Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2017; 5(8D):3248-3256 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2017.v05i08.052

Original Research Article

Clinical Assessment of the Autonomic Nervous System in Type 2 Diabetes Mellitus and Its Correlation with Glycemic Control, Duration and Microalbuminuria

Honnutagi RM[1*], Uday Bhanu C[2], Biradar MS[3], Mantur PG[4], Patil SS[5]
[1]Professor, Department of General Medicine, Sri B.M.Patil Medical College hospital & Research centre, Bijapur, Karnataka (India)
[2]Junior Resident Department of General Medicine, Sri B.M.Patil Medical College hospital & Research centre, Bijapur, Karnataka (India)
[3]Professor, Department of General Medicine, Sri B.M.Patil Medical College hospital & Research centre, Bijapur, Karnataka (India)
[4]Associate Professor, Department of General Medicine, Sri B.M.Patil Medical College hospital & Research centre, Bijapur, Karnataka (India)
[5]Assistant Professor, Department of General Medicine, Sri B.M.Patil Medical College hospital & Research centre, Bijapur, Karnataka (India)

***Corresponding author**
Dr. Honnutagi, R.M,
Email: drhonnutagi@gmail.com

Abstract: The Clinical assessment of the autonomic nervous system in Diabetes mellitus (DM) and its correlation with glycemic control, duration and microalbuminuria, Cross sectional study of 96 adult diabetes patients. Ninety six patients with DM who were on regular treatment with either insulin and/or oral hypoglycemic agents were studied. Cardiovascular autonomic neuropathy (CAN) score was calculated using the clinical test variables. Of the 96 patients 45 had no CAN, 31 had early CAN and 20 had severe CAN. The mean HbA_{1C} was 7.83.The mean of CAN score increased with duration of diabetes. The mean CAN score was higher in patients who had complication of diabetes as compared to patients without complications. The heart rate variability with respiration was found to be 15.84 ± 7.02/min. The mean valsalva ratio was 1.31 ± 0.23.The mean 30:15 ratio was 1.06 ± 0.04.The mean drop in BP on standing was 7.30 ± 7.24 mmHg. The mean rise in diastolic BP on sustained hand grip was 16.04 ± 4.11 mmHg. The prevalence of autonomic neuropathy in DM as assessed by CAN score was 45%.The CAN score did not correlate with the duration of DM. The HbA_{1C} had a significant correlation with the severity of autonomic neuropathy. Occurrence of CAN correlated with the presence of peripheral neuropathy but not with the presence of retinopathy or nephropathy. All individual tests in the battery of CAN score were significantly associated with the presence of autonomic neuropathy, except 30:15 ratio.
Keywords: Cardiovascular autonomic neuropathy, diabetes mellitus, heart rate variability, postural hypotension, valsalva ratio

INTRODUCTION

Diabetic autonomic neuropathy (DAN) significantly affects the quality of life and survival in patients with DM [1,2]. The prevalence rate of DAN varies from 7.7% of newly diagnosed cases to 90% in advanced cases of DM [3]. DAN may be manifested by clinical or subclinical dysfunction of one or more organ system [4]. Extensive clinical investigation of Cardiovascular autonomic neuropathy (CAN) has been performed in the last 20 years due to the availability of several simple non-invasive tests for cardiac autonomic function [5,6]. In our study we attempt to assess the cardiovascular autonomic nervous system in patients with diabetes and correlate it with duration and glycemic control.

MATERIALS AND METHODS

Ninety six type 2 Diabetic patients attending the department of general medicine, Sri B.M.Patil Medical College hospital & Research centre, Bijapur, Karnataka (India) between October 2011 to sept 2013 were included in this study.

INCLUSION CRITERIA

- 2 DM patients who were on regular treatment with insulin and oral hypoglycaemic agents OHA were studied.

EXCLUSION CRITERIA

- Patients on drugs which interferes with autonomic nervous system (ANS) like β blocker tri-cyclic antidepressants etc.
- Patients with acute complications of DM
- Patients with established disease of other major organs.

All the subjects were evaluated with a detailed clinical history, clinical examination and following clinical tests for autonomic neuropathy [1,7].

Heart rate variability in response to deep breathing [1]

The patient is connected to the Electrocardiography (ECG) monitor, lies quietly and breathes deeply at a rate of six breaths per minute Fifteen beats per minute difference or more is normal and 10 beats/min or less abnormal.

Heart rate variability in response to standing [8]

The patient is connected to ECG monitor while lying down and then stands to a full upright position. ECG tracings are used to determine the 30:15 ratios, calculated as the ratio of the longest R-R interval during beats 20-40 to the shortest R-R interval during beats 5-25 **[9]**

Heart rate response to valsalva maneuver [1]

The supine patient, connected to an ECG monitor forcibly exhales for 15 seconds against a fixed resistance (40 mmHg) with an open glottis. The Valsalva ratio is determined from the ECG tracings.

Postural hypotension

Defined as a fall in systolic BP of ≥20 mmHg or diastolic BP of ≥10 mmHg accompanied by symptoms [10]

BP response to sustained hand grip exercise

The patients is asked to squeeze the handgrip dynamometer to isomeric maximum, and then held at 30% maximum for 5 min. A rise in DBP of ≤16 mmHg in the contralateral arm is considered abnormal [1].

ANALYSIS

DAN score values obtained from the various tests were tabulated into a worksheet and a scoring system was applied to each test as recommended by Bellavere *et al.* [11] (Table 1).

Table-1: Bellavere's scoring system

Test	*Score*		
	0 (Normal)	*1 (Borderline)*	*2 (Abnormal)*
Heart rate variability	>15	10-15	<10
Valsalva ratio	≥1.21	1.11-1.20	≤1.10
30:15	≥1.04	1.01-1.03	≤1.00
BP response to standing (mmHg)	≥10	11-29	≥30
BP response to hand grip (diastolic BP) (mmHg)	≤16	11-15	≤10

The sum of the score obtained from each test determined the final classification of the patient's degree of cardiac autonomic neuropathy (CAN). Classification of patients was done according to the total score (Table 2)

Table-2: CAN scoring system

CAN score	Categories
0-1	No autonomic neuropathy
2-4	Early autonomic neuropathy
5-10	Severe autonomic neuropathy

CAN=Cardiovascular autonomic neuropathy

RESULTS

The study comprised of 96 patients of DM meeting the inclusion criteria. The mean age of the patients was 47.64 years, youngest patient being of 23 years and oldest being 66 years. Maximum numbers of patients were in the age group 40-60 years (64%). Thirty nine (40.6%) patients were females and 57 (59.4%) were males; there was no difference in the mean age between the two sexes.

The mean duration of diabetes in our study was 8.4 ± 3.8 years (Females 7.82 ± 2.08, Males 8.11 ± 3.1 years). The commonest symptom seen in our study was postural giddiness (44%) other less common symptoms included urgency, frequency (32%), constipation and diarrhea (24%).

CAN score was applied to all our patients, 45 patients did not have cardiac autonomic neuropathy, 31 had early cardiac autonomic neuropathy and 20 had severe cardiac autonomic neuropathy. The mean CAN score of all patients was 2.04 (Females 1.98, Males 2.26). There was no significant correlation between CAN score and gender of the patient (*P* value of 0.32). The mean CAN score in patients with diabetes of less than four years duration was 1.282; in patients with diabetes of 4-6 years duration was 1.421 and in patients with diabetes of >6 years duration was 2.326. This observation was statistically not significant.

Diabetic nephropathy as evidenced by albuminuria was present in 47 (%) patients, 43 (40%) had micro albuminuria and 6 (4%) had macro albuminuria. Diabetic retinopathy as evidenced by ophthalmoscopy examination was present in 57 (59.3%) patients. Peripheral neuropathy as evidenced by clinical examination and symptoms was present in 51 (53.1%) patients.

The mean CAN score in patients with retinopathy was 2.806, while in those patients, without retinopathy it was 0.789, the mean CAN score of patients with micro albuminuria was 3.250, while in those with macro albuminuria it was 4.0 and in those without albuminuria it was 1.036. Both the observations were not statistically significant.

However very significant correlation was found when the mean CAN score was compared between patients with peripheral neuropathy and those without, i.e. mean CAN score of 3.50 and 0.893 respectively.

CAN scores versus individual variables: CAN score versus heart rate variability (HRV). The mean HRV was found to be 15.8 ± 7.02/min, HRV with respiration had a significant negative correlation with CAN score (R value of -0.808; *P* value 0.001) (Figure 1). CAN score versus valsalva ratio (VR). The mean valsalva ratio was 1.31 ± 0.23, valsalva ratio had a very significant negative correlation with CAN score (R value of -0.540; *P* value 0.001) (Figure 2). CAN score versus BP on standing (BPS). The mean drop in systolic BP on standing was 7.30 ± 7.24 mmHg. Drop in BP on standing had a significant positive correlation with CAN score (R value +0.753; *P* value 0.001) (Figure 3). CAN score versus hand grip response to BP (HGBP). The mean rise in diastolic BP on sustained hand grip was 16.04 ± 4.11 mmHg. HGBP had a significant negative correlation with CAN score (R value -0.779; *P* value 0.001) (Figure 4). Heart rate variability in response to standing. The mean value of 30:15 ratios was 1.06 ± 0.04. 30:15 ratio did not have a significant correlation with CAN score. Glycemic control in our study was assessed using HbA_{1c}, the mean HbA_{1c} in our study was 7.73. There was a highly significant correlation between HbA_{1c} and CAN score (*P* value 0.035) (Figure 5).

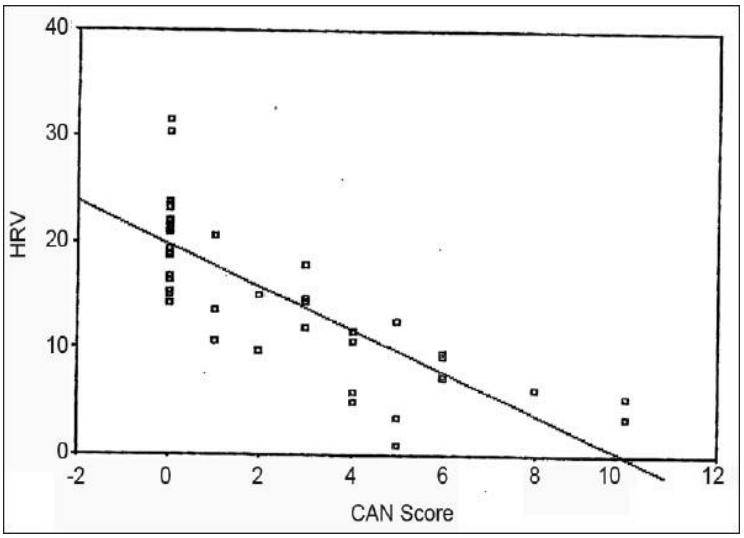

Fig-1: Cardiovascular autonomic neuropathy score vs. heart rate variability

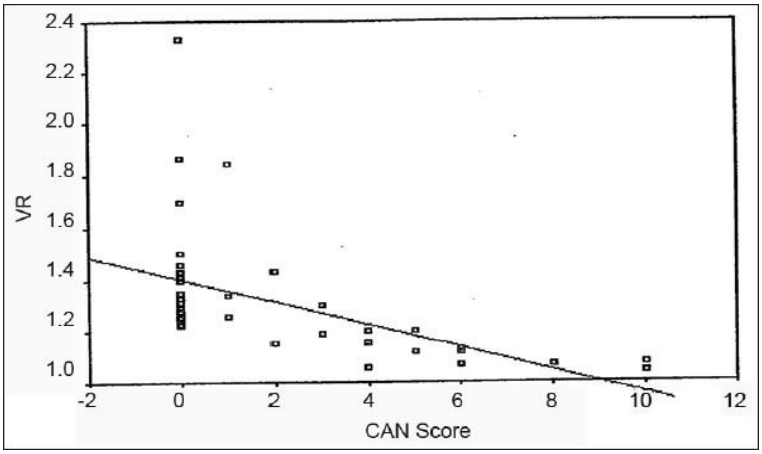

Fig-2: Cardiovascular autonomic neuropathy score vs. valsalva ratio

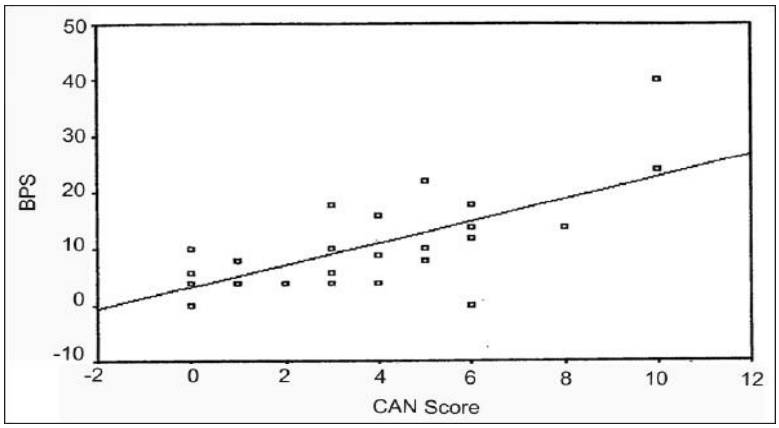

Fig-3: Cardiovascular autonomic neuropathy score vs. BP on standing

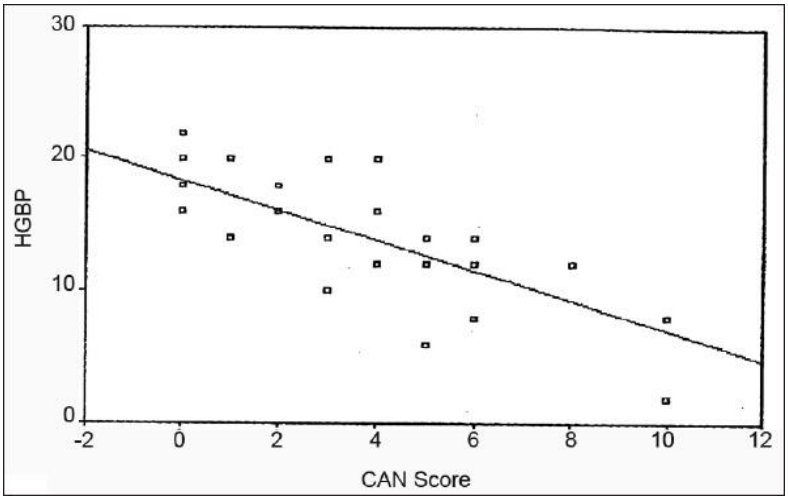

Fig-4: Cardiovascular autonomic neuropathy score vs. hand grip response to BP

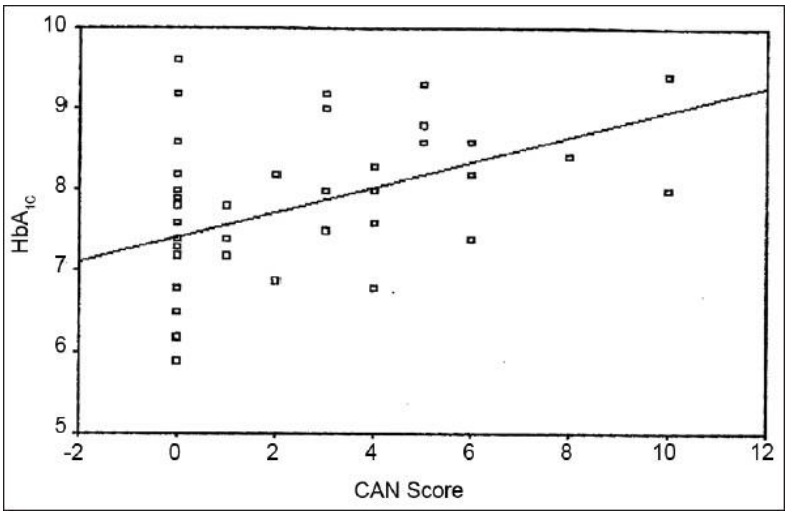

Fig-5: Correlation between HbA1C and Cardiovascular autonomic neuropathy score

DISCUSSION

Diabetic autonomic neuropathy is a chronic, clinically significant but frequently unnoticed complication of diabetes, which steals the patients of their ability to sense hypoglycemia or the chest pain of a heart attack. This in turn leads to significant morbidity and mortality.

The reported prevalence of diabetic autonomic neuropathy varies depending on whether studies have been carried out in the community, clinic or tertiary referral center [12]. The prevalence rates of CAN in several different studies, shows a dramatic variability from a low of 7.7% for newly diagnosed patients with type 1 diabetes, when strict criteria to define CAN were used, to a high of 90% in potential recipients of a pancreas transplant [3].

A number of biochemical mechanisms, including non-enzymatic glycosylation, increase in oxidative stress, neuro- inflammation and activation of the polyol and protein kinase c (PKC) pathways, contribute to the development of diabetic neuropathy. The length of time that nerve fibers are exposed to hyperglycemia and the degree of glycemic variability are critical in up regulating these biochemical pathways. Perhaps one of the most overlooked of all serious complications of diabetes is cardiac autonomic neuropathy [13]. CAN results from damage to the autonomic nerve fibers that innervate the heart and blood vessels and results in abnormalities in heart

control and vascular dynamics [14]. Reduced heart rate variation is the earliest indicator of CAN [15, 16].

In a review of several epidemiological studies among individuals diagnosed with diabetes, it was shown that five-year mortality rate from this serious complication is five times higher for individuals with CAN than for individuals without cardiovascular autonomic involvement [17].

Dysfunction of the ANS is associated with increased risk of mortality in individuals with diabetes. Though the exact pathogenic mechanism is unclear, it is realized that some deaths may be avoidable through early identification of these high-risk patients. In the early 1970s, Ewing *et al.* [7] proposed five simple noninvasive cardiovascular reflex tests (i.e., Valsalva maneuver, heart rate response to deep breathing, heart rate response to standing up, blood pressure response to standing up, and blood pressure response to sustained hand grip) that have been applied successfully by many. The consensus statement published by the expert panel at the 1988 San Antonio Conference recognized strong evidence for three tests of heart rate control (mainly tests of parasympathetic control). The three tests recommended were heart rate response to [1] deep breathing, [2] standing, and the [3] valsalva maneuver. Two tests of blood pressure control were also recommended: blood pressure response to [1] standing or passive tilting and [2] sustained hand grip. These tests were judged suitable for both routine screening and monitoring the progress of autonomic neuropathy [3].

In a study done by Khandelwal *et al.* [18] on diabetic autonomic neuropathy, the mean age of patients was 45.36. Roy *et al.* [19] in a study on autonomic influence of cardiovascular performance in diabetic subjects, found the mean age to be 48.9, Noronha *et al.* [20] studied Indian diabetics and found the mean age 57.8 yrs. Ewing DJ *et al.* [21] in their study of autonomic neuropathy in male diabetic patients found increasing CAN score with age. Similar findings were found by Toyry JP *et al.* [22]. However, in our study there was no significant correlation between CAN score and age of patient (P value -0.32).

In our study, 40.6% of patients were females and 39.4% were males. There was no difference in mean age between two sexes. Noronha JL *et al.* [20] this study had 32% females and 68% males; Roy TM *et al.* [19] studied the prevalence of autonomic neuropathy with 40% females and 60% males, in both the above studies, there was no significant difference in the mean age between the two sexes as in our study. In our study the mean duration of diabetes mellitus was 7.41 ± 3.8 years. The mean duration of diabetes mellitus in females was 6.82 ± 2.08 and in males was 7.42 ± 3.1. This matches with the studies done by Keen *et al.* [23] the mean duration of diabetes being 8.2 ± 2.6 years, and Noronha *et al.* [20] where in, the mean duration of diabetes was 7.2 ± 2 years.

The study done by Roy *et al.* [19] showed that cardiovascular symptoms were the commonest (52%) while urological, gastro-intestinal symptoms were less common (20%). In our study we found prevalence of CVS symptoms urologic symptoms and GIT symptoms were 44%, 32% and 24% respectively. Similar findings were noted by Noronha *et al.* [19] sudomotor symptoms were present in both the above studies with a percentage ranging from 6-16%, however in our study; we did not find any patients with sudomotor symptoms.

CAN score was calculated for all our patients, 40% of patients had no autonomic neuropathy, 20% had early autonomic neuropathy and the rest had severe autonomic neuropathy. Keen *et al.* [23] in his study found the prevalence of autonomic neuropathy in 32% of the patients, Noronha *et al.*, [20] found the prevalence of autonomic neuropathy in 38.5% of the patients with 11% of patients having severe autonomic neuropathy. Toyry *et al.*, [22] found the prevalence of autonomic neuropathy in 22% of the patients. Mean CAN score of our patients was 2.04, with males having CAN score of 2.26 and females 1.98, similar observation were found by Noronha *et al.* [20] in his study, where he found the mean CAN score was 2.23. Roy *et al.* [19] found the mean CAN score in diabetic males to be 2.11.

When we correlated the mean CAN score with the duration of diabetes, we found the mean CAN score was higher in diabetics of more than 6 years duration however, this observation was not statistically significant. This is in contrast to the study done by Toyry *et al.* [22] Whereas Noronha *et al.* [20] found increasing mean CAN score with duration of diabetes but the observation was not statistically significant, like in our study.

We found out that the mean CAN score correlated poorly with the presence of retinopathy or nephropathy. However significant correlation was found when the mean CAN score was compared between patients with peripheral neuropathy and those

without i.e., mean CAN score of 3.50 and 0.893 respectively.

Resting tachycardia which is regarded as the characteristic feature of cardiac denervation was present in patients with severe CAN, who had an average heart rate of 99.8 ± 12.3 beats/min which was significantly higher than the heart rate of patients without CAN ($P < 0.001$). However none of our patients with early CAN have resting tachycardia. Hence it can be considered a manifestation of severe CAN. Patients with severe CAN had a HRV of 18.7 ± 3.6/min and those with early CAN had a HRV of 12 ± 5.4/min. Similar observations were shown by Toyry *et al*. [24].

The DCCT trial [24] found that 1.6% of patients with a five year history of DM had HRV, the rate rose to 6.2% of those with a 5 year history of DM, and to 12% in those who had the disease for more than 9 years. In response to valsalva maneuver HRV is effort dependent, hence we used a manometer to standardizes the valsalva maneuver. We found that the patients with early and severe CAN had a valsalva ratio of 1.33 ± 0.24 and 1.11 ± 0.1 respectively. As the severity of CAN increased the heart rate variability in response to valsalve maneuver decreased. Levitt *et al*. [25] in 1996 concluded that valsalva ratio may be the best method to monitor the progression of autonomic dysfunction in diabetes. Our study showed that the patients with severe CAN had little variation of the RR intervals on standing with an average 30:15 ratio of 1.06 ± 0.04, the ratio was 1.12 ± 0.10 in patients with early CAN. We found that postural hypotension was a late feature of CAN. The patients with severe CAN had an average fall of systolic BP of 16.4 ± 11.11 mmHg. This was also noted by Toyry *et al*. in their study [22]. The patients with severe CAN showed a rise of 10.93 ± 4.83 mm Hg in the diastolic BP in response to sustained handgrip. Patients with early CAN showed a rise of 13 ± 4.51 mm Hg. Similar observation were also noted by, Toyry *et al*. [22] in their study.

The mean HbA_{1c} in our study was 7.73. We found a significant correlation between HbA_{1c} and the degree of cardiac autonomic neuropathy (P value 0.035). Mustonen *et al*. [26] showed in a 4 year follow up study of 32 subjects with type 2 diabetes that poor glycemic control was an important determinant of the progression of autonomic nerve dysfunction. Similarly DCCT (24) provided extensive clinical evidence that good metabolic control of diabetes mellitus reduces diabetic complications specifically DAN. However Khandelwal *et al*. [18] found a poor correlation of the HbA with the CAN score.

In our study we have made an earnest effort to quantify autonomic dysfunction by using the CAN score and also analyze the individual parameters of the entire battery. Handgrip response to blood pressure and HRV with respiration was found to be more sensitive (sensitivity of 74 and 80% respectively). Regarding safety of testing procedures an expert panel from the American Academy of Neurology (AAN) reviewed a number of standardized measures and found that noninvasive autonomic tests were found to have a high value- to- risk ratio [27]. The Valsalva maneuver transiently increases intrathoracic, intraocular and intracranial pressure, creating for example, a small theoretical risk of intraocular hemorrhage and lens dislocation. In the published literature of over 100 studies, there have been no reports of deaths and adverse event due to the procedures.

Tests for autonomic function based on the changes in the heart rate variations and blood pressure regulation can detect cardiovascular complications at early stages of involvement in asymptomatic patients offering a quick diagnosis and subsequent therapy. Vinik *et al*. [12] has observed that power spectral analysis of 24 hour ECG recording may be more sensitive in detecting CAN than simple tests in the early stage of CAN. Regular HRV testing provides early detection and thereby promotes timely therapeutic intervention. Vinik *et al*. [28] recommended that measurement of HRV at the time of diagnosis of type 2 DM and within 5 years of diagnosis of type 1 DM serves as a baseline, with which 1 year interval tests can be compared.

The limitations of our study were that we have used the criteria suggested by Bellavere which test mainly for the parasympathetic component of the ANS to identify the patients with CAN. Probably using tests for sympathetic component also as suggested by Ewing *et al*. [29] along with tests for the parasympathetic component would have helped to identify more patients with CAN. Results would have been more appropriate if sample size could be more and if study design was a prospective study unlike in ours where sample size was small and design was a cross-sectional study.

CONCLUSION

The prevalence of autonomic neuropathy in diabetes mellitus as assessed by CAN score was 40%.

The mean CAN score did not correlates the age and sex of the patient or the duration of diabetes. The glycemic control as assessed by HbA_{1c} had a significant correlation with the severity of autonomic neuropathy i.e. patients with poor glycemic control had a higher CAN score. Occurrence of cardiac autonomic neuropathy correlated with the presence of peripheral neuropathy, however no significant correlation was found with retinopathy and neuropathy. All individual tests in the battery of CAN score were significantly associated with the presence of autonomic neuropathy, except 30:15 ratio.

REFERENCES

1. Vinik AI, Erbas T. Recognizing and treating diabetic autonomic neuropathy. Cleve Clin J Med 2001;68:928-44.
2. Freeman R. The peripheral nervous system and diabetes. Joslin's Diabetes Mellitus. In: Weir G, Kahn R, King GL, editors. Philadelphia: Lippincott; 2002. p. 804-7.
3. Kennedy WR, Navarro X, Sutherland DE. Neuropathy profile of diabetic patients ia a pancreas tramsplantation program. Neurology 1995;45:773-80;25.
4. American Diabetes Association and American Academy of Neurology: Report and recommendations of the San Antonio Conference on diabetic neuropathy. Diabetes 1988;37:1000-4.
5. Ewing DJ. Cardiovascular reflexes and autonomic neuropathy. QJ Med 1978;55:321-7.
6. Clarke BF, Ewing DJ, Campbell IW. Diabetic autonomic neuropathy. Diabetologia 1979;17:195-212.
7. Ewing DJ, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years' experience in diabetes. Diabetes Care 1985;8:491-8.
8. Swing DJ, Campbell IW, Murray H, Neilson JM, Clarke BF. Immediate heart-rate response to standing: Simple test for autonomic neuropathy in diabetes. Br Med J 1978;1:145-7.
9. Ziegler D, Laux G, Dannehl K, Spüler M, Mühlen H, Mayer P, Gries FA. Assessment of cardiovascular autonomic function: Age-related normal ranges and reproducibility of spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses. Diabet Med 1992;9:166-75.
10. Position paper: Orthostatic hypotension, multiple system atrophy (the Shy Drager syndrome) and pure autonomic failure. J Auton Nerv Syst 1996;58:123-4.
11. Bellavere F, Bosello G, Fedele D, Cardone C, Ferri M. Diagnosis and management of diabetic autonomic neuropathy. Br Med J 1983;287:61.
12. Vinik AI, Mitchell BD, Maser RE, Freeman R. Diabetic autonomic neuropathy. Diabetes Care 2003;26:1553-79.
13. Maser RE, Lenhard MJ, DeCherney GS. Cardiovascular autonomic neuropathy: The clinical significance of its determination. Endocrinologist 2000;10:27-33.
14. Schumer MP, Joyner SA, Pfeifer MA. Cardiovascular autonomic neuropathy testing in patients with diabetes. Diabetes Spectr 1998;11:227-31.
15. Ziegler D. Diabetic Cardiovascular Autonomic Neuropathy: prognosis, diagnosis, and treatment.Diabetes Metab Rev 1994;10:339-83.
16. Ziegler D. Cardiovascular autonomic neuropathy: clinical manifestations and measurement. Diabetes Rev 1999;7:300-15.
17. American Diabetes Association and American Academy of Neurology: Proceedings of a consensus development conference on standardized measures in diabetic neuropathy. Diabetes Care 1992;15:1080-107.
18. Khandelwal E, Ashok KJ, Kishore KD. Pattern and prevalence of cardiovascular autonomic neuropathy in diabetics visiting a tertiary care referral centre in India, Indian J Pharmacol 2011;55:19-27.
19. Roy TM, Peterson HR, Snider HL, Cyrus J, Broadstone VL, Fell RD, *et al*. Autonomic influence on cardiovascular performance in diabetic subjects. Am J Med 1989;87:382-8.
20. Noronha JL, Bhandarkar SD, Shenoy PN, Retnam VJ. Autonomic neuropathy in diabetes mellitus. J Postgrad Med 1981;27:1-6.
21. Ewing DJ, Campbell IW, Clarke BF. Assessment of Cardiovascular effects in diabetic autonomic neuropathy and prognostic implications: Ann Int Med 1980;92:308-11.
22. Toyry JP, Niskanen LK, Lansimies EA, Partanen KP, Uusitupa MI. Autonomic neuropathy predicts the development of stroke in patients with non-insulin-dependent diabetes mellitus. Stroke l996;27:1316-8.
23. Keen H. Autonomic neuropathy in diabetes mellitus. Postgrad Med J 1959,35: 272-80.
24. DiabetesControl and Complications Trial Research Group. The effect of intensive diabetes therapy on measures of autonomic nervous system functions in the Diabetes Control and Complications Trial (DCCT). Diabetologia 1998;41:416-23.

25. Levitt NS, Stansberrry KB, Wynchank S, Vinik AI. The natural progression of autonomic neuropathy and autonomic function tests in a cohort of people with IDDM. Diabetes Care 1996;19:751-4.
26. Mustonen J, Uusitipa M, Mantysaari M, Länsimies E, Pyörälä K, Laakso M. Changes in autonomic nervous function during the 4 year follow-up in middle-aged diabetic and non diabetic subjects initially free of coronary heart disease. J Intern Med 1997;241:227-35.
27. American Academy of Neurology Therapeutics and Technology Assessment Subcommitteee Assessment: clinical autonomic testing report.Neurology 1996;46:873-80.
28. Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. Circulation 2007;115:387-97.
29. Ewing DJ, Clarke BF. Diagnosis and management of DAN. Br Med J 1982;285:916-8.