diagnostic power of serum adropin level in differentiating diabetic patients from the control group was higher than the power of serum adropin level in differentiating DPN among diabetic patients.

Conclusion

In conclusion, the decreased circulating serum adropin levels in diabetic patients especially diabetic patients with DPN was negatively correlated with metabolic risk factors, TCSS, as well as electrophysiological tests. The identification of optimum cutoff of serum adropin could help in evaluating diabetes and DPN in an attempt to decrease health hazards related to

neuropathy. Further multicenter studies with bigger sample size are needed to validate our finding.

Recommendation
The results of this study provide new insights into pathogenesis of DPN. We indeed need further large studies that could help in following up the patients and to better understand the clinical significance of adropin in the pathogenesis of other diabetic microvascular complications.

Conflicts of interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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