Electrophysiological changes, plasma vascular endothelial growth factor, fatty acid synthase, and adhesion molecules in diabetic neuropathy

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ABSTRACT

The objectives: To evaluate the electrophysiological changes, blood flow index, vascular endothelial growth factor (VEGF), soluble fatty acid synthase (s-FAS), and intercellular adhesion molecule (I-CAM) in diabetic neuropathy.

Methods: This study was conducted from March 2004 to November 2007 on 60 type II diabetic patients and 30 controls, recruited from the Diabetic Research Center of King Abdul-Aziz University Hospital, Riyadh, Kingdom of Saudi Arabia. Electrophysiological studies were carried out in the Clinical Physiology Laboratory. Patients and controls were of the same age, gender, and weight.

Results: The study included 30 controls (group I), 30 diabetics type II without complications (group II), and 30 with peripheral neuropathy (group III). There was a significant decrease of motor conduction velocity, prolongation of F wave response of median, ulnar, peroneal nerves, significant decrease of median and ulnar sensory conduction velocity, sural nerve conduction velocity and sensory amplitude, showed significant decrease, ankle/brachial index (A/BI) showed insignificant change, also there was a significant increase of plasma VEGF, s-FAS, and ICAM all in group III compared to groups I and II. The results revealed that VEGF and s-FAS are good predictors for median nerve motor conduction velocity, also VEGF is a good predictor of sural nerve sensory conduction velocity in diabetic neuropathy.

Conclusion: The rise of VEGF in diabetic neuropathy may be protective to preserve the nerve blood flow, the significant rise of s-FAS may be causative in advancement of neuropathy, I-CAM high levels suggest its leading role in interaction between endothelium, blood elements, and peripheral nerves. The results showed that human neuropathy is the result of multiple factors, thus, it may be optimistic to believe that reversing one of them, such as s-FAS will halt, or reverse nerve damage. Targeting multiple mechanisms simultaneously, by administering combination treatments of VEGF, and anti-apoptotic drugs may be prospective.


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Received 23rd April 2008. Accepted 22nd July 2008.

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Diabetic polyneuropathy (DPN) is responsible for 50-75% of non-traumatic amputations. The main symptoms are numbness, tingling, burning, and shooting pain in the legs, whereas the physical manifestations include reduced pain, touch and vibratory perception in a symmetrical stocking glove distribution. Sensorimotor diabetic neuropathy involves both sensory and motor function, where paresthesias occur along with decreased strength in the lower limb muscles. Currently, the factors recognized in the pathogenesis of DPN are vascular insufficiency, loss of growth factor tropism, and autoimmune destruction of small unmyelinated nerves (C fibers). Hyperglycemia, which has emerged as a major risk factor for the development of diabetic neuropathy, may affect the peripheral sensory nerves through several mechanisms. First, the increased intracellular sorbitol accumulation can cause direct neuronal damage, or decrease neuronal blood flow and peripheral nerve hypoxia. Second, the activation of protein kinase C that can affect the sodium, potassium, adenosine triphosphatase (ATPase), and enzymes that are important for maintaining cellular membrane potential and nerve conduction, it can also induce vasoconstriction and reduce neuronal blood flow. Third, the auto-oxidation of glucose causes increased production of reactive oxygen species, and the formation of advanced glycation end products to induce endothelial damage. 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. Vascular endothelial growth factor (VEGF) is a polypeptide that is mitogenic for endothelial cells, and induces angiogenesis and vasculogenesis. It is a potent stimulator of microvascular permeability, and a chemotactic factor for endothelial cells and monocytes. Moreover, it enhances endothelial functions that mediate the inhibition of vascular smooth muscle cell proliferation, enhanced endothelial cell survival, suppression of thrombosis and anti-inflammatory effects. Very little information is available regarding the role of VEGF in the development of diabetic neuropathy. Defective regulation of apoptosis or programmed cell death may play a role in the etiology of many diseases, such as, cancer, viral infection, autoimmune disease, degenerative disease of the central nervous system, and diabetic neuropathy. Fatty acid synthase (Fas) system, believed to be the first initiator of apoptosis, is composed of Fas Ligand (FasL), a type II transmembrane glycoprotein receptor, and Fas antigen (Fas/Apo-1/CD95), a type I transmembrane glycoprotein receptor. Cross-linking of Fas by FasL triggers apoptosis in various target cells. In type 2 diabetes, characterized by the association of relative insulin deficiency and insulin resistance, apoptosis of pancreatic B cells is now suggested. Recent studies concluded that the serum from type 2 diabetic patients with neuropathy contained immunoglobulins, able to induce apoptosis in neuronal cells, also long term exposure of nerves to sera from diabetics patients in vivo could contribute to development of neuropathy. Disturbed nerve regeneration in diabetes has been ascribed at least in part, to all or some of the decreased levels of neurotrophic factors, altered cellular signal pathways, or abnormal expression of cell adhesion molecules. There is a lack of evidence concerning the potential role of vascular adhesion molecules in diabetic peripheral neuritis. The aims of this study was to evaluate the electrophysiological changes, blood flow index, plasma levels of VEGF, soluble fatty acid synthase (s-FAS), and intercellular adhesion molecule (ICAM) in diabetic peripheral neuropathy.

Methods. This study was conducted from March 2004 to November 2007 in the Clinical Physiology Laboratory and Diabetes Research Center, King Abdul-Aziz University Hospital, Riyadh, Kingdom of Saudi Arabia. All biochemical parameters were measured in the Biochemistry Laboratory, Physiology Department, King Khalid University Hospital, Riyadh, Saudi Arabia. The Ethics Committee of the hospital approved the study, and all the procedures were performed in accordance with the institutional guidelines, and Helsinki declaration. Before the study, informed consent was obtained from the participants. Measurement kits for VEGF, s-FAS, and ICAM-1 kits were purchased from R & D Co. (Minneapolis, USA). The present study was conducted in 90 adult females, of the same age group (mean age 45±3.9 years), in which, 60 were type II diabetics of the same duration of diabetes (10±2.1 years), and were divided into 3 groups, according to the following experimental design: group I (includes 30 healthy female controls), group II (includes 30 type II diabetics without complication), group III (includes 30 type II diabetics with peripheral neuropathy). All patients were recruited from the Diabetic Research Center of King Abdul-Aziz University Hospital. All patients and controls were subjected to full history.
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taking, thorough clinical examination, and laboratory investigations to establish the diagnosis, and to exclude other associated pathological conditions. Patients must fulfill the following criteria: they were all type 2 diabetes mellitus, metabolic control was assessed on the basis of glycosylated hemoglobin (HbA1c) level, they were on oral hypoglycemics (metformin, or gliclazide), all patients did not have current or past evidence of any cardiovascular, respiratory, hepatic or renal disorder. Patients treated with vasoactive medication (angiotensin-converting enzyme inhibitors, statins, aspirin, nonsteroidal anti-inflammatory drugs, angiotensin II antagonists), also folic acid, or vitamins treatment was stopped at least 3 days before blood withdrawal. Patients were also excluded based on the following criteria: subjects with type I diabetes, also with micro albuminuria, and high serum creatinine, aspartate transaminase, and alanine transaminase, creatinine levels >30 µg/l, urinary albumin >15 mg/l, hepatic impairment, gout, or hyperuricemia, high cholesterol (total cholesterol >7 mmol/l), thyroid dysfunction (thyroid-stimulating hormone >3.8 µU/l, or free thyroxine >20 pmol/l). Also, patients with autonomic neuropathy were excluded, the latter was assessed as the presence of any history of abnormal bowel or bladder function, impaired heart rate response to postural change or Valsalva maneuver, orthostatic intolerance, gastroparesis.

Sample collection. All controls and diabetic patients had fasted for 8 hours, and 10 ml of blood was collected in ethylene diamine tetraacetic acid tubes for plasma collection, and stored at -70°C. Sera was also separated by centrifugation of clotted blood, labeled, and stored. The following parameters were measured in all subjects: estimation of fasting and post-prandial serum glucose level, estimation of HbA1C. Whole blood was used for the assessment, and was analyzed by high pressure liquid chromatography (nondiabetic range 4-6%), liver function tests, kidney function tests, complete urine analysis, and detection of microalbuminuria. Specific investigations include: VEGF, s-Fas, and ICAM-1, all were measured by indirect enzyme immunoassay as described previously. Tests for the sensations of vibration (260 Hertz fork), light touch, pain (pin prick), temperature, and joint position in the index finger and big toe bilaterally, were carried out. The 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 Kimura in the Department of Clinical Neurophysiology. Nerve conduction studies were performed with standard surface recording techniques using an electromyography type Spirit Nicolet Viking (Nicolet-Biomedical Inc, Madison-WI, USA) with standard filter settings. Electromyographic settings of the machine for motor nerves were: frequency (8 Hertz [Hz]-8kiloHertz [kHz]), sweep speed (5 m/sec/division), gain (1000uV), stimulation intensity (400 V), and duration (0.1 msec). Electromyographic settings for sensory nerves were: frequency (8 Hz-1.6 kHz, sweep speed (5msec/division), gain (10uV), stimulation intensity (208 V), duration (0.05 msec). Unilateral motor NCS’s were performed on the median, ulnar, and peroneal. Also, median, ulnar, and sural sensory NCS’s were performed antidromically. All stimulation and recording were performed using surface electrodes. Measurements of distance, response latencies, and amplitude were carried out in a standard fashion using onset latencies, and base line to peak amplitude. Measurements from the initial positive peak to negative peak were used for sensory responses (peak-peak amplitudes). Testing was standardized for temperature, side of testing, stimulation protocol, averaging sensory potentials, marking latencies and amplitudes, and providing information for the core laboratory. Patients with clinical neuropathy were diagnosed on the basis of: 1) 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, 2) abnormal quantitative sensory tests for vibration, tactile, thermal warming, and cooling thresholds. Clinical neuropathy is consistent with 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, the nerve being stimulated supramaximally at the wrist. The fixed distal distance for motor NCV was 80 mm above the recording electrode, the nerves were stimulated also at the elbow (the distal latency of the CMAP was measured from the onset of the stimulus to the initial CMAP negative deflection). For common peroneal conduction velocity, the nerve was stimulated at anterior surface of the ankle, and at poplitealfossa.

Sensory nerve conduction. The sensory nerve action potential (SNAP) of the right median was measured by the antidromic technique on the index finger, with ring electrodes placed 2.5 cm apart, and of ulnar nerves 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, 14
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Lower limb blood flow. Microvascular blood flow was accurately measured noninvasively using Doppler flowmetry (MD6-system-Hokanson-USA). Doppler measurements of the ankle/brachial pressure index (A/BI), plethysmographic blood flow helped to diagnose patients with peripheral occlusive artery disease (POAD). 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. Ankle/brachial pressure index values <0.9 were used as indicators of significant PAOD. In relation to A/BI, subjects were divided into 2 groups: a) group I, having severe lower limb atherosclerotic ischemia (A/BI<0.5), and b) group II, having mild to moderate lower limb ischemia (A/BI 0.5-0.9). Quantitative Doppler waveform analysis was performed on the brachial and dorsalis pedis using the Dopplex Assist, with an 8-megaHz Doppler probe. Qualitative waveform analysis was performed by visual interpretation of continuously displayed waveforms. The on-screen loss of reverse flow (loss of diastolic signal) was used as an indicator of significant arterial disease. All patients were rested supine for 15 minutes, using the Vasoguard Doppler probe, systolic pressures were measured in the right brachial artery, right dorsalis pedis and posterior tibial arteries, then in the left side. Pressures were measured twice and A/BI was calculated. The lowest leg A/BI was used in the analysis.

Statistical analysis. All data are 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. Spearman rho correlation coefficient was applied to find 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, VEGF, s-FAS, and ICAM (independent variable). A 5% level is chosen as a level of significance in all statistical significance tests used.

Results. On the screening day, diabetic patients fasting blood glucose (FBG) was 9.1±2.8 mmol/l, HbA1c 7.9±1.7%, and all other screening measures were normal, including lipid profile (total cholesterol 4.7±0.5 mmol/l) and duration of diabetes 10±2.1 years. Controls age was 45±4 years. On the screening day, FBG was 5.4±0.1 mmol/l, HbA1c 5.4±0.1%, and total cholesterol 4.2±0.3 mmol/l. 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, 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 in group III (65.1±3.97) compared to groups I (41.4±1.183) and II (66.64±0.937). Concerning the median nerve F wave response, there was a significant prolongation of median nerve F wave response in group III (32.067±0.798) compared to groups I (25.8±0.99) and II (25.433±0.77). Also, there was significant decrease of ulnar nerve motor conduction velocity in group III (40.533±1.18) compared to groups I (54.1±1.296) and II (54.233±1.755). Concerning ulnar nerve F wave response, there was significant prolongation of ulnar nerve F wave response in group III (31.867±1.06) compared to groups I (26.5±0.97) and II (26.3±4.58). Also common peroneal nerve motor conduction velocity was significantly decreased in group III (40.39±6.42) compared to groups I (55.1±3.97) and II (54.64±2.37). There was significant prolongation of peroneal nerve F wave response in group III (31.57±1.2), for group I it was 28.3±0.82 and 28.98±0.75 for group III. Concerning median nerve sensory conduction velocity, there was a significant decrease in group III (40.533±1.187) versus 51.1±0.803 in group I and 50.93±0.784 in group II. Also, there was a significant decrease in the ulnar nerve sensory conduction velocity in group III, it was 40.533±1.118 versus 52.86±2.145 for group I and 53.33±2.249 for group II. Sural nerve sensory conduction velocity showed significant delay in group III (39.0±4.67), compared to groups I (47.76±1.135) and II (48.267±0.739). Concerning sural nerve sensory amplitude it showed significant decrease in group III (27.33±0.72), it was 34.967±0.88 for group I, and 35.2±0.96 for group II. Ankle brachial pressure index showed insignificant change in group III (0.986±0.074) compared to groups I (1.003±0.08) and group II (1.06±0.096). There was a significant increase of plasma VEGF, s-FAS, and ICAM in group III compared to group I (p=0.032), and group II (p=0.001), however, there was insignificant change between controls and diabetics without neuropathy (p=0.063). There was a negative correlation between median nerve motor conduction velocity and s-Fas (r= -0.765, p=0.001), and ICAM.
peripheral neuropathy. In contrast, following VEGF found to be markedly attenuated resulting in severe blood flow, and the number of vasa nervorum were models. In 2 different animal models of diabetics, nerve of an angiogenic growth factor was studied in animal vasa nervorum and can be reversed by administration pernicious disorder. VEGF may constitute a novel treatment strategy for this diabetic neuropathy, so the angiogenic growth factor microvascular complications. The finding that VEGF is a good predictor of motor nerve conduction velocity in neuropathy could be explained by the concept that VEGF can stimulate angiogenesis, enhance collateral vessel formation, and increase the permeability of the microvasculature. Vascular endothelial growth factor-dependent cell survival and VEGF-induced synthesis of nitric oxide and prostaglandin are likely to be key mediators of its vascular protective effect. The significant increase of VEGF in diabetics with neuropathy observed in this study, also the positive correlation between plasma VEGF and motor nerve conduction velocity, and the finding that VEGF is a good predictor of motor nerve conduction velocity in neuropathy could be explained by the VEGF compensatory rise to manage microvascular complications. These findings that implicate microvascular disruption is the basis for diabetic neuropathy, so the angiogenic growth factor VEGF may constitute a novel treatment strategy for this pernicious disorder. The hypothesis that experimental diabetic neuropathy results from destruction of the vasa nervorum and can be reversed by administration of an angiogenic growth factor was studied in animal models. In 2 different animal models of diabetics, nerve blood flow, and the number of vasa nervorum were found to be markedly attenuated resulting in severe peripheral neuropathy. In contrast, following VEGF gene transfer, vascularity and blood flow in nerves of treated animals were similar to those of non-diabetic controls, constitutive overexpression of VEGF resulted in restoration of large and small fiber peripheral nerve function. Very little information is available regarding the role of VEGF in the development of diabetic neuropathy. In streptozotocin-induced diabetic rats, VEGF expression has been reported to be increased in the sciatic nerve and dorsal root ganglia. Treatment with insulin or nerve growth factor can prevent the increases in VEGF expression. Furthermore, VEGF gene therapy was shown to prevent or reverse the establishment of axonal loss and myelin degeneration, that was observed in the untreated animals with similar degrees of hind limb ischemia. Blood flow at the nerve level, was also found to be preserved at normal levels in the VEGF-treated animals where it was considerably reduced in the untreated ischemic animals. Finally, it was also reported that VEGF stimulated the migration, and prevented the hypoxia-induced apoptosis of Schwann cells in vitro, which exhibited VEGF receptors. Therefore, we suggest that VEGF, in addition to restoring blood flow by inducing angiogenesis, may directly promote the survival of peripheral nerve cells. Such characteristics would make VEGF an ideal agent for preventing or restoring nerve dysfunction in diabetes. The finding of the present study that the s-Fas levels were higher in the diabetics with neuropathy, and the negative correlation between plasma s-Fas and motor nerve conduction velocity confirm a causative role of s-Fas, the shear stress induced by diabetes stimulates apoptosis, which may result in a dynamic process of tissue remodelling. This could have been of importance, as s-Fas has recently been suggested to be involved in the advancement of diabetic neuropathy. Increased serum concentrations of s-Fas are considered to reflect activation of the Fas/FasL system in vivo which is an important inducer of apoptosis. The high s-Fas in neuropathy may be a causative by necrosis or apoptosis induced via activation of tumor necrosis factor (TNF) and the Fas/FasL system. The rise of s-Fas could be by over expression of Fas receptor by peripheral nerve cells. Free oxygen radicals, caspase 8, and growth factors, could all regulate Fas expression in nervous system, accumulation of free oxygen radicals in neurons could act as a molecular switch turning Fas function to death, oxidation stress induces expression of Fas and Fas ligand in neural cells in vitro. The finding that there was significant increase of plasma ICAM in diabetic neuropathy. Also the negative correlation between plasma ICAM and motor nerve conduction velocity in neuropathy could be elucidated by the fact that ICAM has a role in atherosclerotic peripheral artery disease in diabetes, elevated levels of I-CAM and endothelial adhesion molecule 1 (E-selectin) in type 2 diabetes occur early in the course of asymptomatic
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peripheral artery occlusive disease, and this is related to glycemic control,\textsuperscript{15} this suggest that I-CAM has a role in atherosclerotic peripheral artery disease and in neuropathy in diabetes, there is an increasing body of evidence to implicate a leading role of adhesion molecules in the interaction between endothelium, blood elements, low density lipoproteins, and peripheral nerves,\textsuperscript{16} all these effects enhance neuropathy as they affect blood flow to peripheral nerves.

In conclusion, human neuropathy is the result of multiple factors, so it may be too optimistic to believe that reversing one of them will halt or reverse nerve damage. The notion of targeting multiple mechanisms simultaneously by administering combination treatments is therefore winning converts among clinical investigators. The findings of the present study revealed that VEGF is a good predictor for motor nerve conduction velocity in neuropathy. The possible use of VEGF, which can have a direct impact on both the nerve blood flow and the nerve cells, offers distinct advantages over other therapeutic approaches that target either of these tissues separately. So it could be a good candidate for clinical use in the future. Cautious attention should be paid, however, to the possible adverse effects of VEGF, particularly the development of proliferative retinopathy, and the possible mitogenic effects of VEGF in tumor development should also be kept in mind. The rise of s-FAS in neuropathy could indicate that apoptosis is the cause of reduced neural integrity in diabetes.

Concerning limitations of the study some patients came to blood collection without fasting, some patients ignore and escape from the study and there are few patients have other diabetic complications other than neuropathy.

Acknowledgments. I would like to express my gratitude to the Dean and Vice Dean of the Deanship of Scientific Research of King Saud University for sponsoring this study.

References