

The association of adiponectin gene expression and serum levels with susceptibility to peripheral polyneuropathy in Egyptian patients by women with hypothyroidism

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Received 10 September 2018

Accepted 29 November 2018

The Egyptian Journal of Internal Medicine 2019, 31:302–313

Background

Hypothyroidism has numerous comorbidities including degenerative neurological disease and insulin resistance. Adiponectin is an adipokine secreted by adipose tissue with insulin-sensitizing, anti-inflammatory, and antioxidant properties. This study was designed to explore the expression pattern of Adiponectin and its serum level in hypothyroidism and peripheral neuropathy (PN). The aim was to also evaluate the association between the expression pattern of adiponectin and its serum level with the clinical and electrophysiological tests of PN in hypothyroid patients.

Participants and methods

This cross-sectional controlled study enrolled 110 hypothyroid patients and 80 control group participants. All participants were subjected to a complete neurological examination and nerve conduction study (NCS). Adiponectin level was measured using an enzyme-linked immunosorbent assay. Adiponectin expression levels were estimated using real-time PCR.

Results

The results showed lower values of serum adiponectin and adiponectin expression levels in hypothyroid patients, especially patients with PN. NCS in the studied group showed that motor and sensory nerve conduction in the median and posterior tibial nerves were significantly decreased in both hypothyroid patients with or without PN compared with the euthyroid group. In addition, serum adiponectin and adiponectin expression were negatively correlated with Toronto Clinical Scoring System as well as cardiometabolic risks and positively correlated with NCS of the median, sural, and tibial nerves. The diagnostic power of adiponectin expression is better than that of adiponectin serum levels.

Conclusion

Hypothyroid patients with PN had lower values of serum adiponectin and adiponectin expression levels than hypothyroid patients without PN; the diagnostic power of combined adiponectin serum and expression levels was thus highly significant, and they could be a useful diagnostic biomarker of PN.

Keywords:

adiponectin expression, hypothyroid, nerve conduction studies, polyneuropathy

Egypt J Intern Med 31:302–313

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1110-7782

Introduction

Hypothyroidism is the second common endocrine disease accounting for 2–15% of diseases in the general population [1]. There is compelling evidence suggesting that hypothyroidism is associated with significant alterations both in the neuromuscular system and in brain functions [2]. The neurological manifestations of hypothyroidism are varied and include peripheral neuropathy (PN), entrapment neuropathy, mental dysfunction, abnormal movement, and myxedema coma [3,4].

Omics studies have indeed demonstrated that the neurological dysfunction associated with hypothyroidism may be a result of hormonal imbalance

or may be related to the immune mechanisms associated with thyroid diseases [5]. Increasing evidence points to critical roles of weight gain in hypothyroidism, which may be a contributory factor for neuropathy. In addition, in hypothyroidism there is deposition of mucopolysaccharides in the tissues surrounding the nerves [6,7].

Thyroid stimulating hormone (TSH) stimulates the production of adipokines in human abdominal adipose tissue and preadipocytes by affecting the function of the

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TSH receptor [8]. Adiponectin, an adipose tissue-secreted protein, has been well recognized to exhibit insulin-sensitizing, anti-inflammatory, and anti-atherosclerotic properties, which are mediated through its receptors [7,8].

Hypothyroidism is associated with different comorbidities including obesity, dyslipidemia, insulin resistance and hyperglycemia. The complex etiopathogenesis and heterogeneous clinical manifestations of hypothyroidism make it commonly misdiagnosed. Moreover, the pathological link between hypothyroidism and PN is not clear. Thus, we aimed, in the current study, to explore the expression pattern of adiponectin and its serum level in hypothyroidism and PN. Furthermore, we aimed to evaluate the association between the expression pattern of adiponectin and its serum level with the clinical and electrophysiological tests of PN in hypothyroid patients.

Participants and methods

Participants

This cross-sectional controlled study was conducted in the Outpatient Clinic of Neurology Department at Zagazig University Hospital. This study included 190 unrelated participants. One hundred ten hypothyroid patients from Diabetes and Endocrinology Outpatient Clinic of Internal Medicine Department of Zagazig University Hospitals and 80 euthyroid controls who were matched to cases by age, sex, and ethnic origin were recruited to participate in the study.

The enrolled hypothyroid participants were then classified into hypothyroid patients without PN and hypothyroid patients with PN. Patients with elevated TSH and low thyroid hormone levels were diagnosed as clinical hypothyroid patients.

All participants were subjected to thorough history taking; data were collected through a predesigned structured questionnaire to collect information about age. The enrolled participants were subjected to full clinical assessment including anthropometric variables including BMI and waist circumference (cm)/hip circumference (cm) (WHR) and neurological examination. There is no gold standard for the diagnosis of PN. The expert panel of San Antonio conference recommends that it should be made on the basis of neuropathic symptoms, signs, and nerve conduction studies (NCSs) [9].

Neurological examination was performed using the 10 g Semmes-Weinstein monofilament, 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 was also measured, using a biothesiometer, to define the presence of diabetic neuropathy with a cut-off vibration perception threshold of more than 25 V for the diagnosis of loss of protective sensation.

Exclusion criteria included current psychiatric disorder that might affect the reliability of their response to the study questionnaire, diabetes mellitus, hypertension, and liver or kidney disease. Individuals were also excluded if they took medications known to affect endocrine parameters, metabolism, or inflammation at the start of this study and during the preceding 6 months (sexual steroids) or immediately preceding month (anti-inflammatory drugs). There was no concurrent minor infection reported during the study or during the month preceding the study. Participants with any 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. We also excluded pregnant patients. The ethical committee of Faculty of Medicine, Zagazig University, approved our study protocol, and all participants signed the written informed consent.

Severity of neuropathy

The severity of neuropathy was graded according to the 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 [10].

Nerve conduction study

The motor nerve conduction velocity (MNCV) of the median nerve, the ulnar nerve, and the common peroneal nerve (CPN) and the sensory conduction velocity (SNCV) of the median nerve, the ulnar nerve, the posterior tibial nerve (PTN), and the sural nerve of all participants were carried out for all participants with the Micromed machine in the neurology outpatient clinic. According to the revised criteria of Dyck *et al.* [11], abnormal NCS 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 waveforms.

Blood sampling and laboratory biomarkers

After an overnight fast, blood samples were taken from the participants. Fasting plasma glucose (FPG) was measured by glucose oxidase method (Spinreact; Sant Esteve de Bas, Girona, Spain). Fasting serum insulin (FSI) levels were estimated by an enzyme-linked immunosorbent assay (ELISA) kit (Ray Bio, Norcross, Georgia, USA). Insulin resistance was assessed using the homeostasis model assessment method, homeostasis model assessments of insulin resistance (HOMA-IR), and was calculated as $\text{FSI} (\mu\text{U/ml}) \times \text{FBG} (\text{mg/dl}) / 405$. Total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides levels were measured by enzymatic methods (Spinreact). Friedewald formula was used for calculation of the low-density lipoprotein (LDL)-cholesterol level [12].

Immunochemical assays

The thyroid function tests including FT3, FT4, antithyroglobulin antibodies [anti-triglycerides (TG)], anti-thyroid peroxidase antibodies (anti-TPO), and TSH were measured using chemiluminescence immunoassay (CLIA) assay kit (Immunospec Corporation, Canoga Park, California, USA). The normal reference range for FT3 is 1.8–4.6 pg/ml, for FT4 it is 1.0–1.8 ng/dl, and for TSH it is 0.3–4.2 IU/ml. FSI concentrations were measured using high-sensitivity ELISA kit (Biosource Europe S.A., Nivelles, Belgium). Serum adiponectin concentrations were measured using ELISA (Enzyme-Linked Biotechnology Co. Ltd., Shanghai, China).

Adiponectin gene expression

Total RNA was isolated from whole blood using PAXgene tubes with on-column DNase treatment according to the manufacturer's protocol (PreAnalytiX). RNA was isolated using Trizol (Invitrogen) and an RNeasy Mini Kit (Qiagen, Valencia, USA) with on-column DNase treatment. Spectrophotometry was used to determine RNA concentrations, and RNA samples were stored at -80°C before starting the cDNA synthesis. The mRNA gene expression levels of adiponectin were generated using a custom RT2 Profiler PCR Array run on an ABI 7900HT (Mayo Clinic Medical Genome Facility). Genes were normalized to the mean of three housekeeping genes and to experimental controls and were expressed as relative quantification.

Statistical analyses

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 statistics (mean \pm SD) and were analyzed using t test. Comparison of several means was carried out by one-way analysis of variance, followed by least significance difference test for multiple comparisons between groups. Pearson's correlation coefficient was used to assess the association between adiponectin gene expression and serum level with anthropometric measures as well as electrophysiological parameters in patients. Receiver operating characteristic (ROC) analysis was performed to assess the potential accuracy of adiponectin gene expression and serum level, the area under the curve (AUC), and the cut-off values for diagnosis of PN among hypothyroid patients. We considered P to be significant at less than 0.05 with a 95% confidence interval (CI).

Results

Anthropometric and biochemical characteristics of the studied groups

Hypothyroid patients had significantly higher values of systolic and diastolic blood pressure, as well as lipid profile (TG, TC, and LDL), compared with the control group. Furthermore, hypothyroid patients had significantly higher values of anthropometric measures including BMI and waist/hip ratio, in addition to FPG, FSI, HOMA-IR, TSH, and TCSS compared with the control group ($P < 0.001$). In contrast, obese patients had significantly lower values of HDL and FT4 compared with the control group (Table 1).

General characteristics of hypothyroidism groups with or without neuropathy

In hypothyroid patients with PN, there were statistically significant higher values of diastolic blood pressure, BMI, waist/hip ratio, TG, LDL, FSI, HOMA-IR, and FPG compared with hypothyroid patients without PN. On the contrary, hypothyroid patients with PN had significantly lower values of FT4 and HDL compared with hypothyroid patients without PN (Table 2).

Comparison of serum adiponectin and adiponectin expression levels in the studied groups

Our results show that hypothyroid patients with PN had statistically significant lower values of serum adiponectin and adiponectin expression (9.07 ± 2.946 and 1.22 ± 0.378 , respectively) compared with that of hypothyroid patients without PN (11.92 ± 1.676 and

Table 1 Anthropometric and biochemical characteristics of the studied groups.

Variables	Euthyroid group (n=80)	Hypothyroid group (n=110)	P value
Age	43.4±7.98	42.45±7.63	0.455
SBP (mmHg)	116.8±15.48	150.6±17.84	< 0.001*
DBP (mmHg)	75.8±9.001	88.85±8.56	< 0.001*
BMI (kg/m ²)	21.2±2.014	35.41±4.21	< 0.001*
Waist/hip ratio	0.86±0.012	9.012±0.183	< 0.001*
Total cholesterol (mg/dl)	185.61±18.87	217.11±8.87	< 0.001*
Triglycerides (mg/dl)	93.62±16.75	222.54±15.0	< 0.001*
LDL cholesterol (mg/dl)	102.677±26.79	127.65±2.7	< 0.001*
HDL cholesterol (mg/dl)	47.91±4.26	35.76±0.548	< 0.001*
FPG (mg/dl)	87.62±6.276	86.93±10.03	< 0.001*
FSI (μU/ml)	5.4±1.82	13.18±6.37	< 0.001*
HOMA-IR	1.27±0.40	5.7±4.14	< 0.001*
TCSS	0.57±0.011	6.358±4.33	< 0.001*
FT4 (ng/dl)	1.19±0.047	0.69±0.22	< 0.001*
TSH (μU/ml)	2.52±1.951	17.52±3.15	< 0.001*

DBP, diastolic blood pressure; FPG fasting plasma glucose; FSI, fasting serum insulin; FT4, free thyroxine; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessments of insulin resistance; LDL-c, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TCSS, Toronto Clinical Scoring System; TSH, thyroid stimulating hormone. * $P < 0.05$.

Table 2 Laboratory and anthropometric parameters of hypothyroid patients by women

Variables	Hypothyroid without PN (N=73)	Hypothyroid with PN (N=37)	P value
Age	38.98±7.98	49.95±7.63	0.295
SBP (mmHg)	152.54±15.48	148.23±17.84	0.302
DBP (mmHg)	84.7±9.001	96.05±8.56	< 0.001*
BMI (kg/m ²)	34.2±2.014	33.41±4.21	< 0.001*
Waist/hip ratio	0.94±0.03	1.13±2.69	< 0.001*
TC (mg/dl)	215.76±18.87	220.56±8.87	0.294
Triglycerides (mg/dl)	175.62±16.75	233.54±15.0	< 0.001*
LDL cholesterol (mg/dl)	131.22±26.79	120.48±2.7	< 0.001*
HDL cholesterol (mg/dl)	36.82±4.26	33.76±0.548	< 0.001*
FPG (mg/dl)	77.62±6.276	86.93±10.03	< 0.001*
FSI (μU/ml)	11.4±1.82	22.18±6.37	< 0.001*
HOMA-IR	6.57±0.40	8.7±4.14	< 0.001*
TCSS	2.37±1.011	9.358±2.33	< 0.001*
FT4 (ng/dl)	0.79±0.047	0.49±0.22	< 0.001*
TSH (μU/ml)	7.76±5.29	14.42±3.15	< 0.001*

DBP, diastolic blood pressure; FPG, fasting plasma glucose; FSI, fasting serum insulin; FT4, free thyroxine; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessments of insulin resistance; LDL-c, low-density lipoprotein cholesterol; PN, peripheral neuropathy; SBP, systolic blood pressure; TCSS, Toronto Clinical Scoring System; TSH, thyroid stimulating hormone. * $P < 0.05$.

1.89±0.26, respectively) and euthyroid participants (15.45±2.087 and 2.456±0.334, respectively), as shown in Fig. 1a and b, respectively.

Electrophysiological tests of the studied groups

Nerve conduction velocities in the studied group showed that MNCV in median and PTNs were significantly decreased in both hypothyroid patients with or without PN compared with the control group. Moreover, SNCV in the median nerve and ulnar nerve was significantly decreased in hypothyroid patients with PN. However, SNCV in the ulnar nerve was significantly decreased in hypothyroid patients with PN (Table 3).

As regards amplitudes, CMAP amplitude in median and PTNs was significantly decreased in both hypothyroid patients with or without PN compared with the control group. SNAP amplitude in the median nerve and PTN was significantly decreased in both hypothyroid patients with or without PN compared with the control group (Table 3).

Correlations between serum adiponectin and adiponectin expression levels with Toronto Clinical Scoring System as well as electrophysiological parameters in patients with hypothyroidism

Concerning serum adiponectin and adiponectin expression levels, our results demonstrated a significantly negative correlation with TCSS. On the contrary, there were significant positive correlations with electrophysiological tests: MNCV, SNCV, CMAP amplitude and SNAP amplitude of the median, sural and PTNs (Table 4).

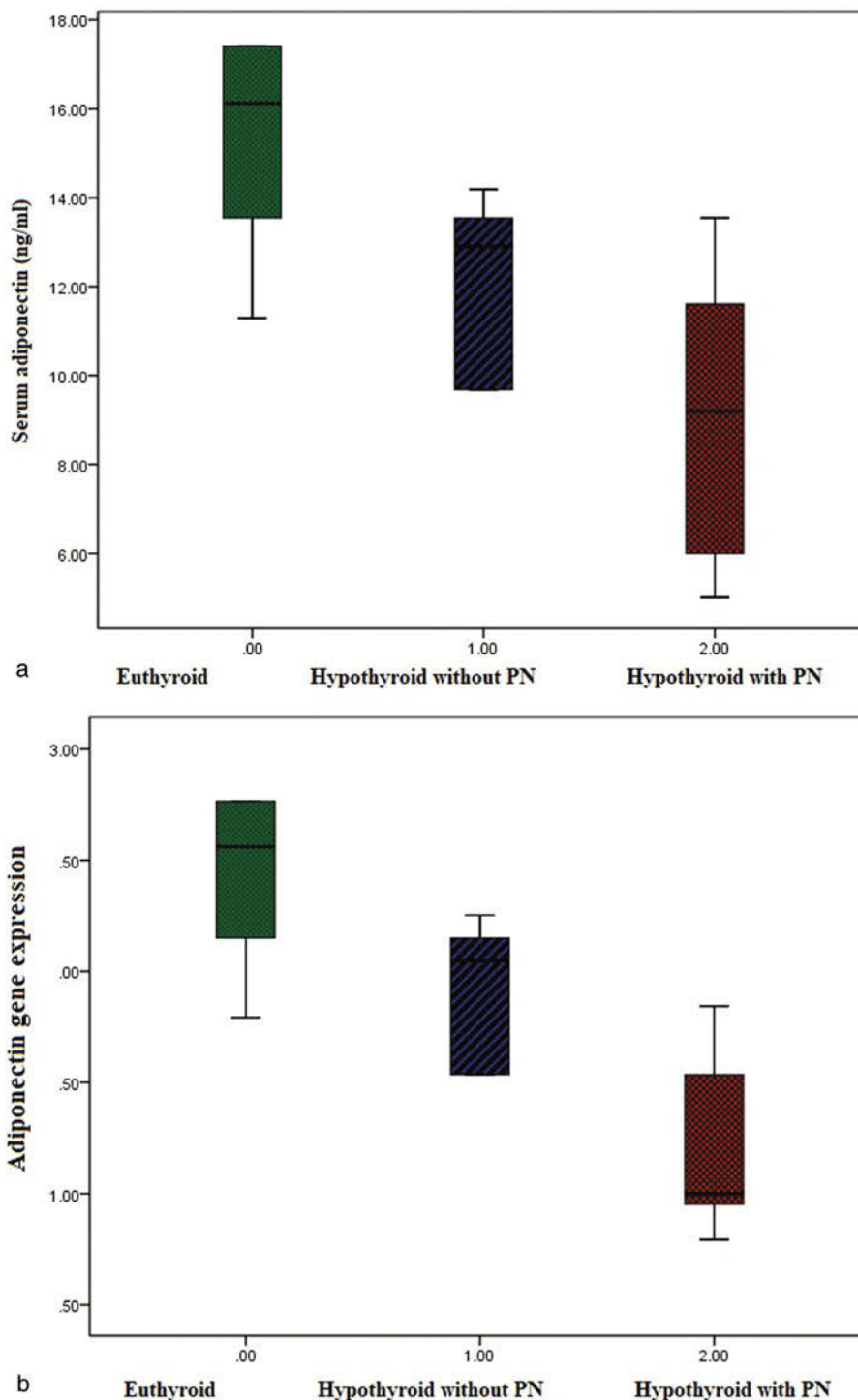
Correlations between serum adiponectin and adiponectin expression levels with other studied parameters in hypothyroid patients

Our results demonstrated significant negative correlations between serum adiponectin and adiponectin expression and cardiometabolic parameters including BMI, TC, TG, FPG, FSI, and systolic and diastolic blood pressure as well as HOMA-IR (Table 5). Even more important, our results demonstrated statistically significant positive correlations between serum adiponectin and adiponectin expression level (Fig. 2).

Linear regression analyses in obese patients to assess the main independent parameters associated with serum adiponectin and adiponectin expression levels

Linear regression analysis test revealed that serum adiponectin levels were independently correlated with FT4, FSI, FPG, and HOMA-IR ($P < 0.001$). Interestingly, adiponectin expression levels were

Fig. 1



(a) Comparison of serum adiponectin levels in the studied groups. (b) Comparison of adiponectin expression levels in the studied groups.

independently correlated with TCSS and TG (Table 6).

Logistic regression analysis in hypothyroid women

Logistic regression analysis test revealed that, after adjusting for age, BMI, and lipid profile, the only biomarker independently correlated with PN among hypothyroidism patients was serum adiponectin level (Table 7).

Accuracy of serum adiponectin and adiponectin expression levels for discriminating hypothyroidism group from control euthyroid group by receiver operating characteristic analysis

We investigated the potential diagnostic value of serum adiponectin and adiponectin expression by ROC tests (Fig. 3a and b). When we discriminate hypothyroid patients from the control group, the cut-off values of serum adiponectin and adiponectin expression were 13.38 and 2.21, respectively, and the AUC values

Table 3 Comparison of clinical and electrophysiological tests of the studied groups

Electrophysiological parameters	Euthyroid (n=80)	Hypothyroid without peripheral neuropathy (n=73)	Hypothyroid with peripheral neuropathy (n=37)	P_1	P_2
TCSS	0.565±0.21	3.34±2.37	9.28±2.96	<0.001*	< 0.001*
MNCV (m/s)					
Median	53.27±1.89	59.48±11.35	45.2±1.3	<0.001*	< 0.001*
PTN	54.09±10.3	58.7±11.27	46.6±6.32	<0.001*	< 0.001*
CPN	52.17±9.8	54.95±11.62	56.6±8.97	0.015	0.316
SNCV (m/s)					
Median	51.29±9.79	58.3±11.77	42.36±4.65	<0.001*	<0.001*
PTN	51.50±10.42	53.65±8.15	51.68±5.26	0.106	0.696
Ulnar	53.20±9.83	54.45±5.23	44.04±8.26	0.457	<0.001*
Sural	50.33±9.87	52.39±4.93	50.93±5.56	0.071	0.331
CMAP amplitude (mV)					
Median	7.92±0.48	6.59±1.31	4.5±0.5	<0.001*	<0.001*
PTN	8.94±1.48	7.35±1.31	5.6±0.5	<0.001*	<0.001*
CPN	5.95±1.48	6.26±1.67	6.09±1.39	0.337	0.818
SNAP amplitude (µV)					
Median	10.88±1.97	8.24±1.87	6.54±1.38	<0.001*	<0.001*
Sural	8.65±1.85	9.41±2.34	8.31±1.75	<0.001*	0.404
PTN	7.52±1.93	5.74±1.85	4.01±1.39	<0.001*	<0.001*
Ulnar	10.23±1.94	8.44±1.68	6.64±1.89	0.084	0.507

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

Table 4 Pearson correlation between adiponectin gene expression and serum level with Toronto Clinical Scoring System as well as electrophysiological parameters in hypothyroid group by women

Electrophysiological parameters	Adiponectin serum level		Adiponectin gene expression	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
TCSS	-0.578	<0.001*	-0.618	<0.001*
MNCV				
Median	0.197	<0.01*	0.319	<0.001*
PTN	0.263	<0.001*	0.249	<0.001*
CPN	0.097	0.183	0.094	0.181
SNCV				
Median	0.174	<0.001*	0.357	<0.001*
PTN	0.222	<0.001*	0.295	<0.001*
Ulnar	0.013	0.789	0.566	0.650
Sural	0.208	0.835	0.113	0.393
CMAP amplitude				
Median	0.317	<0.001*	0.506	<0.001*
PTN	0.274	<0.001*	0.506	<0.001*
CPN	0.063	0.489	0.005	0.949
SNAP amplitude				
Median	0.373	<0.001*	0.393	<0.001*
PTN	0.373	<0.001*	0.3240	<0.001*
Sural	0.072	0.325	0.093	0.265
Ulnar	0.302	0.444	0.172	0.175

CMAP, compound muscle action potential; CPN, common peroneal nerve; MNCV, motor nerve conduction velocity; PTN, posterior tibial nerve; SNAP, sensory nerve action potential; TCSS, Toronto Clinical Scoring System.

were 0.903 (95% CI=0.860–0.46), and 0.915 (95% CI=0.876–0.954), respectively. In addition, the sensitivities and the specificities were (93.6 and 73.7%) and (95 and 76%), respectively.

ROC analysis revealed that combined serum adiponectin and adiponectin expression levels were useful in discriminating the hypothyroidism group from the control group (AUC=0.908, 95% CI:

Table 5 Pearson correlation coefficient between adiponectin gene expression and serum level with other studied parameters in hypothyroid patients by women

	Adiponectin serum level	Adiponectin gene expression
BMI		
<i>r</i>	-0.791	-0.699
<i>P</i>	<0.001*	<0.001*
Waist circumference (cm)		
<i>r</i>	-0.496	-0.487
<i>P</i>	<0.001*	<0.001*
SBP (mmHg)		
<i>r</i>	-0.541	-0.645
<i>P</i>	<0.001*	<0.001*
DBP (mmHg)		
<i>r</i>	-0.575	-0.497
<i>P</i>	<0.001*	<0.001*
TC (mg/dl)		
<i>r</i>	-0.573	-0.522
<i>P</i>	<0.001*	<0.001*
Triglycerides (mg/dl)		
<i>r</i>	-0.610	-0.712
<i>P</i>	<0.001*	<0.001*
HDL cholesterol (mg/dl)		
<i>r</i>	0.879	0.882
<i>P</i>	<0.001*	<0.001*
LDL cholesterol (mg/dl)		
<i>r</i>	-0.466	-0.459
<i>P</i>	<0.001*	<0.001*
FPG (mg/dl)		
<i>r</i>	-0.675	-0.623
<i>P</i>	<0.001*	<0.001*
FSI (μ U/ml)		
<i>r</i>	-0.625	-0.597
<i>P</i>	<0.001*	<0.001*
HOMA-IR		
<i>r</i>	-0.689	-0.711
<i>P</i>	<0.001*	<0.001*

DBP, diastolic blood pressure; FPG, fasting plasma glucose; FSI, fasting serum insulin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessments of insulin resistance; LDL, low-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol.

0.867–0.949, $P < 0.001$), with a sensitivity of 94.7% and specificity of 75.7 (Fig. 3c).

Accuracy of serum adiponectin and adiponectin expression levels for discriminating PN among hypothyroid patients by receiver operating characteristic analysis

We further investigated the potential diagnostic value of serum adiponectin and adiponectin expression by ROC test (Fig. 4a and b). In hypothyroid patients, when we discriminate patients with PN from patients without PN, the cut-off values of serum adiponectin and adiponectin expression were 12.25 and 1.667, respectively, and the AUC values were 0.785 (95% CI=0.692–0.879) and 0.892 (95% CI=0.828–0.957), respectively. In addition, the sensitivities and the

specificities were (86.9 and 55%) and (77.8 and 63.2%), respectively.

ROC analysis revealed that combined serum adiponectin and adiponectin expression levels were useful in discriminating PN among hypothyroid patients (AUC=0.814, 95% CI: 0.732–0.897, $P < 0.001$), with a sensitivity of 86.5% and specificity of 54.2 (Fig. 4c).

Discussion

There is mounting evidence suggesting that hypothyroidism may cause slowing of nerve conduction. The pathophysiological role of hypothyroidism in PN could be due to mechanical and metabolic reasons including insulin resistance, which is the most likely pathophysiology to explain the occurrence of median nerve impairment among obese individuals [5–7].

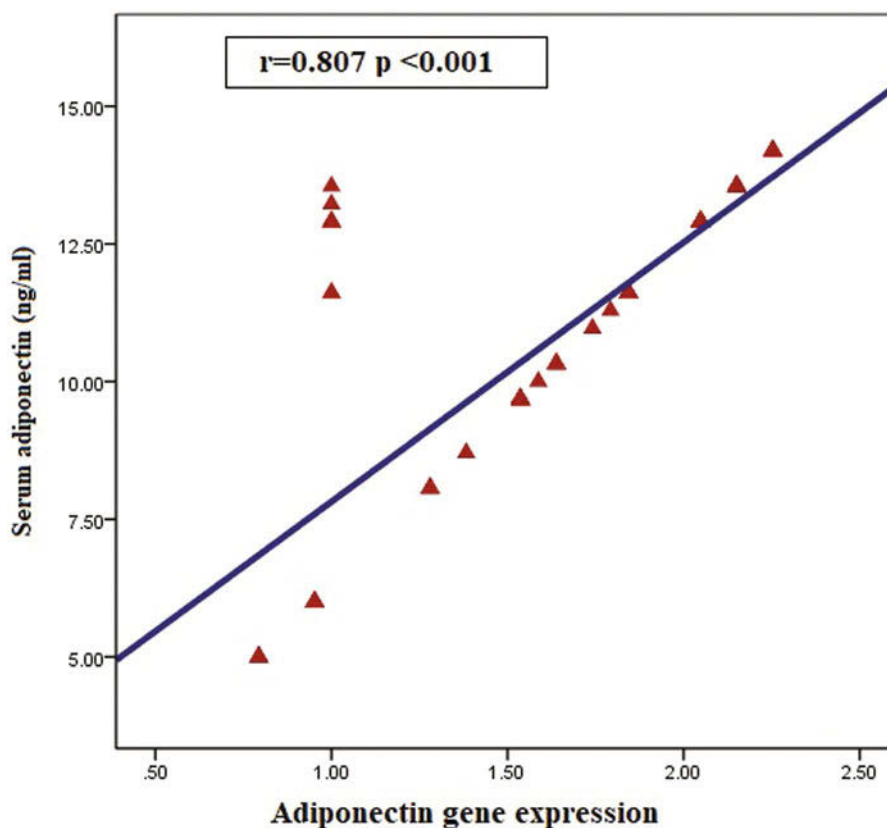
Despite the growing evidence that the symptoms of PN are not a reliable indicator for the presence of neuropathy in the disease course, as about 50% of patients with neuropathy are asymptomatic, therefore, they are prone to insensate foot complications [13]. Thereby, early recognition of the high-risk population is enormously important. To address this need, we have focused on estimating the levels of adiponectin expression and its serum level in hypothyroidism and PN. To the best of our knowledge, this study was the first study to evaluate the association between expression pattern of adiponectin, and its serum level with the clinical and electrophysiological tests of PN as well as clinical and laboratory characteristics of hypothyroid patients.

According to our study, the prevalence of PN was 33.6% among the studied hypothyroid patients, as we assessed our patients by both clinical scoring (TCSS) and electrophysiological tests.

Similar to our findings, the prospective cohort study on neurologically asymptomatic patients with primary hypothyroidism was conducted to evaluate the frequency and pattern of neurophysiological changes in this group of patients. The interesting findings of the study are that about 52% of the patients had some abnormality by NCS, predominantly of the motor demyelinating pattern, and 30% of patients had median mononeuropathy consistent with carpal tunnel syndrome [14].

Our study revealed clear evidence that hypothyroid patients had significantly higher values of TCSS compared with the euthyroid group. In addition,

Fig. 2



Correlation between serum adiponectin levels and adiponectin gene expression in patients with hypothyroidism.

Table 6 Linear regression analyses to test the influence of the main independent variables against adiponectin gene expression and serum levels (dependent variable) in obese patients by women

Model	Unstandardized coefficients	SE	Standardized coefficients	<i>t</i>	<i>P</i> value	95% CI	
	<i>B</i>		β			Lower bound	Upper bound
Adiponectin serum level							
(Constant)	30.358	5.601		5.420	<0.001*	19.24	41.472
TC	-0.008	0.005	0-.122	-1.528	0.130	-0.018	0.002
TG	-0.004	0.004	-0.135	-1.061	0.291	-0.012	0.004
FPG	-0.01-	0.004	-0.360	-3.860	<0.001*	-0.023	-0.007
FSI	-0.219	0.050	-0.410	-4.385	<0.001*	-0.318	-0.120
HOMA-IR	0.050	0.070	0.072	-0.524	<0.001*	-0.411	-0.388
TCSS	-0.123	0.065	-0.243	-1.897	0.061	-0.251	0.006
TSH	0.024	0.146	0.013	0.167	0.867	-0.266	0.315
FT4	-3.211	1.315	-0.187	-2.442	<0.05*	-5.811	-0.602
Adiponectin gene expression							
(Constant)	4.230	0.918		4.607	<0.001*	2.409	6.052
TC	-0.001	0.001	-0.192	-1.41	0.161	-0.002	-0.000
TG	-0.036	0.008	-0.429	-4.425	<0.001*	-0.053	0.020
FPG	0-.006	0.011	-0.057	-0.562	0.575	-0.029	0.016
FSI	-0.003	0.010	-0.035	-0.287	0.774	-0.022	0.017
HOMA-IR	-0.007	0.024	-0.025	-0.307	0.760	0-.056	0.041
TCSS	-0.484	0.224	-0.178	-2.156	<0.05*	-0.929	-0.039

CI, confidence interval; FPG, fasting plasma glucose; FSI, fasting serum insulin; HOMA-IR, homeostasis model assessments of insulin resistance; TC, total cholesterol; TCSS, Toronto Clinical Scoring System; TG, triglycerides; TSH, thyroid stimulating hormone.

nerve conduction velocities in the studied group showed that MNCV in median and PTNs was significantly decreased in both hypothyroid patients

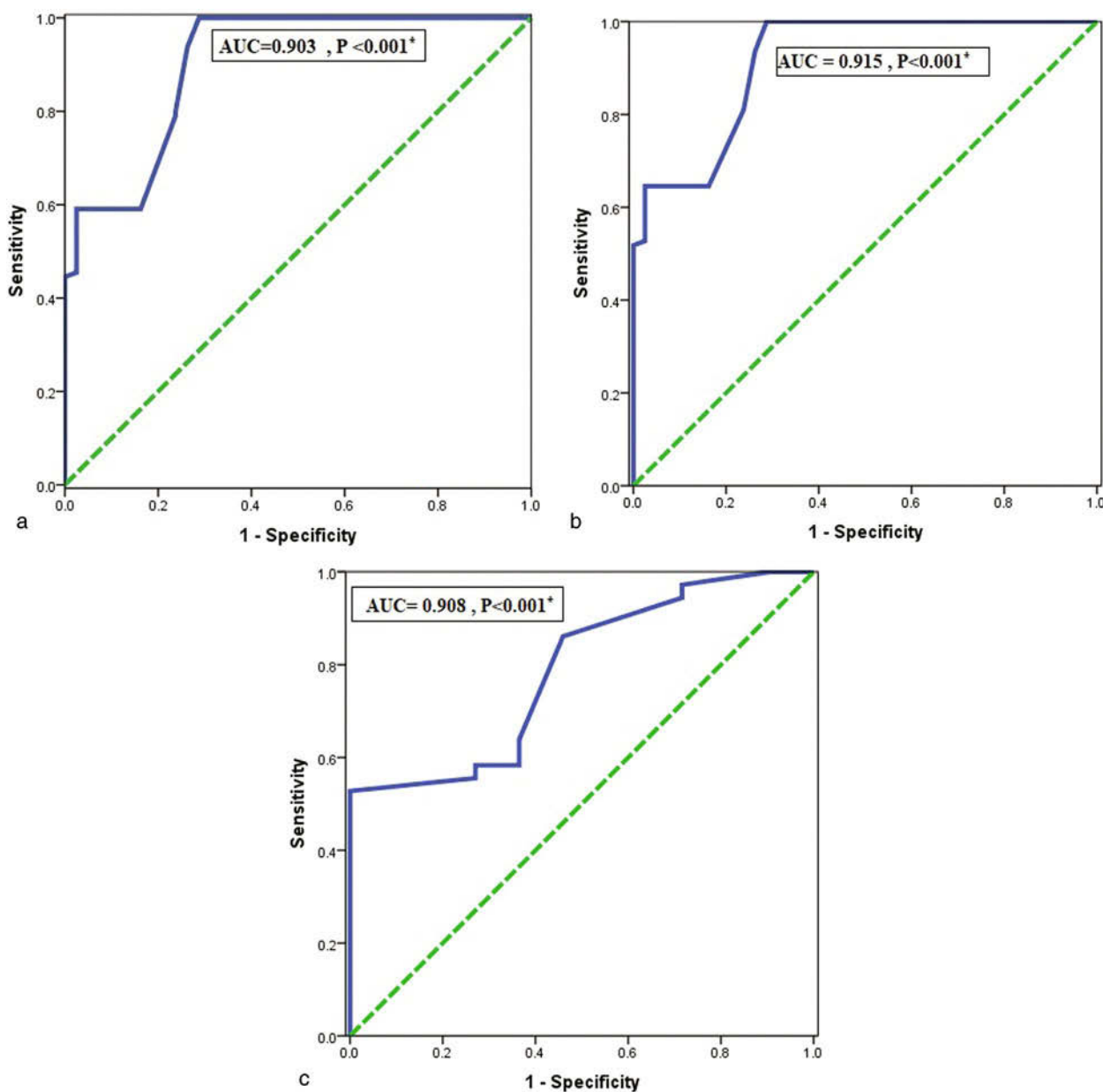
with or without PN compared with the euthyroid group. Moreover, SNCV in the median and ulnar nerves was significantly decreased in hypothyroid

Table 7 Logistic regression analysis evaluating the main independent variables associated with peripheral neuropathy among hypothyroid women

	B	SE	t	P value	Odds ratio	95% CI	
						Lower	Upper
Step 1 ^a							
Serum adiponectin	-0.399	0.124	10.377	<0.001*	0.671	0.527	0.855
FT4	-2.550	2.110	1.460	0.227	0.078	0.001	4.885
TSH	0.129	0.225	0.331	0.565	0.879	0.565	1.365
Constant	179.73	139.873	1.651	0.199	1.140		

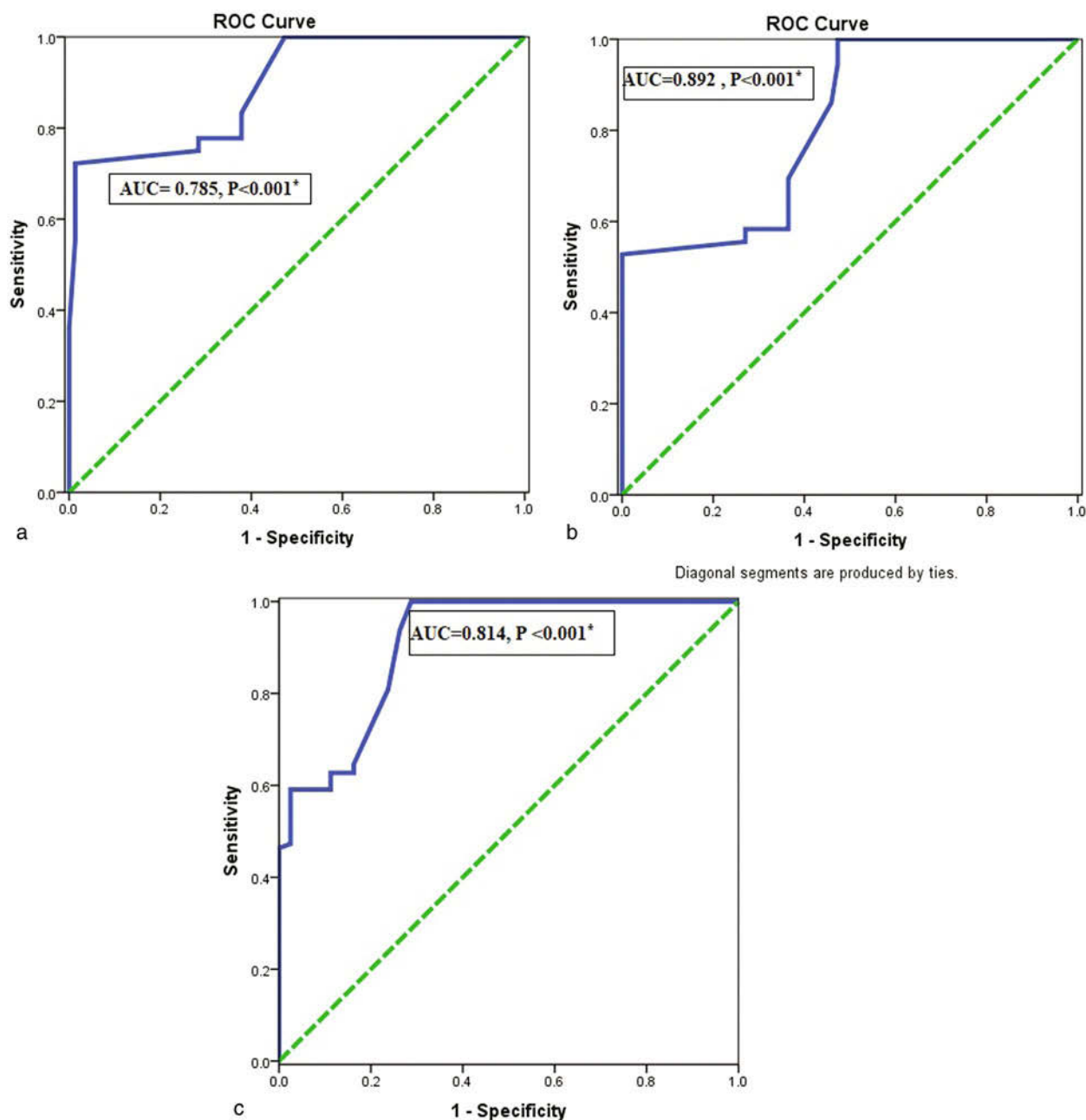
CI, confidence interval; TSH, thyroid stimulating hormone.

Fig. 3



(a) ROC curve of serum adiponectin levels for discriminating hypothyroid from euthyroid. (b) ROC curve of adiponectin expression levels for discriminating hypothyroid from euthyroid. (c) ROC curve of combined serum adiponectin levels and adiponectin expression for discriminating hypothyroid from euthyroid. ROC, receiver operating characteristic curve.

Fig. 4



(a) ROC curve of serum adiponectin levels for hypothyroid with PN from hypothyroid without PN. (b) ROC curve of NFκβ/P65 activity for discriminating hypothyroid with PN from hypothyroid without PN. (c) ROC curve of combined serum adiponectin levels and adiponectin expression for discriminating hypothyroid with PN from hypothyroid without PN. PN, peripheral neuropathy; ROC, receiver operating characteristic curve.

patients with PN. However, SNCV in the ulnar nerve was significantly decreased in hypothyroid patients with PN.

According to Kececi and Degirmenci [15], the entrapment neuropathy and polyneuropathy in hypothyroid patients can be reversible in a period of 3 months if appropriate hormone replacement treatment is administered.

Supporting our results, Mahadule *et al.* [16] found that hypothyroid patients had significantly prolonged

distal motor latencies, reduced CMAP amplitudes and slowed MNCV for bilateral median, ulnar and PTNs.

The mechanisms involved in the development of neuropathy in hypothyroidism is that the thyroid hormones' deficiency leads to deficiency of ATP; reduced ATPase and Na-K pump activity in hypothyroidism cause subsequent alteration of pump-dependent axonal transport and may lead to PN [6,17].

The results presented herein are innovative, as this study performs a robust estimation of serum adiponectin and adiponectin expression. Our study revealed that hypothyroid patients with PN had statistically significant lower values of serum adiponectin and adiponectin expression compared with that of hypothyroid patients without PN and euthyroid controls.

To the best of our knowledge, this study is the first study that has explored the correlation of serum adiponectin and adiponectin expression with clinical scoring (TCSS) and electrophysiological tests. Noteworthy, our results confirmed that serum adiponectin and adiponectin expression level had a significant negative correlation with TCSS. As regards electrophysiological tests, there were positive correlations between serum adiponectin and adiponectin expression level with electrophysiological tests: MNCV, SNCV, CMAP amplitude and SNAP amplitude of the median, sural and PTNs.

An interesting study by Ji *et al.* [18] observed an increased risk of PN in diabetic patients, by downregulating adiponectin expression, which resulted in significantly reduced circulating adiponectin plasma levels. Accumulating evidence has shown that the adiponectin gene serves as a protective factor in preventing diabetes progression by suppressing inflammatory responses and increasing insulin sensitivity [19]. In order to better elucidate the correlations, we further analyzed our results by linear regression analysis test, which revealed that serum adiponectin levels were independently correlated with FT4, FSI, FPG, and HOMA-IR. Interestingly, adiponectin expression levels were independently correlated with TCSS and TG. However, the logistic regression analysis test revealed that after adjusting for age, BMI, and lipid profile, the only biomarker independently correlated with PN among hypothyroidism was serum adiponectin level

Similar to our results, current evidence indicated that adiponectin levels were correlated negatively with adiposity [20], insulin resistance [21], and metabolic syndrome [22].

Accordingly, we analyzed our data by ROC to estimate the cut-off, AUC, sensitivity and specificity of serum adiponectin and adiponectin expression. Our results detected that the diagnostic power of adiponectin expression levels was significantly higher than serum adiponectin levels in differentiating hypothyroid

patients from the euthyroid group as well as discriminating PN among obese patients. Nonetheless, the AUC, sensitivity and specificity of combined serum adiponectin and adiponectin expression levels improved the diagnostic power of serum adiponectin levels in differentiating hypothyroid patients from the euthyroid group as well as discriminating PN among obese patients.

In conclusion, our results showed lower values of serum adiponectin and adiponectin expression levels in hypothyroid patients, especially patients with PN. In addition, serum adiponectin and adiponectin expression were negatively correlated with TCSS as well as cardiometabolic risks and positively correlated with MNCV, SNCV, CMAP amplitude and SNAP amplitude of the median, sural and PTNs; the diagnostic power of adiponectin expression is better than adiponectin serum levels.

Financial support and sponsorship

Nil.

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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