Evaluation of plasma levels of adrenomedullin and ghrelin, and their correlation with electrophysiological changes in diabetic neuropathy

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ABSTRACT

الأهداف: تقويم مستويات بلازما هرمون الأدرينوميدالين والجريلين وعلاقتهم مع التغيرات الكهرومغناطيسية لدى المرضى المصابين بالاعتلال العصبي السكري.

الطريقة: أجريت هذه الدراسة خلال الفترة من مارس 2008م حتى نوفمبر 2010م في قسم الفيسولوجيا الطبية، مستشفى الملك عبدالعزيز، جامعة الملك سعود، الرياض، المملكة العربية السعودية على 90 إمرأة قسموا على النحو التالي: مجموعة الشاهد (المجموعة الأولى) 30 امرأة، 30 امرأة مصابة بالسكري تم التحكم به (المجموعة الثانية)، و30 امرأة مصابة بالاعتلال العصبي (المجموعة الثالثة). تم قياس كلا من القياسات الكيميائية والكهروفيزيائية لمستويات بلازما هرمون الأدرينوميدالين والجريلين.

النتائج: أظهرت الدراسة انخفاض مهم إحصائي لسرعة العصب الحسي، والحركي، واستطالة استجابة موجة للعصب المتوسط، والزبدي، والشظوي، والربكي مع الاعتلال العصبي. أظهر مؤشر الكاحل/العضد تغير في جميع المجموعات بالمقارنة مع مجموعة الشاهد. كان هنالك انخفاض بشكل احصائي هرمون الأدرينوميدالين والجريلين في المجموعة الثالثة بالمقارنة مع المجموعة الأولى. أظهرت النتائج أن هرمون الأدرينوميدالين والجريلين مرتبطين بشكل ايجابي مع الأعصاب المحيطية الحركية وسرعة التوصيل الحسية.

خاتمة: يشير تغير تركيز مستويات هرمون الأدرينوميدالين والجريلين إلى دورها الفيزولوجيا المرضي وانخفاضها عامل مسبب ولديه آثار عصبية وعضلية محصنة لذلك يشير انعدامها إلى اصابات عصبونية وتطور الاعتلال عصبي، وتدعو الحاجة إلى توضيح دورها الدقيق الذي تلعبه في الاعتلال العصبي السكري.

Objective: To evaluate plasma levels of adrenomedullin (AM) and ghrelin and their correlation with the electrophysiological changes in diabetic neuropathy.

Methods: The current study was conducted from March 2008 to November 2010 at the Clinical Physiology Department, King Abdulaziz University Hospital, King Saud University, Riyadh, Kingdom of Saudi Arabia on 90 females divided into 30 controls (group I), 30 controlled diabetic patients (group II), and 30 with peripheral neuropathy (group III). All electrophysiological and biochemical measurements of AM and ghrelin were investigated.

Results: There was a significant decrease of motor and sensory nerve conduction velocity and prolongation of F wave response of median, ulnar, peroneal, and sural nerves in diabetic patients with neuropathy. Ankle/brachial index (A/BI) showed insignificant change in all groups compared with the control group. There was significant decrease of plasma levels of AM and ghrelin in group III compared with group I. The results revealed that AM and ghrelin were positively correlated with peripheral nerves motor and sensory conduction velocities.

Conclusion: The altered concentration of AM and ghrelin in diabetic neuropathy could indicate a pathophysiological role. The decline of plasma levels of AM and ghrelin in diabetic neuropathy may be a causative factor, they have neuroprotective and vasculoprotective effects, so their lack could induce neuronal injury and advancement of neuropathy, but the precise role of AM and ghrelin in the pathogenesis of diabetic neuropathy is still to be elucidated.

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Diabetic polyneuropathy (DPN) is the most common complication of diabetes mellitus, occurring in more than 50% of patients who have been hyperglycemic for several years.[1] Diabetic neuropathy causes dysfunction of small and large nerve fibers, and negatively impacts quality of life in diabetic patients.[1] Sensorimotor diabetic neuropathy involves both sensory and motor function, where paraesthesias occur along with decreased strength in the lower limb muscles.[2] Small-fiber peripheral neuropathy is characterized by deficits in pain and temperature perception, and a predisposition to foot ulceration. Large-fiber dysfunctions include loss of proprioception, deficits in nerve conduction, and weakness of distal limbs.[3] Currently, the factors recognized in the pathogenesis of DPN are vascular insufficiency, loss of growth factor trophism, and autoimmune destruction of small unmyelinated nerves (C fibers).[4] Hyperglycemia may affect the peripheral sensory nerves through several mechanisms to induce diabetic neuropathy. The increased intracellular sorbitol accumulation, decreased neuronal blood flow, and peripheral nerve hypoxia in hyperglycemia can cause direct neuronal damage.[5] The auto-oxidation of glucose causes increased production of reactive oxygen species, and the formation of advanced glycation end products to induce endothelial damage. Also, the activation of protein kinase C and enzymes that are important for maintaining cellular membrane potential and nerve conduction can induce vasoconstriction and reduce neuronal blood flow.[6] Type 2 diabetes is associated with accelerated atherosclerotic changes characterized by abnormal vascular function, which may ultimately contribute to the clinical manifestations of neuropathy, and micro- and macrovascular disease. These abnormal vasomotor responses may be related to insulin resistance, hyperinsulinemia, hyperglycemia, endothelial dysfunction, dyslipidemia, or changes in sensitivity to norepinephrine.[6]

Adrenomedullin (AM) is a hypotensive factor produced by human adrenal pheochromocytoma cells, smooth muscles, and endothelial cells of the vasculature. It has been reported to cause natriuresis and diuresis, and inhibits angiotensin II-induced aldosterone production.[7] Elevated blood AM concentrations are observed in heart failure and myocardial infarction during sepsis and endotoxin shock. Adrenomedullin exerts its hypotensive effect and an antiproliferative effect through a c-AMP-dependent process; also, it suppresses oxidative stress through a c-AMP signaling pathway.[8] The recent work of Maki et al[9] stated that AM has angiogenic and vasoprotective effects on prevention of cognitive decline after chronic cerebral hypoperfusion in mice. They reported that AM's angiogenic role is mainly via vascular endothelial growth factor induction.[10] Adrenomedullin exerts a wide range of vascular actions (mostly protective). These include endothelium-dependent and -independent vasodilatation, anti-oxidative stress effects, stimulation of endothelial nitric oxide production, anti proliferation of vascular smooth muscle, and adventitial fibroblast cells.[11] There is a lack of knowledge of AM regulation, production, and release at the systemic level in diabetic neuropathy.

Ghrelin peptides exist in 2 major molecular forms, n-octanoylated ghrelin (acyl-ghrelin), and des-n-octanoyl ghrelin (des-acyl-ghrelin). The acyl-ghrelin form is an acylated 28-amino-acid peptide, initially isolated from human and rat stomach as an endogenous ligand for the growth hormone (GH) secretagogue receptor (GHS-R).[12] An accumulating body of evidence has shown that des-acyl-ghrelin exhibits biological activities on adipocytes, myocytes, neuronal precursor cells, osteoblasts, and myelocytes. Many of these activities are associated with cell fate, such as promotion of cell survival and/or apoptosis as well as cell proliferation.[13] It acts on the pituitary to stimulate growth hormone release and on the hypothalamus to enhance food intake.[13,14] Acylation of ghrelin is necessary for the binding of ghrelin to the GHS-R. The wide distribution of GHS-Rs in the nervous system, visceral organs, skin, bone, and blood vessels suggests a potentially more broad array of actions for ghrelin on cardiovascular effects, gastric movement, gastric acid secretion, and on the sympathetic nerve activity.[15,16] Ghrelin plays an important role in glucose homeostasis; it has a physiological role in modulating GH secretion, insulin secretion, and glucose metabolism.[17] Ghrelin has direct effects on pancreatic islet function. Pharmacologic studies indicate the important obesogenic/diabetogenic properties of ghrelin.[17] There is a shortage of evidence regarding ghrelin regulation, production, and release at the systemic level in diabetic neuropathy.

The present study aimed to investigate the plasma levels of AM and ghrelin in diabetic patients with and without neuropathy, and to correlate their levels with the electrophysiological measures of nerve impairment and sensory loss, which are the gold standard for determining motor and sensory nerve function. We studied motor and sensory nerve conduction velocities (NCVs) of the median, ulnar, peroneal, and sural nerves. We also examined sensory impairment by quantitative assessment of thermal sensitivity and other neurological examinations.

Methods. This study was conducted from March 2008 to November 2010 in the Clinical Physiology Laboratory, King Abdulaziz University Hospital, Riyadh, Kingdom of Saudi Arabia. The patients were recruited from the Diabetes Research Center, at King Abdulaziz University Hospital. This study was conducted as a continuation of a previous study, published in 2008.[18] All measurements of biochemical data were evaluated in the Biochemistry Laboratory of the Physiology Department, King Khalid University Hospital, Riyadh, Saudi Arabia. The Ethics Committee of King Khalid University Hospital approved the study, and all procedures were performed according to the guidelines of the Institutional Review Board (IRB). The study protocol followed the ethical guidelines of the most recent Declaration of Helsinki. Written consent was obtained from the participants prior to the start of the study.

Measurement kits for AM (AMEK-010-08) and ghrelin (EK-031-30) were purchased from Phoenix Pharmaceuticals Inc., Burlingame, California, USA.

Study design. The present study was conducted on 90 age matched adult females (mean age 45±3.9 years), of which, 60 were type II diabetics with the same duration of diabetes (10±2.1 years), and divided into 3 groups. Group I (controls, included 30 healthy female controls), group II (included 30 type II diabetics without complications), group III (included 30 type II diabetics with peripheral neuropathy). All patients and controls were subjected to full history taking, thorough clinical examination, and laboratory investigations to establish the diagnosis, and to exclude other associated pathological conditions. All patients fulfilled the following criteria: They were all of type 2 diabetes mellitus, metabolic control was assessed on the basis of glycosylated hemoglobin (HbA1c) level (non-diabetic range 4.0-6.0%), they were on oral hypoglycemics (metformin, or gliclazide), and all patients did not have current or past evidence of any cardiovascular, respiratory, hepatic, or renal disorder. The exclusion criteria included subjects with type I diabetes, high total cholesterol >7 mmol/L, hepatic impairment, and high liver enzymes such as aspartate transaminase, and alanine transaminase. Kidney impairment such as micro albuminuria, high serum creatinine >30 µg/l, urinary albumin >15 mg/l, gout, or hyperuricemia, thyroid dysfunction (thyroid-stimulating hormone >3.8 mU/l, or free thyroxine >20 pmol/l). Also, patients with autonomic neuropathy with abnormal bowel or bladder function, impaired heart rate response to postural change or Valsalva maneuver.

Patients treated with drugs that affect biochemicals measurements such as folic acid, vitamins, vasoactive medications (angiotensin II antagonists, angiotensinconverting enzyme inhibitors, statins, aspirin, nonsteroidal anti-inflammatory drugs), were also stopped at least one day before blood withdrawal.

Sample collection. Controls and diabetic patients fasted for 8 hours before a plasma collection blood sample of 10 ml was collected in ethylene diamine tetraacetic acid tubes and stored at -70°C. Clotted blood was centrifuged for sera collection, labeled, and stored. Fasting and post-prandial serum glucose levels and HbA1C were estimated.[19,20] Whole blood was used for HbA1C, and analyzed by high pressure liquid chromatography (non diabetic range: 4-6%).[18,19] Liver and kidney function tests were estimated.[21]

Specific investigations. The AM (AMEK-010-08) and ghrelin (EK-031-30) plasma levels were measured by indirect enzyme immunoassay as described previously.[20,21] Quantitative sensory tests for the sensations of light touch, pain (pin prick), temperature, and joint position in the index finger and big toe bilaterally were carried out. A questionnaire on symptoms of peripheral neuropathy was completed by all patients, and by all control subjects. The tendon reflexes in the quadriceps, gastrocnemius, and biceps muscles were classified, either as present or absent. The nerve conduction tests were performed as described by previous reports[22] in the Department of Clinical Physiology with standard surface recording techniques using an electromyography type Spirit Nicolet Viking (Nicolet-Biomedical Inc, Madison, WI, USA). Unilateral motor nerve conduction studies (NCS) were performed on the median, ulnar, and peroneal nerves. Sensory NCS were performed antidromically on median, ulnar, and sural nerves. For motor nerves, the electromyographic settings of the machine were: frequency (8 Hertz [Hz]-8 kiloHertz [kHz]), sweep speed (5 msec/division), gain (1000uV), stimulation intensity (400 V), and duration (0.1 msec). While the settings for sensory nerves were: frequency (8 Hz-1.6 kHz), sweep speed (5 msec/division), gain (10uV), stimulation intensity (208 V), and duration (0.05 msec). Measurements of distance, response latencies, and amplitude were carried out in a standard fashion using onset latencies, and base line to peak amplitude. Measurements of peak-peak amplitudes were used for sensory responses.[21] Patients with clinical neuropathy were diagnosed on the basis of: abnormal electrodiagnostic tests with decreased nerve conduction velocity (NCV), or decreased amplitudes, or prolonged sensory or motor distal latencies, or prolonged F-wave motor latency of median, ulnar or peroneal nerves, and

abnormal quantitative sensory tests for vibration, tactile, thermal warming, and cooling thresholds. Clinical neuropathy was considered in the presence of peripheral sensorimotor neuropathy plus either abnormal nerve conduction in at least 2 peripheral nerves.

Motor nerves conduction. The compound muscle action potentials (CMAP) of the right median and ulnar nerves were obtained using supramaximal stimulation of the nerves at the wrist and elbow, with the distal distance for motor NCV 80 mm above the recording electrode.[22] For common peroneal conduction velocity, the nerve was stimulated at the anterior surface of the ankle, and at the popliteal fossa.

Sensory nerve conduction. The sensory nerve action potential (SNAP) of the right median nerve was measured by the antidromic technique on the index finger with ring electrodes placed 2.5 cm apart. For the ulnar nerves, this was measured by the antidromic technique on the small or ring finger with ring electrodes placed 2.5 cm apart. The fixed distal distance for sensory NCV was 20 mm proximal to the distal wrist crease. The sural SNAPs were recorded behind the lateral malleolus on the posterior lateral aspect of the leg, 14 cm from the active electrode with a fixed distal distance of around 140 mm above the recording electrode.[23] Conduction velocities were calculated for motor and sensory nerves. Supramaximal stimulation, with 10 supramaximal stimuli per nerve, and the minimal reproducible latency of at least 3 responses were measured for F-waves of the median, ulnar, and peroneal motor nerves.[23]

Lower limb blood flow. Doppler measurements of the ankle/brachial pressure index (A/BI), and plethysmographic blood flow were used help to diagnose patients with peripheral occlusive artery disease (POAD). Microvascular blood flow was accurately measured noninvasively using Doppler flowmetry (MD6 System, Hokanson, Washington, USA).[24] Both foot pulses were palpated, and classified as present or absent. Absence of one or both foot pulses was used as an indicator of arterial disease. The A/BI values <0.9 were used as indicators of significant PAOD.[25] Quantitative Doppler waveform analysis was performed using an 8-megaHz Doppler probe on the brachial and dorsalis pedis. All patients were rested supine for 15 minutes, and using the Vasoguard Doppler probe (MD6 System, Hokanson, Washington, USA) systolic pressures were measured in the right brachial artery, right dorsalis pedis, and posterior tibial arteries and repeated on the left side. Pressures were measured twice and the A/BI was calculated. The lowest leg A/BI was used in the analysis.[25]

Statistical analysis. The data were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) computer program. All data were reported as mean±SD. Two-way ANOVA was performed to compare between the study groups with differences considered significant when $p<0.05$. Post ANOVA test analysis including Tukey-Kramer multiple comparisons test was used for comparison between the different study groups. A difference of $p<0.05$ was considered significant, and confidence interval of 95%. Spearman rho correlation coefficient was applied to find the strength of correlation between continuous quantitative variables. In addition, linear regression analysis was used to examine the relationship between median nerve motor, sural nerve sensory conduction velocity in neuropathy, AM, and ghrelin (independent variables). A 5% level was chosen as a level of significance in all statistical significance tests used.

Results. All demographic features and biochemical measurements are shown in **Table 1**. The diabetic patients fasting blood glucose (FBG), HbA1c, and BMI showed a significant increase in groups II and III when compared with group I (controls). Mean arterial blood pressure, lipid profile including total cholesterol and triglycerides, and kidney function including blood urea and creatinine showed an insignificant change in group II and III compared with controls.

All patients in group III had peripheral neurological symptoms (tingling or numbness). Some of the patients had loss of sense of light touch, pain, or joint position, and 24 patients had abnormal vibration sense, whereas 6 patients had normal vibration sense. Five control females had occasional numbness of the soles of the feet with no signs of PDN, 15 patients (with PDN) had absent joint reflexes in the lower extremities. There was a significant decrease of median nerve motor conduction velocity, and a significant prolongation of median nerve F-wave response in group III compared with groups I and II. There was significant decrease of ulnar nerve motor conduction velocity, and a significant prolongation of ulnar nerve F-wave response in group III compared with groups I and II. The common peroneal nerve motor conduction velocity was significantly decreased, and there was a significant prolongation of peroneal nerve F-wave response in group III compared with group I and group II. Also, there was a significant decrease in the median and ulnar nerve sensory conduction velocity in group III compared with group I and group II. Sural nerve sensory conduction velocity showed a significant delay, and sural nerve sensory amplitude showed a significant decrease in group III compared with groups I and II (**Table 2**).

Table 1 - Demographic and biochemical features of type II diabetic patients and matched controls.

Features	Group I: controls (n = 30)	Group II: diabetics without complications (n = 30)	Group III: diabetics with neuropathy (n = 30)	*P*-values
Mean age (years) 50±1	51±0.34	51±0.1	50±2.1	0.06
Fasting blood glucose level (mmol/dl)	5.1±0.03	8.6±1.55	9.1±2.8	0.001* 0.02†
HbA1c (%)	5.4±0.1	6.9±1.7	8.8±0.2	0.0001* 0.032†
BMI (kg/m^2)	25±4*	29±1.3	30±0.5	0.001* 0.23†
Mean arterial blood pressure (mm Hg)	85±1.1	89±0.43	90±0.22	0.065
Lipid profile				
Triglycerides (mmol/l)	1.7±0.4	1.9±0.06	2.01±0.09	0.084
Cholesterol (mmol/l)	4.7±0.5	5.3±1.1	5.5±1.2	0.062
Kidney function				
Urea (mmol/l)	4.5±0.6	5.1±0.16	5.9±0.8	0.072
Creatinine (µmol/l)	87.9±4.6	95.1±8.16	99.79±3.8	0.084

Results are expressed as mean ± standard deviation, *significant changes in group II compared with control group I, †significant changes in group III compared with control group I, HbA1c - glycosylated hemoglobin, BMI - body mass index

Table 2 - Nerve conduction studies in the controls, diabetics without complications, and diabetics with neuropathy.

Studies	Group I: controls (n = 30)	Group II: diabetics without complications (n = 30)	Group III: diabetics with neuropathy (n = 30)	*P*-values
Median nerve				
Motor conduction velocity (m/sec)	65.1±3.97	66.64±0.937	41.4±1.183	0.01*, 0.002†
F-wave (msec)	25.8±0.99	25.433±0.77	32.067±0.798	0.002*, 0.041†
Ulnar nerve				
Motor conduction velocity (m/sec)	54.1±1.296	54.233±1.755	40.533±1.18	0.01*, 0.012†
F-wave	26.5±0.97	26.3±4.58	31.867±1.06	0.001*, 0.03†
Common peroneal nerve				
Motor conduction velocity (m/sec)	55.1±3.97	54.64±2.37	40.39±6.42	0.01*, 0.002†
F-wave	28.3±0.82	28.98±0.75	31.57±1.2	0.001*, 0.041†
Sensory response				
Median nerve sensory conduction velocity (m/sec)	51.1±0.803	50.933±0.784	40.533±1.187	0.001*, 0.02†
Ulnar nerve sensory conduction velocity (m/sec)	52.86±2.145	53.33±2.249	40.533±1.118	0.002*, 0.001†
Sural nerve sensory conduction velocity (m/sec)	47.76±1.135	48.267±0.739	39.0±4.67	0.01*, 0.002†
Sural nerve sensory amplitude (uv)	34.967±0.88	35.2±0.96	27.33±0.72	0.01*, 0.002†

Results are expressed as mean ± standard deviation, *significant changes in group III compared with control group I, †significant changes in group III compared with control group II

The A/BI showed an insignificant change in group III compared with groups I and group II. There was a significant decrease of plasma AM in group III compared to group I and group II, however, there was an insignificant change between controls (group I) and controlled diabetic patients without neuropathy group II. Regarding plasma ghrelin levels, there was a significant decrease of its level in group III compared with group I and group II. Also, there was significant decrease of ghrelin level in group II compared with controls (group I) (**Table 3**).

There was a positive correlation between both median and peroneal nerve motor conduction velocity and plasma levels of AM. However, there was a positive weak correlation between mean sural nerve sensory conduction velocity and plasma AM. There was also

Table 3 - Plasma levels of adrenomedullin (AM) and ghrelin in type II diabetic patients and matched controls.

Variable	Group I: controls (n = 30)	Group II: diabetics without complications (n = 30)	Group III: diabetics with neuropathy (n = 30)	*P*-values
Plasma AM (pmol/ml)	2.66±0.62	2.656±0.587	1.03±0.653	0.032* 0.001†
Plasma ghrelin (pmol/ml)	178.66±45.29	120.66±47.46	83.3±45.207	0.001* 0.021† 0.043‡

Results are expressed as mean ± standard deviation, *significant changes in group III compared with control group I, †significant changes in group III compared with control group II, ‡group II compared with group I

Table 4 - Correlation between nerve conduction velocities, plasma levels of adrenomedullin (AM) and ghrelin in diabetic patients with neuropathy.

Variable	Correlation co-efficient	*P*-values
Mean median nerve motor conduction velocity and plasma AM	0.546	<0.002
Peroneal nerve motor conduction velocity and plasma AM	0.653	<0.0005
Mean median nerve motor conduction velocity and plasma ghrelin	0.633	<0.05
Peroneal nerve motor conduction velocity and plasma ghrelin	0.561	<0.01
Mean sural nerve sensory conduction velocity and plasma AM	0.391	<0.001
Mean sural nerve sensory conduction velocity and plasma ghrelin	0.691	<0.001

a positive correlation between median and peroneal nerve motor conduction velocity, and mean sural nerve sensory conduction velocity and plasma ghrelin level (**Table 4**).

Linear regression analysis was carried out for AM (as an independent variable), and median motor and sural nerves sensory conduction velocity (as dependent variables). The analysis revealed AM as a predictor of median motor and sural sensory conduction velocity in the neuropathy group (ß=-0.56, p=0.022) (ß=0.313, p=0.026). Linear regression analysis was carried out for ghrelin (as an independent variable), and median motor and sural nerves sensory conduction velocity (as dependent variables). The analysis revealed also that ghrelin is a predictor of median motor (p=0.002) and sural sensory (p=0.006) conduction velocity in the neuropathy group.

Discussion. Among diabetic patients, peripheral neuropathy is common and ultimately accounts for significant morbidity. Foot ulceration initiated by trauma is one of the poor consequences of such sensory defects involving the lower extremities.[1] Hyperglycemia may affect the peripheral sensory nerves through several mechanisms that can cause direct neuronal damage, or decrease neuronal blood flow and peripheral nerve hypoxia.[5] It causes activation of protein kinase C, and enzymes can also induce vasoconstriction and reduce neuronal blood flow.

Recent studies[18] examined the role of growth factors and certain biological markers in diabetic neuropathy, but thus far limited studies have evaluated AM plasma levels in diabetic neuropathy. The findings of the present study revealed a decrease of plasma AM levels, and a positive correlation between plasma AM levels and motor nerves conduction velocity in patients with diabetic neuropathy. The results show that AM is a predictor of motor and sensory nerve function.

The study results also indicate that low AM could impair peripheral nerves motor and sensory function and conduction velocity, and prove that there is a cross linkage between lack of AM and the incidence of DPN. The decline in plasma AM may have a causative effect in diabetic neuropathy, which may be dependent on the development of microangiopathy.[26] The lack of AM in the DPN group in this study denotes a loss of its protective effects, as AM suppresses oxidative stress through the c-AMP signaling pathway.[8] Also, as AM has angiogenic and vasoprotective effects, its angiogenic role is mainly through vascular endothelial growth factor induction.[10]

A previous study[27] stated a significant increase of plasma AM levels and a negative correlation between b-wave absolute latency of the electroretinogram, and plasma AM in diabetic retinopathy. They postulated that AM could be compensatorily neuroprotective and vasculoprotective in diabetic complications.

Adrenomedullin may play a protective role in diabetic neuropathy as it has a vasodilator effect on the neural vessels, and it has apoptosis survival properties on the endothelial cells.[10] It also has an antioxidant effect as shown in the study of Katsuki et al,[28] who evaluated the relationship between the plasma levels of 8-epi-prostaglandinF2α (8-epi-PGF2α), the most reliable marker for the assessment of oxidative stress, and AM in patients with type 2 diabetes. They showed that oxidative stress stimulates secretion of AM from the endothelium and vascular smooth muscle cells, and that AM mRNA expression is increased by activation of the nuclear factor-κB pathway. They postulated that there was a significant positive correlation between increased oxidative stress and elevated plasma levels of AM in patients with type 2 diabetes, and stated that enhanced oxidative stress may regulate the plasma levels of AM in hypertensive patients. Shimosawa et al[29] reported that endogenous AM may protect from organ damage by inhibiting oxidative stress production. So, depending on these reports, we can conclude that low AM plasma levels reported in the present study may lead to exacerbated oxidative stress-induced vasoconstriction, and thus, may play a causative role in neuronal injury and DPN. This could be explained on the basis that this peptide plays an important role in physiological and pathological conditions compensating the effects of vasoconstrictive molecules and oxidative stress.[29] Low AM levels in DPN patients stated in this study could be due to low insulin in diabetics with DPN, as previous studies reported that acute hyperinsulinemia may stimulate AM production from pancreatic islets, and the increased blood levels of AM may compensate for the decreased vasodilatory effect of insulin in patients with type 2 diabetes mellitus. The trend of lower levels of AM in patients with neuropathy in this study raises the intriguing possibility that low AM may be somehow involved in the induction of diabetic neuropathy.

The findings of the present study of a significant decrease of plasma ghrelin levels in diabetic patients with and without neuropathy compared with controls could be explained as a causative relation, as most publications addressing the relationship between ghrelin and insulin resistance and/or diabetic states suggest that a correlation between ghrelin and insulin resistance and/or diabetes mellitus exists.[17] High leptin, and low ghrelin signals may synergize with each other to increase insulin resistance and the clustering of metabolic abnormalities and eventually type 2 diabetes mellitus.[17] Recently, Tong et al[30] suggested that circulating ghrelin suppresses glucose-stimulated insulin secretion, and deteriorates glucose tolerance in healthy subjects. Their findings raised the possibility that endogenous ghrelin has a role in physiologic insulin secretion, and that ghrelin antagonists could improve beta-cell function. Also, compensatory hyperinsulinemia due to insulin resistance was associated with significantly reduced ghrelin concentrations.[31] Our findings are in accordance with the work of Sangiao-Alvarellos et al,[32] who showed that fasting plasma concentrations of total ghrelin were lower among subjects with type 2 diabetes, and an inverse correlation between ghrelin and insulin concentrations, as well as insulin sensitivity was observed. Ghrelin may have a role in hepatic glucose metabolism.[33] It could also be speculated that low blood ghrelin levels might affect the growth hormone/insulin like growth factor-1-axis, which in turn might increase insulin resistance and lead eventually to the development of type 2 diabetes mellitus.[34] However, the theoretical link between low ghrelin and pathogenesis of type 2 diabetes remains highly speculative.

The finding of the present study of low ghrelin levels, and its positive correlation with motor and sensory nerves conduction velocity in DPN group, leads us to postulate an etiological role of low ghrelin in DPN. This is in agreement with the study of Kyoraku et al,[35] who showed for the first time that ghrelin alleviated experimental diabetic sensorimotor neuropathy in rats. From a therapeutic point of view, they postulated that delayed treatment with ghrelin completely restored motor and sensory nerve conduction velocity to control values in mice with established disease. So, there is a growing body of evidence suggesting that there is a cross linkage between low ghrelin levels and the incidence of DPN due to loss of the anti-inflammatory and anti-oxidative effect of ghrelin. Diabetes compromises antioxidant defense mechanisms. Ghrelin administration to human umbilical vein endothelial cells and human polymorphonuclear cells suppressed reactive oxidative species generation. Ghrelin administration ameliorated the diabetes-induced elevation of oxidative stress marker 8-iso-PGF2α levels, which may also be involved in the therapeutic effect of ghrelin.[32] Collectively, we can conclude that altered ghrelin levels in type II diabetes with DPN and loss of its anti-inflammatory, anti-oxidative effect, and promotion of nerve cell survival and proliferating effect[12] may indicate an intensive causative relation with DPN.

Study limitations. Some patients attended for blood collection without fasting, and some patients refused blood collection by venipuncture because of fear and distress of needles, and hence were excluded from the study. There were also a few patients with other diabetic complications together with neuropathy, and so they were also excluded from the study.

In conclusion, the altered concentration of AM in diabetic neuropathy could indicate a certain interaction between AM and neuronal function. The decline of plasma levels of AM in diabetic neuropathy may be a causative factor, as AM has neuroprotective and vasculoprotective effects; its lack could stimulate neuronal injury. Also, altered plasma ghrelin levels may be a causative factor in advancement of neuropathy, but it is still uncertain whether the decreased release of AM and ghrelin is a causative mechanism or a coincidental event in diabetic neuropathy. Although the precise role of AM and ghrelin in the pathogenesis of diabetic neuropathy is still to be elucidated, the results of the present study add to the fact that diabetic neuropathy is the result of multiple factors, thus, it may be optimistic to believe that reversal of either AM or ghrelin may ameliorate diabetic neuropathy. However, further studies are warranted to highlight a prospective new approach in the alleviation of diabetic neuropathy.

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