Correlation of acetylator phenotype with peripheral, autonomic and central neuropathy in Northern Indian non-insulin-dependent diabetes mellitus patients

Abstract Objectives: Genetic susceptibility to diabetic neuropathy has been suspected and tentatively explored; however, diabetic autonomic and central neuropathies are poorly investigated areas. Previous trials correlating types of diabetes and diabetic neuropathy with acetylator dimorphisms have not been conclusive. The present study was designed to test peripheral neuropathy, autonomic neuropathy and integrity of central conduction pathways in patients of non-insulin-dependent diabetes mellitus (NIDDM), and to correlate the findings with the acetylator phenotype.

Methods: Twenty-six patients of NIDDM with stable glycaemic control and 11 age- and sex-matched control subjects were recruited, clinically examined and investigated with glycaemic and lipid profile, renal function tests, nerve conduction studies (sensory and motor), auditory brain stem evoked responses (ABERs) and somatosensory evoked potentials (SEPs). Acetylator status of the subjects was determined by sulphadimidine test.

Results: Out of 26 NIDDM patients, eight (30.7%; group 1A) were slow acetylators and 18 (69.3%; group 1B) were fast acetylators. The distribution of slow and rapid acetylators in both the groups was similar. Glycaemic and lipid profiles and 24-h urinary albumin excretion in groups 1A and 1B were also similar. Motor nerve conduction velocity, latency of F wave, sensory nerve conduction and amplitudes of sensory nerve action potentials were not different between fast and slow acetylator NIDDM patients. On testing for ABERs, there were no statistically significant differences in peak latencies of waves I, III and V; interpeak latencies (IPLs) I–III, III–V and I–V; amplitude of waves I, III and V on both sides between NIDDM patients and controls. However, peak latencies of wave III ($P < 0.01$), wave V ($P < 0.005$), IPLs I–III and I–V ($P < 0.005$), IPLs III–V ($P < 0.05$), and amplitudes of wave I ($P < 0.05$) and wave V ($P < 0.05$) on the left side were significantly different in slow acetylator NIDDM patients. Increase on the right side for the same group was statistically significant for IPLs I–III and I–V ($P < 0.05$). SEPs showed no statistically significant difference between NIDDM patients and controls, and slow and fast acetylator NIDDM patients.

Conclusions: No significant association of acetylator status with peripheral neuropathy in NIDDM subjects was observed in the present study. However, central neural conduction, primarily tested by ABERs, was significantly delayed in slow acetylators compared with fast acetylator NIDDM patients. Hence, there may be a predisposition to neuropathy in this group of patients, and such a predisposition may be better detected by studying central rather than peripheral nervous conduction pathways in NIDDM patients.

Key words Non-insulin-dependent diabetes mellitus (NIDDM) · Acetylator phenotype · Central neuropathy

Introduction

Diabetic neuropathy encompasses several distinct syndromes of focal and diffuse peripheral somatic and autonomic neuropathy. Recently, central nervous system dysfunction, termed as “central neuropathy”, has added yet another dimension. The pathogenesis of neuropathy has been a matter of debate. A small percentage of patients develop neuropathy regardless of the duration of their diabetes and its adequate control, while others manifest with severe neuropathy at the presentation. The
cause of marked variations in the course and extent of neuropathy in the presence of a presumably common metabolic abnormality is unknown; however, genetic susceptibility has been suspected.

A number of drugs (isoniazid, procainamide, hydralazine, sulfonamides and dapsone) are metabolized in the liver by the enzyme N-acetyltransferase (NAT). The rate of activity of this enzyme is genetically determined. Variation in the individual acetylation capacity is attributed to NAT variants of liver, intestinal mucosa and other tissues. Populations can be divided into slow and fast acetylators with implications for the dosage and adverse drug reactions of various drugs and for the pathogenesis of various diseases and their complications. Polymorphisms of NAT have been linked to drug toxicity in those having slow acetylator phenotype. For example, slow acetylators are more likely to develop neuropathy when treated with isoniazid [1]. Furthermore, attenuation of drug effect is observed in individuals having fast acetylator phenotype. Acetylator phenotype has been linked to diabetes mellitus and diabetic peripheral neuropathy [2], procainamide-induced systemic lupus erythematosus [3], hyperthyroidism [4] and various cancers [5–7]. McLaren observed a significantly higher proportion of fast acetylators in the group of patients with insulin-dependent diabetes mellitus (IDDM) without neuropathy compared with those with neuropathy and normal subjects [2]. Others have not consistently recorded these findings [8–10]. No satisfactory explanation is available for development of diabetic neuropathy in slow acetylators; however, it has been proposed that similar to isoniazid-induced neuropathy, there is interaction of some metabolite with the genetic tendency. Moreover, high blood glucose concentrations may influence acetylator status as well [11].

No correlation, however, has so far been performed between acetylator phenotype and diabetic autonomic and central neuropathy. Furthermore, there is a marked geographical variation in population distribution of acetylator phenotype [12–14]. Even in northern India, prevalence of fast acetylators has ranged from 27% to 80% in the various studies [15–18]. However, estimation of acetylator polymorphisms in diabetes, and its correlation to microvascular complications has never been performed in Asian Indian non-insulin-dependent diabetes mellitus (NIDDM) patients. The aim of the present study was to perform acetylator status by sulphanilamide test in Asian Indian NIDDM patients and correlate it with their clinical and metabolic profiles and microvascular complications, particularly peripheral, autonomic and central neuropathy.

apparent auditory pathology, pregnancy, and advanced renal, hepatic and respiratory dysfunction. Patients with history of recurrent hypoglycaemia in the past, and hypoglycaemia or diabetic ketoacidosis 72 h prior to the day of examination were not included in the study. Patients receiving methylodopa, reserpine, phenytoin or other neurotoxic drugs were not recruited. Eleven healthy non-diabetic matched subjects, not differing in age and sex distribution compared with the patient group were taken as the control group. Informed consent was obtained in all the cases.

A detailed history and clinical examination with special reference to peripheral and autonomic neuropathy were recorded. Fundus examination was performed by an expert ophthalmologist. Clinical tests for autonomic neuropathy included heart rate response to Valsalva maneuver, immediate heart rate response to standing, heart rate variation during deep breathing and response of blood pressure to standing. These tests were performed according to the standard guidelines. A mean of three readings was calculated in all the above four tests of autonomic nerve functions.

Nerve conduction studies were performed on DISA Neuromatic 2000 C (DISA Electronics, Denmark). The study included amplitude of compound muscle action potential (CMAP), motor nerve conduction velocity (MNCV; amplifier setting: sweep speed: 2 msec–div.⁻¹, sensitivity: 5 nV–div.⁻¹, filter setting: 30 Hz–10 KHz) and F wave latency (amplifier setting: sweep speed: 5–10 msec–div.⁻¹, sensitivity: 200 µV–div.⁻¹, filter setting: 20 Hz to 10 KHz) in right median, ulnar, tibial and common peroneal nerves. For sensory nerve conduction studies, amplitude of sensory nerve action potential (SNAP) and sensory nerve conduction velocity (SNCV; amplifier setting: sweep speed: 2 msec–div.⁻¹, sensitivity: 20–50 µV–div.⁻¹, filter setting: 20 Hz to 2 KHz) in the right median, ulnar and sural nerves studied.

Auditory brain stem evoked responses (ABERs) and somatosensory evoked potentials (SEPs) were recorded in a shielded, sound attenuated room under standard conditions using a Nicolet-1170 clinical averaging system (Nicolet Instrument Corp., Madison, Wis., USA). For recording ABERs, 100-μsec rarefaction click was delivered at 70 dB above the hearing level, and the rate of stimulation was set at 11.1 per second. A band pass of 150–300 c/sec was used. Percutaneous silver disc electrodes were placed at the vertex and both mastoids, with the common reference electrode being on the mastoid contralateral to the ear stimulated. Two thousand and forty-eight responses collected within first 10 msec were averaged. Latencies of waves I to V and interpeak latencies (IPLs) of wave I to III, III to V and I to V in milliseconds, and amplitudes (μV) of waves I, III and V were recorded.

SEPs were obtained for stimulation of median nerve at wrist by square wave stimulation of 100-μsec duration, 5-Hz frequency and at threshold level. A band pass of 10 Hz to 2 KHz was used. A total of 512 responses collected within the first 30 msec were recorded. Latency and amplitude of wave P20 were noted. The same procedure was repeated on the median nerve on the other side. Acetylator status of the subjects was determined by sulphanilamide acetylation test. Sulphanilamide was given in a dosage of 44 mg·kg-body weight⁻¹ in the fasting state by mouth. Estimation of total and free sulphanilamide in serum was done by the modified Bratton-Marshall technique [19]. The subjects were classified as slow acetylators if the proportion of acetylated sulphanilamide in the serum sample collected 6 h after the oral dose of sulphanilamide was less than 25%.

Materials and methods

The study was conducted on 26 consecutive patients of 35–60 years of age, who had been suffering from NIDDM for 5 years or more. Patients with the following were excluded: peripheral neuropathy due to other causes, family history of neuromuscular disorders, apparent auditory pathology, pregnancy, and advanced renal, hepatic and respiratory dysfunction. Patients with history of recurrent hypoglycaemia in the past, and hypoglycaemia or diabetic ketoacidosis 72 h prior to the day of examination were not included in the study. Patients receiving methylodopa, reserpine, phenytoin or other neurotoxic drugs were not recruited. Eleven healthy non-diabetic matched subjects, not differing in age and sex distribution compared with the patient group were taken as the control group. Informed consent was obtained in all the cases.

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Results

The mean with (SD) age in both the groups was comparable. The mean duration of diabetes was 9.7 (3.7) years (Table 1). Out of 26 NIDDM patients, eight (30.7%; group 1A) were slow acetylators, while 18 (69.3%; group 1B) were fast acetylators. Seven (63.63%) rapid acetylators and four (30.37%) slow acetylators were recorded in the control group. The distribution of