Original Article

Autonomic nervous system function in type 2 diabetes using conventional clinical autonomic tests, heart rate and blood pressure variability measures

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ABSTRACT

Background: There are currently approximately 40.9 million patients with diabetes mellitus in India and this number is expected to rise to about 69.9 million by the year 2025. This high burden of diabetes is likely to be associated with an increase in associated complications. **Materials and Methods:** A total of 23 (15 male and 8 female) patients with type 2 diabetes of 10-15 years duration and their age and gender matched controls (n=23) were recruited. All subjects underwent detailed clinical proforma, questionnaire related to autonomic symptoms, anthropometry, peripheral neural examination and tests of autonomic nervous system including both conventional and newer methods (heart rate and blood pressure variability). **Results:** Conventional tests of cardiac parasympathetic and sympathetic activity were significantly lower in patients with diabetes compared to the controls ($P<0.05$). The diabetic patients group had significantly lower high frequency and low-frequency HRV when expressed in absolute units ($P<0.05$) and total power ($P<0.01$) compared to the controls. **Conclusion:** Data from the current study demonstrated that diabetics had both cardiac sympathetic and cardiac parasympathetic nervous system involvement. The presence of symptoms and involvement of both components of the autonomic nervous system suggest that dysfunction has been present for a while in these diabetics. There is a strong need for earlier and regular evaluation of autonomic nervous system in type 2 diabetics to prevent further complications.

Key words: Autonomic, diabetes, heart rate, neuropathy, variability

INTRODUCTION

There are currently approximately 40.9 million patients with diabetes mellitus in India and this number is expected to rise to about 69.9 million by the year 2025.[1] This high burden of diabetes is likely to be associated with an increase in associated complications.[2] Autonomic nervous system dysfunction is one of the significant complications of diabetes mellitus and this is generally associated with a poor prognosis.[3] Cardiac parasympathetic involvement precedes sympathetic damage.[4]

In keeping with the recommendations of the American Diabetes Association (1992),[5] five standard cardiovascular reflex tests are used to assess cardiovascular autonomic function. These include changes in heart rate during deep timed breathing, Valsalva manoeuvre and standing up to assess cardiac parasympathetic activity and blood pressure responses to standing up and sustained handgrip to evaluate sympathetic nervous activity.[5] Though these methods are used widely, they may be associated with difficulties in patient compliance, particularly those who are elderly. The tests may also lack the sensitivity to detect subtle changes.[6] Our own studies indicate that correlations between the various conventional tests of cardiac parasympathetic activity are low.[7] This may, in

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part, be due to methodological issues and, in part, to the fact that these tests have different afferent pathways while evoking a common efferent pathway. Further, each of the tests does not exclusively reveal impairment of one or the other arm of the autonomic nervous system.[8] The development of newer, more sensitive methods like heart rate variability, systolic blood pressure variability and baroreflex sensitivity are believed to have a greater ability to differentiate vagal and sympathetic modulation of the autonomic nervous system compared to conventional tests in a variety of conditions.[9,10]

There have only been a few Indian studies on autonomic nervous system changes in diabetics.[11,12] These studies have used different methods, some at variance with current recommendations.[10] None have used the newer, more sensitive techniques that we have discussed for the current study. The impetus for detecting early autonomic neuropathy using sensitive methods are emerging data that indicate that the progress of early diabetic autonomic neuropathy can be arrested with adequate glycemic control.[2] The current study thus aimed to evaluate cardiovascular autonomic activity using heart rate and blood pressure variability, baroreflex sensitivity and conventional cardiovascular autonomic tests in patients with type 2 diabetes with a disease duration of 10 to 15 years, in whom autonomic neuropathy would be expected to be present because of the duration of the disease.

MATERIALS AND METHODS

A total of 23 (15 male and 8 female) patients with type 2 diabetes of 10-15 years duration were recruited from Diabetic Clinic of the Hospital and their age and gender matched controls (n=23) were recruited from in and around the medical college. A minimum sample of n=20 was calculated to detect a difference of approximately 10% between control and diabetic patients with power of 0.8 and alpha at 0.05.

Subjects with a history of asthma, hypertension, cardiovascular disease and those on chronic medication were excluded from the study. None of the subjects were current smokers and all had alcohol consumptions of less than two drinks per day. Prior to the recruitment details of the procedure was discussed and an explanatory statement was provided to the subject after which written informed consent was obtained. This study was approved by the Institution Ethical Review Board.

The study subjects were required to respond to a detailed clinical proforma, answer a questionnaire related to autonomic symptoms and undergo detailed measurements of anthropometry. They also underwent peripheral neural examination based on (i) the presence or absence of foot pain, numbness, tingling, weakness, imbalance and upper limb symptoms (total score=6) (ii) 8 reflex scores involving knee and ankle reflexes (iii) 5 physical examination scores involving presence or absence of pinprick, temperature, light touch, vibration and position sense. Following which they were graded as no neuropathy (score <5), mild neuropathy (score 6-8), moderate neuropathy (score 9-11) and severe neuropathy (score >12). The total possible score was 19.[13] Retinopathy and nephropathy were ruled out from their medical records; all patients had undergone assessments for these in the previous year. All subjects were studied in the fasting state, in the morning. Subjects were instructed to have their last meal prior to 9 pm on the night before experimentation and to avoid heavy physical activity the evening prior to experimentation. They were also instructed to refrain from all caffeinated beverages for 12 h prior to the experiment.

Cardiovascular autonomic tests
Continuous lead II ECG (Nihon Kohden RM-6000 Polygraph system, Japan) and beat to beat blood pressure (Portapres, TNO, The Netherlands) was measured for 10 minutes after a mandatory 30 minutes rest. Automated cuff blood pressure was also recorded on the right arm at the end of the rest period, after the change of posture and sustained isometric contraction (automated Welch Allyn, Welch Allyn Inc., NY, USA); heart rate for each manoeuvre, described below, was calculated using 10 consecutive RR intervals immediately prior to manoeuvre, while the maximal heart rate was determined by scanning the RR intervals following each manoeuvre. The subsequent test was performed only after the heart rate returned to the basal level. Details of the tests are given below.

Cough
Subjects were asked to cough maximally once. This was repeated a second time after the heart rate returns to basal values. The increment in heart rate (maximal heart rate following cough-basal heart rate) was used in the analysis as an index of vagally mediated withdrawal to the heart.

Maximum hand grip
The immediate heart rate response to a single maximal hand grip (Smedley's Dynamometer, TTM, Tokyo) sustained to a count of three was determined. The test was performed twice.

Timed deep breathing
The subjects were asked to breathe maximally at six breaths per minute (5 seconds in and 5 seconds out) based on verbal prompts from the investigator. The maximum-minimum

heart rate during each 10-second breathing cycle was measured and the highest difference during six successive breathing cycles was used in the analysis.

Heart rate and blood pressure response to standing

Subjects were instructed to stand immediately without any support. Blood pressure was recorded using an automated sphygmomanometer immediately after standing. Continuous ECG was recorded from which a 30:15 ratio was calculated. It is the ratio of the longest RR interval around the 30th beat after standing up, to the shortest RR interval around the 15th beat during standing.

Valsalva manoeuvre

The subject was required to maintain a pressure of 40 mm Hg for 10 seconds after application of a nose clip. The Valsalva ratio was calculated as the ratio of the longest RR interval within 20 beats of the manoeuvre, to the shortest RR interval during the manoeuvre.

Sustained isometric contraction

Handgrip was maintained at 30% of the maximum voluntary contraction for 3 minutes, using a dynamometer. Heart rate and blood pressure responses were measured at the end of 3 minutes, prior to release of the dynamometer. The delta change in heart rate and blood pressure were calculated.

Spectral analysis of heart rate variability and systolic blood pressure variability

ECG and beat-to-beat systolic blood pressure (SBP) were recorded continuously for a period of 10-12 minutes, with the subject supine, awake and resting as described earlier.[7] Briefly, the data were digitied on-line at 1000 Hz using an IBM compatible PC and a data acquisition package (CVMS, World Precision Instruments Inc., Sarasota, FL, USA) incorporating a signal manifold and a C10-AD 16 Jr A/D card that was installed within the computer. The data acquisition system included a threshold peak detection from which the RR intervals and beat to beat SBP would be determined. Data segments of 128 seconds duration were sampled at 2 Hz to create 256-point data sets. For each 10- to 12-minute recording, eight data sets of 256 points overlapping by half will be processed. The linear trend was removed from each data set to avoid its contribution to low-frequency power. A Hanning window in the time domain would be used to attenuate 'spectral leakage'. Spectral analysis was performed using a fast Fourier transform. The frequency resolution was 0.0078 and the highest frequency evaluated was 0.4 Hz. The spectra obtained for the different data sets were averaged to reduce variance and to sharpen reproducible central peaks. Power was calculated in two bands. The 0.04-0.15 Hz band

of RR power is also referred to as low-frequency band and is believed to reflect, at least in part, sympathetic nerve activity to the heart, while 0.15-0.4 Hz band referred to as the high frequency band and reflects parasympathetic nerve activity to the heart. The low-frequency component of SBP variability was expressed in absolute units, and is believed to reflect vasomotor sympathetic nerve activity. In addition to the absolute power, data for heart rate variability were also presented as normalised units as recommended, where the power in the two bands is expressed as a percentage of the total power minus the power of the very low-frequency band (0.0-0.04 Hz).[14]

The alpha coefficient was determined from the spectral analysis of systolic blood pressure and RR variability, as the squared ratio of RR and SBP power in the 0.07-0.14 Hz range.[15]

Statistical Analysis

Data were normally distributed based on the Kolmogorov-Smirnov Z test for normality. Descriptive data are expressed as mean ± standard deviation. Vagal and sympathetic activity were compared between the diabetes group and their controls using an independent 't' test. The null hypothesis was rejected if $P < 0.05$.

RESULTS

The characteristics of the two study groups are summarized in Table 1. There were no differences in the anthropometric measurements (both primary and derived parameters) between diabetic patients and the controls. The resting heart rate, systolic and the diastolic blood pressure were similar in both the groups.

Tests of the autonomic nervous system indicated varied responses between the two groups. Tests of cardiac

Table 1: Baseline characteristics of the control group and patients with type 2 diabetes

	Control group (n=23)	Diabetes group (n=23)
Age (years)	59±10	58±8
Height (m)	1.59±0.09	1.60±0.09
Weight (kg)	57.7±10.3	62.4±11.9
Body mass index (kg/m²)	22.6±3.5	23.8±3.4
Percentage fat (%)	28.7±4.3	27.3±5.4
Waist hip ratio	0.92±0.07	0.93±0.09
Basal heart rate (bpm)	69.2±9.8	72.2±6.4
Basal systolic blood pressure (mm Hg)	124.8±14.5	133.1±16.8
Basal diastolic blood pressure (mm Hg)	79.0±9.0	79.2±6.6

All mean ± standard deviation, comparisons between the groups using an independent 't' test. All data presented above were comparable between the two groups

parasympathetic activity including the heart rate response to cough, maximum hand grip, timed deep breathing, standing 30:15 ratio and Valsalva ratio were significantly lower in patients with diabetes compared to the controls (*P*<0.05) [Table 2]. Tests of cardiac sympathetic activity systolic blood pressure response to standing (*P*<0.05) and heart rate (*P*<0.05) response to sustained isometric contraction were lower in the diabetic group compared to controls [Table 2].

Patients with diabetes had significantly lower high-frequency and low-frequency HRV when expressed in absolute units (*P*<0.05) and total power (*P*<0.01) compared to the controls [Table 3]. These differences disappeared when the data were represented in normalised units. The control group had a wider spread of data compared to patients with diabetes, for both low- and high-frequency power spectra in absolute units. In contrast to the changes in the heart rate variability, low-frequency SBP variability was comparable between the two groups as was the alpha coefficient, an indicator of the baroreflex sensitivity [Table 3].

Out of 23 diabetic patients recruited, 16 diabetics completed the peripheral nervous system examination and autonomic questionnaire. A total of 7 diabetics could not spare time for the same. To rule out a response bias, conventional autonomic tests and newer methods of autonomic nervous activity were compared between responders and non responders. Data of autonomic function were comparable between responders and non-responders.

Peripheral nervous examination (n=16) revealed that 38%

(n=6) had mild peripheral neuropathy and 25% (n=4) had moderate peripheral neuropathy. Out of 10 diabetics who tested positive for peripheral neuropathy 9 presented with at least one autonomic symptom andcardiac sympathetic involvement and 6 diabetics had cardiacparasympathetic involvement based on conventional clinical autonomic tests. Peripheral nervous examination revealed negative results for six diabetics, out of which four had cardiac parasympathetic involvement and five had sympathetic involvement.

The autonomic symptom questionnaire (n=17) indicated the following results; 18% (n=3) had gustatory symptoms, 29% (n=5) postural giddiness, 29% (n=5) were dyspeptic, 12% (n= 2) had diarrhoea/bloating/constipation, 47% (n=8) urinary symptoms and 47% (n=8) of males were impotent. A total of 82% (n=14) of the diabetics presented with at least one autonomic symptom.

Eighty one percent (n= 9) of the diabetics who presented with at least one symptom also had one cardiac parasympathetic test positive and all who presented with at least one symptom had cardiac sympathetic involvement [Table 4].

Discussion

The data of this study show that long standing older patients with diabetes have considerable autonomic dysfunction when assessed both conventionally and by more recently developed methods. Conventional tests in our study group uncovered involvement of both components of autonomic nervous system, though the parasympathetic cardiac involvement was predominant. This is in keeping with the development of autonomic neuropathy which typically involves the parasympathetic

Table 2: Comparison of conventional cardiovascular autonomic tests in control subjects and patients with type 2 diabetes

	Control group (n=23)	Diabetes group (n=23)
Cough: delta HR (bpm)	12.8±5.3	9.8±3.5*
Maximum hand grip: deltaHR (bpm)	14.0±5.7	10.6±4.2*
Timed deep breathing: delta HR (bpm)	14.8±7.5	10.2±4.3*
Blood pressure responses to standing: delta SBP (mm Hg)	4.07±11.1	-9.7±14.9**
Lying to standing: 30:15 ratio	1.36±0.19	1.17±0.12**
Valsalva manoeuvre (Valsalva ratio)	1.62±0.26	1.32±0.22**
Sustained isometric contraction: delta DBP (mmHg)	8.9±11.1	1.9±8.4
Sustained isometric contraction: delta HR (bpm)	11.0±16.4	2.0±5.0*

HR: Heart rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, bpm: Beats per minute. All mean ± standard deviation, Comparisons between the groups using an independent 't' test. *P<0.05, **P<0.01

Table 3: Comparison of indices of heart rate, systolic blood pressure variability and baroreflex sensitivity in control subjects and patients with type 2 diabetes.

	Control group (n=23)	Diabetes group (n=23)
Low frequency (LF) absolute units	270.4±243.1	127.5±124.5*
High frequency (HF) absolute units	236.6±259.6	82.5±92.8*
Total power	835.2±580.9	374.1±337.7**
Low-frequency normalized units	63.6±24.8	72.8±20.8
High-frequency normalized units	46.9±19.8	43.9±14.6
LF/HF ratio	1.97±1.71	2.04±1.32
Low-frequency systolic blood pressure (mm Hg)	5.37±5.1	3.53±3.16
Alpha coefficient (ms/mm Hg)	8.51±4.21	8.11±3.95

All mean ± standard deviation, comparisons between the groups using an independent 't' test. *P<0.05, **P<0.01

Table 4: Demonstrating the relation between the ANS symptom and conventional autonomic tests in the diabetic group

		Number of ANS symptoms positive				
		0	1	2	3	4
Any cardiac parasympathetic test	Positive (n=11)	2	2	6	0	1
	Negative (n=6)	0	2	1	2	1
Any cardiac sympathetic test	Positive (n=15)	0	4	7	2	2
	Negative (n=2)	2	0	0	0	0

Number of sample with autonomic symptom response=17, ANS: Autonomic nervous system

fibers before the sympathetic nerve fibers.[16] The heart rate variability data suggest that autonomic modulation of the heart is affected in long standing diabetes; while there was a reduction in both the high-frequency (conventionally suggestive of cardiac parasympathetic) and low-frequency (conventionally suggestive of cardiac sympathetic) power spectra of heart rate variability, it is unclear how this should be interpreted since the data were no different between the two groups when these were normalized for total power. Given that a reduction in heart rate variability indices has been linked to increased all-cause and cardiac mortality,[9,17,18] it is tempting to suggest that patients with diabetes who have a low HRV are at an increased risk of mortality. A practical problem with the clinical application of HRV, however, is that normal cutoffs have not been established, although there is general consensus on how HRV should be assessed and reported.[14] It is also not known whether HRV indices improve in patients with diabetes with better glycemic control or who adopt life style behaviors such as increased physical activity, which can independently affect HRV in otherwise healthy individuals.[19-21]

Patients with diabetes in this study were older individuals with long standing diabetes. However, aging also reduces autonomic responsiveness HRV[22,23] and it will be interesting to determine whether studies such as this one but performed in younger individuals might better be able to uncover subtle autonomic deficits without the confounding influence of aging. While HRV indices in the frequency domain have been the subject of considerable interest in research settings, there is a need for continued research using simple clinical indices of HRV such as time domain indices[24,25] or indices derived from the ubiquitous 12 lead ECG,[26] since these are likely to be used by clinicians in their daily practice. The absence of large datasets on autonomic nervous dysfunction in diabetes, contrary to that available for complications such as retinopathy, nephropathy and peripheral neuropathy is a reflection of the difficulties of autonomic testing in a routine clinical setting as well as perhaps the inadequate

priority accorded to autonomic symptoms by the standard practicing physician.[27,28]

Studies using heart rate variability in a large population have demonstrated similar changes of reduced heart rate variability measures, reported in the present study. However, data on autonomic changes especially in an Indian diabetic population are very limited. A study on 631 individuals aged 50-75 years of the town of Hoorn, in the Netherlands, indicated that HRV (measured using R-R interval, SDNN, low-frequency power (LF), and high-frequency power (HF) from 3-min records) was lower among diabetic subjects compared with those with normal fasting glucose, after adjusting for age and sex.[29] In the Framingham Study of 1919 individuals, HRV, as measured using SDNN, LF, HF, and LF/HF from 2-hour recordings, was again lower among diabetic subjects.[30] In a subset of 1933 individuals from the Atherosclerosis Risk in Communities (ARIC) study cohort, HF from 2-minute records was lower among diabetic than nondiabetic subjects, and there was an inverse association between HF and fasting insulin.[31] In a study using the full ARIC cohort, individuals with increased heart rate or decreased LF were at an increased risk of developing diabetes.[32] Though a reduction in heart rate variability measures have been demonstrated consistently in established diabetes, what is not evident is at what point in the natural course of diabetes these measures of HRV were performed. It is our view that HRV should be incorporated in the routine screening process in a diabetic patient and it should be performed on a timely basis and as early as possible. In situations where spectral analysis is not possible, HRV from a simple 12 lead ECG is a possible alternative as we have demonstrated earlier,[26] although this would need to be evaluated prospectively in diabetics.

In conclusion, data from the current study demonstrated that diabetics had cardiac sympathetic and cardiac parasympathetic nervous system involvement. The presence of symptoms and the involvement of both components of the autonomic nervous system suggests that autonomic dysfunction has been present for a while in these diabetics.

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