Subclinical autonomic neuropathy in Saudi type 2 diabetic patients

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ABSTRACT

Objective: To compare the results of autonomic function tests obtained from diabetic patients who had no symptoms or signs of somatic or autonomic neuropathy with those obtained from control subjects.

Methods: We studied 32 diabetic Saudi patients (17 males, 15 females) and 34 control subjects (17 of either gender) at King Khalid University Hospital, Riyadh, in the period 2004-2005. The mean age of patients was 50.3+/-5.04, and of controls was 49.9+/-5.86 years. In diabetics, the mean duration of the disease was 8.7±3.1 years (range 5-15 years), and the mean glycated hemoglobin was 7.76 ±1.14. The same observer performed the autonomic function tests.

Results: In diabetics, the resting heart rate (beats/min) was 80.5±4.13, mean orthostasis ratio was 1.06±0.035, mean Valsalva ratio was 1.19±0.036, mean forced sinus arrhythmia was 12.66±0.8 beats/min, mean diastolic blood pressure increase in response to isometric exercise was 13.03±1.36 mm Hg, and sympathetic skin response was present in only 18 (56.3%) out of 32 patients. These results were significantly different from the control group ($p<0.001$).

Conclusions: Diabetic patients, with no symptoms or signs of neuropathy, can have impaired autonomic function. We consider this subclinical autonomic neuropathy.

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The prevalence of diabetes mellitus is on the rise worldwide, Saudi Arabia is no exception.[1,2] One of the major complication of diabetes is neuropathy, which may involve somatic peripheral nerves, or autonomic nerves, or both. It can be subclinical in the initial stages, later manifesting itself clinically. Subclinical diabetic somatic neuropathy, diagnosed with nerve conduction studies in asymptomatic diabetic patients with no other cause of neuropathy, has been recognized since the early 1970s,[3,4] and has been reported recently from Saudi Arabia.[5,6] There is also a noticeable increase in the frequency of physicians ordering nerve conduction studies to exclude the presence of neuropathy in their diabetic patients. Subclinical diabetic autonomic neuropathy, however, can easily be overlooked in its early stages.[7] It develops insidiously, with vague symptoms and pursues a progressive course, that eventually ends in serious complications such as foot ulcers.[8] Therefore, there is an urgent need for tests that can reveal subclinical autonomic neuropathy. This is especially important since the early intensive treatment of diabetes has been shown to prevent the progression of neuropathy and other complications.[9,10] Autonomic function tests provide the best method for the objective assessment of the integrity of the autonomic nervous system. They are non-invasive, sensitive, specific, and reproducible.[11] The objective of this study is to see whether autonomic dysfunction could be detected in asymptomatic type 2 diabetic patients.

Methods. Patients included in the study were 32 consecutive type 2 diabetic patients (17 males, 15 females) who attended the diabetes clinic in King Khalid University Hospital, Riyadh, in the period 2004-2005. The duration of diabetes since first diagnosed was 5-15 years. The control group comprised 34 healthy subjects (17 of either gender), age- and sex-matched to the patients. The age range for both groups was 40-61 years. The mean HbAc (%) value was 5.35±1.05 in controls and 7.76+/-1.14 in diabetic patients. Consent was obtained from both patients and controls. In accordance with the criteria for standardized classification of diabetic neuropathy,[12,13] the symptom profile was examined and complete neurological examination carried out in both patients and controls. The information gathered consisted of:

A. Clinical database and general laboratory investigations.
B. Autonomic function tests.

A. Clinical database and investigations. This entailed taking full medical history, performing a comprehensive clinical examination and ordering the relevant laboratory investigations. The exclusion criteria in patients included a duration of diabetes of less than 5 years. Any patient who had evidence of cardiac, hepatic, endocrine, or neurological disease was excluded from the study. Patients who had a history of transient, treated urinary tract infection were included, but those with raised blood urea or creatinine level were excluded from the study. Patients with points in the history suggestive of autonomic involvement were also excluded from the study. These included unexplained chronic diarrhea (for which no medical cause was found), erectile failure, gustatory sweating (when no spicy food was taken), post-prandial vomiting and bloating (for which no medical cause was discerned), syncope and falling when arising quickly from the sitting position, and unexplained bladder dysfunction.[14] Information regarding medications taken by the subjects was procured. A list of drugs was inquired about, in order to exclude from the study subjects who were using them. The list included anticholinergics, antidepressants, lithium, nitrofurantoin, phenytoin, amiodarone, isoniazid, flecainide, beta-adrenergic blockers, hydralazine and calcium channel blockers.[15] The subjects had no history of exposure to alcohol, toxic chemicals or history of vitamin deficiencies. A complete physical examination, including neurological examination, was undertaken. An ophthalmologist performed ophthalmoscopy. Patients with evidence of retinopathy were excluded from the study. Laboratory investigations included complete blood count (CBC), blood urea, potassium, lipids, creatinine, and 24-hour urine protein examination. Patients whose 24-hour urine collection contained more than 0.5 g of protein were excluded from the study. Glycated hemoglobin (HbA_{1c}) was measured by column chromatography (Glyco-Tek Laboratories, USA). Using this method, the normal level of HbA1c was taken as 3.9-6.7%.

B. Autonomic function tests. The subject's arm was connected to a sphygmomanometer (Accoson Medical Instruments, England), and ECG leads placed on the chest and connected to a machine (Biopac MP30, USA) that derived instantaneous heart rate from R-R intervals. After allowing him to settle down for 5 minutes, the subject's resting heart rate and blood pressure were recorded. Then the orthostasis test, Valsalva maneuver, forced sinus arrhythmia, and isometric exercise test was carried out, allowing the patient to relax for at least 5 minutes between each test and the next. The subject was then allowed to relax for 10 minutes before undertaking the sympathetic skin response test.

The orthostasis test. After recording the blood pressure and heart rate in the supine position, the subject was asked to stand unaided. The shortest R-R interval at around the 15th heartbeat and the longest R-R interval at around the 30th beat after standing were measured. The orthostasis ratio was determined by dividing the longest R-R interval by the shortest one.

Valsalva maneuver. The subject, while seated, was asked to blow into a mouthpiece connected to a manometer, and to maintain the pressure at 40 mm Hg for 15 seconds. The longest R-R interval during blowing into the mouthpiece and the shortest R-R interval shortly after stopping blowing were determined. Valsalva ratio was recorded as the ratio of the longest R-R interval to the shortest R-R interval. The test was repeated 3 times, and the mean of the 3 Valsalva ratios was recorded.

Forced sinus arrhythmia. While the heart rate (beats/min) was continuously monitored, the subject took 6 consecutive maximal inspirations and expirations at a rate of 6 breaths per minute, paced by the investigator. The maximum increase in heart rate (beats/min) from the resting value was determined.

Isometric exercise test (sustained handgrip test). Before the test, the mean of 3 consecutive diastolic blood pressure (BP) measurements was taken as the baseline value. The subject was then instructed to practice, on a dynamometer, whose scale was visible to him, to maintain his handgrip as powerfully as he could. After that, the subject was asked to maintain 30% of his maximal voluntary contraction force for 5 minutes. The BP was measured at one-minute intervals during the maneuver. The difference between the highest diastolic BP during the maneuver and the baseline value was determined.

Sympathetic skin response (SSR). A Nicolet Viking EMG machine (Nicolet, USA) with SSR software was used. Ten mm disc electrodes were attached to the volar and dorsal surfaces of the right hand. The low frequency filter was set at 0.5Hz and the high frequency filter at 2 kHz. Square pulse stimuli of 0.1ms duration and 250V amplitude were applied to the left wrist, and the response was recorded.

Statistical analysis. Using SPSS version 13.0 software, the student's t-test for independent variables was used to compare the diabetic group and control group with respect to age and gender distribution, resting heart rate, orthostasis, Valsalva ratio, sinus arrhythmia, and isometric exercise tests. The Chi square test was employed to compare the presence of the sympathetic skin response in diabetic patients and in controls. The level of significance was set at $p<0.05$.

Results. **Table 1** shows a summary of the results of autonomic function tests. All parameters of autonomic

Table 1 - Comparison between the control subjects and diabetic patients in terms of sex, age, HbAc (%), and autonomic function parameters.

Variable	Controls (n=34)		Diabetic patients (n=32)		Statistical significance
	Male	Female	Male	Female	
Gender	17	17	17	15	NS
Age (years, mean ± SD)	49.9 ± 5.86		50.3 ± 5.04		NS
HbA_{1c} (%)	5.35 ± 1.05		7.76 ± 1.14		p<0.001
Resting heart rate (beats/min)	73.4 ± 2.64 (range 68–78)		80.5 ± 4.13 (range 72 – 89)		p<0.001
Orthostasis ratio	1.21 ± 0.051		1.06 ± 0.035		p<0.001
Valsalva ratio	1.36 ± 0.048		1.19 ± 0.036		p<0.001
Forced sinus arrhythmia (beats/min)	17.97 ± 1.78		12.66 ± 0.80		p<0.001
Isometric exercise test (mm Hg)	20.53 ± 2.11		13.03 ± 1.36		p<0.001
Sympathetic skin response present	34 (100%)		18 (56.3%)		p<0.001

function tests were significantly different (p<0.001, **Table 1**) in diabetics and controls. The resting heart rate value was higher in diabetics than in the controls, while the increase in heart rate in response to forced sinus arrhythmia and rise in diastolic BP during isometric exercise were lower in the diabetics group. Orthostasis and Valsalva ratios were significantly lower in diabetics. The sympathetic skin response was present in all 34 control subjects, but only in 18 out of the 32 diabetic patients.

Discussion. One of the most important findings of this study is the significant difference in autonomic function between asymptomatic diabetic patients and age- and gender-matched healthy controls.

The resting heart rate. A normal resting heart rate reflects the integrity of autonomic innervation of the heart. Under resting conditions, vagal activity is the main determinant of heart rate. The vagal supply to the heart comes mainly from both the dorsal motor nucleus (DMN) of the vagus nerve and from nucleus ambiguous (NA), both of which receive inputs from nucleus tractus solitarius (NTS) via intervening modulating synapses.[16] The vagus nerve, being the longest autonomic nerve, is susceptible to early damage by factors implicated in the pathogenesis of diabetic neuropathy, such as accumulation of glycosylation products in endoneural blood vessels leading to nerve ischemia, direct neuronal damage by accumulation of sorbitol, neurohormonal growth factor deficiency, and activation of autoimmune mechanisms.[17] Under conditions of complete mental and physical rest, an adult heart rate of 80 beats/minute or more is suspicious, and a rate of 100 beats/min or more indicates considerable cardiac vagal denervation as a result of established autonomic neuropathy.[18]

In diabetic patients, the mean resting heart rate was 80.5+/-4.13 beats/min, which is significantly higher than that recorded in healthy controls (73.4+/-2.64 beats/min), and this points to a defect in their autonomic cardiac vagal control.

Orthostasis test. Upon standing up from the lying position, approximately 700 ml of blood moves from the chest to be pooled in the abdomen and lower limbs, and thereby decreases venous return. This decreases baroreceptor discharges and causes an early reflex tachycardia. Shortly thereafter, the other baroreflexes causes peripheral and splanchnic vasoconstriction, and increases catecholamine levels.[19] This results in increased venous return and in a delayed reflex bradycardia. The early compensatory tachycardia is maximal around the 15th heartbeat after standing, and the delayed reflex bradycardia is maximal around the 30th beat. The orthostasis ratio should normally be 1.04 or more. In advanced autonomic neuropathy, it is fixed at 1.[20] In control subjects. the obtained ratio was 1.21+/-0.051, and in diabetic patients, it was 1.06+/-0.035, which is significantly lower than the controls. It is clear, therefore, that the asymptomatic diabetic patients in our study had not yet reached the stage of established autonomic neuropathy (indicated by a ratio of 1.04 or less), but had a degree of reduced efficiency in their autonomic regulatory mechanisms in response to changes in posture.

Valsalva ratio. The Valsalva maneuver evaluates the baroreflex arc and its sympathetic as well as parasympathetic responses.[21] The Valsalva ratio should normally be 1.21 or more, and a ratio of 1.1 or less indicates autonomic neuropathy. In our study, diabetic patients had a ratio of 1.19 +/-0.036, which was significantly lower than that in controls, but still above 1.10, at which established autonomic neuropathy could be diagnosed.

Forced sinus arrhythmia. Normally, during this test, the heart rate should increase from the resting value by 15 beats/minute or more. An increase of 11-14 beats/min is considered as borderline, and an increase of only 10 beats/in or less indicates functional cardiac vagal denervation.[16,21] In the current study, the diabetic patients exhibited a modest increase in heart rate (12.66+/-0.80 beats/min), which is significantly less than that in controls (17.97+/-1.78 beats/min). This lends further support to the existence of impaired autonomic control in these patients.

Isometric exercise test (sustained handgrip test). During this test, the diastolic BP should normally rise by more than 16 mm Hg.[22] A rise of 11-15 mm Hg is considered borderline, and a rise of 10 mm Hg or less is taken as evidence of autonomic neuropathy. In the current study, the diastolic BP rise in diabetic patients was 13.03+/-1.36 (within the borderline range). However, it was significantly less than that in controls (20.53+/-2.11). This is suggestive of a degree of autonomic dysfunction.

Sympathetic skin response (SSR). Our experience in Saudi subjects showed that SSR was obtainable from all normal individuals tested (unpublished data). The SSR is lost when the small, unmyelinated, sympathetic C fibers in the skin degenerate as a consequence of autonomic neuropathy.[23] The SSR was obtainable in all 34 (100%) control subjects, but in only 18 (56.3%) out of 32 diabetic patients. The finding that SSR was absent in 14 out of the 32 diabetic patients indicates that even in asymptomatic patients, diabetes may be causing degenerative changes in the autonomic innervation of the skin.

Diagnostic yield of the various tests. Diagnostic yield expresses the percentage of patients who had a positive test indicating impairment of autonomic function. Forced sinus arrhythmia had the highest diagnostic yield (100%). The diagnostic yield for other tests was as follows: Valsalva ratio 69%, SSR 44%, orthostasis ratio 19%, resting heart rate 19%, and isometric handgrip tests 15%.

This study demonstrates that diabetic patients, even if still asymptomatic, can develop autonomic dysfunction. The subjects in this study are going to be followed up further to see if, in the future, their autonomic function tests deteriorate further, and whether they will develop frank clinical autonomic neuropathy.

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