Indian Heart Journal 69 (2017) 52–56

Contents lists available at ScienceDirect

Indian Heart Journal

journal homepage: www.elsevier.com/locate/ihj

Original Article

Comparative study of cardiac autonomic status by heart rate variability between under-treatment normotensive and hypertensive known type 2 diabetics

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ARTICLE INFO

Article history:
Received 17 November 2015
Accepted 27 July 2016
Available online 2 August 2016

Keywords:
Dysautonomia
Heart rate variability
Hypertension
Normotensive
Type 2 diabetes

ABSTRACT

Background: Co-existence of hypertension is known in three quarter of Indian type 2 diabetics, this duo having adverse additive effect on cardiovascular health including dysautonomia. Latter can be measured by simple 5 min heart rate variability (HRV) using simple electrocardiogram, which if reduced indicates cardiac risk.

Objective: We compared HRV parameters between hypertensive and normotensive type 2 diabetics, looking for significant difference if any.

Materials and methods: 98 hypertensive and 40 normotensive type 2 diabetics treated as outpatients were evaluated for disease control and risk stratification. Five min resting HRV was measured by Variowin HR, software based instrument, using standard protocols to record time domain, frequency domain and Poincare plot parameters. They were compared between groups for difference.

Results: Mean age was 56 and 51 years, duration 6 years and 4 years respectively in hypertensive (HT) and normotensive (NT) group of type 2 diabetics, which did not significantly differ in distribution of risk factors. There was poor glycaemic control (one third) in both groups and good pressure control in HT group. Both groups revealed all reduced HRV parameters with significant difference in-between only for LF/HF ratio (1.29 in HT vs 2.61 in NT group).

Conclusion: Our findings of HRV suggest that in type 2 diabetics with poor glycaemic and good pressure control, hypertension as a co-existing factor does not make significant difference in cardiac dysautonomia emphasizing residual risk despite antihypertensive treatment and need for early HRV screening, strict glycaemic control and other interventions.

1. Introduction

India stands second globally in type 2 diabetes mellitus (T2DM) with an alarming future prediction[1] and there is co-existence of hypertension (HTN) and T2DM in 75% cases.[2] Taken together they have a threatening synergistic effect[3] with majority having sub-optimal disease control[4,5] In an earlier study we have also demonstrated a similar relation.[6] Cardiac autonomic neuropathy is common yet overlooked complication which contributes to residual risk for cardiovascular morbidity and mortality.[7] Heart rate variability (HRV), measured by simple 5 min recording provides reliable status of cardiac autonomic balance.[8] Reduced HRV is seen in both HTN and T2DM individually and known to be an independent risk factor for cardiovascular health.[9] But only few studies like Takahashi et al.[10] have focused their synergistic effect, perhaps none from India. Indian hypertensives are very peculiar[4] and we undertook this study to assess the effect of coexisting hypertension on HRV in known type 2 diabetics.

2. Materials and methods

2.1. Study design

This case control study was conducted in the Department of Medicine with the help of Department of Physiology, Government Medical College, Bhavnagar, Gujarat, India during a period from 15th October 2014 to 15th January 2015.

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http://dx.doi.org/10.1016/j.ihj.2016.07.013

2.2. Study subjects

After approval from the Institutional ethical Committee and written informed consent from each subject, volunteers were recruited for this study. Of total number of subjects that attended the Out Door Clinic, all the adult subjects were screened for presence of type 2 diabetes. Subjects coming to clinic with record of treatment of diabetes were also included in this screening for confirmation. Of total number of patients with type 2 diabetes, observed during the recruitment period ($n = 300$), 138 patients were randomly selected for this study. Sample size was calculated by software Raosoft (Raosoft, Inc. free online software, Seattle, WA, USA). A sample of 138 subjects for a population of 6 lakhs with 7.33% prevalence of type 2 diabetes mellitus in our region gave us 95% confidence level, leaving 5% margin of error.

2.3. Inclusion and exclusion criteria

We included type 2 diabetic patients, with minimum duration of one year and known glycaemic control, aged 30–70 years, of either sex, taking regular treatment (through chart review), not taking insulin, ready for written consent.

Exclusion criteria were; those patients with less than one year duration of diabetes ($n = 12$), taking irregular treatment of diabetes ($n = 18$), age more than 70 years ($n = 127$), patients having cancer ($n = 0$), chronic dysentery ($n = 0$), chronic renal failure ($n = 3$), type 1 diabetes ($n = 9$), on pace maker ($n = 0$), past history of intervention, drug therapy influencing autonomic function ($n = 11$) and non-volunteers ($n = 1$) were excluded.

2.4. Collection of data

All the data were collected by a personal interview by a trained physician via validated questionnaires that included symptoms of cardiac autonomic neuropathy, investigations done, treatment received, salt, alcohol and tobacco intake and physical activity

Specific emphasis was given to identify following 10 risk factors including diabetes itself: (1) hypertension, (2) hyperlipidaemia, (3) smoking, (4) cardiovascular disease (CVD), (5) family history, (6) age $>$ 52 years, (7) male gender, (8) fasting blood sugar (FBS) $>$130 mg/dL, (9) body mass index (BMI) $>$25 kg/m^2, (10) type 2 diabetes mellitus.

Salt intake was the sum of the salt used during preparation of food and added at the table by each subject.[11,12] Added ingestion was from salt added to manufactured foods was taken into account. The physician also measured body weight on each visit independently in light under clothing to the nearest of 0.1 kg, after removing shoes of the subjects. Height was measured without shoe by standing close to scale.

After a 5-min rest, a blood pressure was recorded in a sitting position, on the right arm with a standard mercury manometer. Every subject had two readings, with the average of these reading recorded as the resting blood pressure. To minimize measurement errors, one individual was assigned to measure blood pressure for all the subjects in both the groups. In accordance with the WHO guidelines, if a blood pressure of more than $\geq$140/90 mmHg was recorded, a repeat measurement was obtained after a 5-min rest, with the subject in a supine position.

Physical activity was assessed by a questionnaires detailing occupational, household, and spare time physical activity. Sedentary lifestyle assessment was based on occupational or household activity, along with spare time activity measures as reported earlier.[11] Alcohol and tobacco consumption were recorded by questionnaires.[12] Tobacco consumption was defined as tobacco intake, in any form, for example, chewing or smoking.[12] Alcohol intake was considered in presence of drinking of alcohol at least once per week.[12]

2.5. Definition of disease control

Prehypertension is diagnosed in presence of a systolic pressure from 120 to 139 mmHg or a diastolic pressure from 80 to 89 mmHg. Readings greater than or equal to 140/90 mmHg are considered hypertension.

Hypertension was also diagnosed as per self-reported use of medications and available records of treatment for high blood pressure during the 2 weeks preceding the clinic examination. Participants also brought to the examination all medications they had taken in the preceding 2 weeks.

We defined glycaemic control as per criteria laid by American Diabetes Association 2014[13] and good glycaemic control was defined as (1) HbA1c $\leq$ 7 mg%, (2) FBS $\leq$ 126 mg% and (3) PP2BS $\leq$ 180 mg%.

2.6. Measurement of HRV

The time domain variables and frequency domain variables were measured and taken for comparison by window based software VarioWin HR.[14] Assessment of heart rate variability was carried out between 8.30 and 12.00 am in an isolated examination room. Patients were requested to avoid coffee, tea, cola drinks and smoking for 12 h and alcoholic beverages for 24 h before procedure. We recorded ECG for the analysis of beat-to-beat heart rate variability after supine rest for at least 5 min while the subject was in supine position and breathing freely. The ECG was recorded from the precordial leads and transferred on-line to a microcomputer for the analysis of heart rate variability. Only stationary time series of approximately 5-min durations free of arrhythmia and artefacts were used.

2.7. HRV parameters

In time-domain analysis of HRV parameters included are RR interval, standard deviation of all RR intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent RR intervals (RMSSD), standard deviation of successive differences (SDSD) and pNN50, which is the percentage of consecutive RR intervals that differ by $>$50 ms.[15]

The frequency-domain analysis of HRV consisted of the power of high frequency (HF), (0.15–0.40 Hz); low frequency (LF), (0.04–0.15 Hz); and very low frequency (VLF), (below 0.04 Hz) power ranges. LF and HF were presented also in normalized units and as a ratio.[15]

Poincare plot analysis included SD1 and SD1 which are standard deviation of RR interval along major and minor axis respectively. Scatter index was represented as ratio of SD1 to SD2 which reflected the HRV in a non-linear manner.

2.8. Statistical analysis

The data was transferred on Excel spreadsheet and descriptive analysis was expressed as mean $\pm$ standard deviation. All calculations were accomplished by Graph Pad in Stat 3 software (demo version free software of GraphPad Software, Inc. California, USA). We calculated the statistical significance of difference in mean distribution of various parameters amongst various subgroups by Mann–Whitney test or unpaired student t test for quantitative data and by Fisher's exact test for qualitative data. Difference was considered statistically significant with $p < 0.05$.

3. Results

Two groups did not significantly differ in gender distribution, height, weight, BMI and glycaemic control. However, hypertensive diabetics (HTDM) had older age (57 years vs 52 years, $p = 0.005$), blood pressure (systolic 136 vs 124, diastolic 83 vs 79, mean 101 vs 94. $p < 0.05$ for all) and duration of type 2 diabetes (6 years vs 4 years, $p = 0.018$) as compared to normotensive ones (NTDM) yet pressure control was comparatively good (half having normal mean blood pressure) in former group (Table 1).

HTDM and NTDM groups had statistically insignificant difference of distribution of risk factors where high BMI, female gender, older age, positive family history and poor glycaemic control were present in large proportion of subjects in both groups (Table 2).

Comparison of time domain, frequency domain and non-linear parameters of heart rate variability revealed that there is reduced HRV in hypertensive (HTDM) and normotensive (NTDM) type 2 diabetics. Though reduced in both groups, NTDM group had better profile of frequency domain parameters than HTDM group (LFnu – 283 ms vs 262 ms, HFnu – 360 ms vs 262 ms, LF:HF ratio 2.61 vs 1.29, $p > 0.05$ for all) although they had higher heart rate (88 vs 83). Except for LF/HF ratio ($p = 0.002$) all parameters did not differ between HTDM and NTDM groups ($p > 0.05$) (Table 3).

Similarly, NTDM subjects, like HTDM subjects had reduced values but no significantly better profile of time domain HRV parameters (SDNN 24 vs 26, RMSSD 20 vs 21, SDSD 18 vs 17, NN50 13 vs 9, Triangular HRV index 13 vs 9, $p > 0.05$ for all) (Table 3).

Similarly, comparison of HRV parameters based on Poincare plotting revealed almost reduced but comparable results in NTDM and HTDM group (SD1 14 vs 13, SD2 26 vs 25, scatter index 0.48 vs 0.52, $p > 0.05$ for all), with all lacking statistical significance (Table 3).

4. Discussion

Type 2 diabetes mellitus (T2DM) has reached epidemic proportion in India[1] and hypertension (HTN) is prevailing in nearly one out of three urban Indian.[4] Co-existence of T2DM and

Table 1
Demographic and clinical characteristics of type 2 diabetic subjects under study ($n = 138$).

General features	HT group	NT group	HT vs NT	Total
	mean ± SD	mean ± SD	*p* value	mean ± SD
Age (years)	57.07 ± 8.36	51.61 ± 9.61	0.005*	55.5 ± 9.09
Gender – male/female/total	47/51/98	21/19/40	0.71	68/70/138
Duration of DM (years)	6.21 ± 5.38	3.97 ± 4.05	0.018*	5.66 ± 5.15
Height (cm)	159.5 ± 9.23	160.8 ± 9.54	0.083	160.58 ±9.74
Weight (kg)	67.5 ± 10.67	67.35 ± 10.31	0.76	67.50 ± 10.68
BMI (kg/m^2)	26.63 ± 4.59	25.59 ± 4.15	0.22	26.31 ± 4.51
Glycemic control – value	mean ± SD-HT	mean ± SD-NT	*p* value	mean ± SD
1. HbA1c (mg%)	8.17 ± 1.99	7.63 ± 1.5	0.53	8.10 ± 0.93
2. FBS (mg%)	158.14 ± 57.05	173.91 ± 66.97	0.28	162.0 ± 59.65
3. PP2BS (mg%)	251.13 ± 103.34	238.63 ± 89.73	0.69	245.76 ± 98.15
Glycemic control – prevalence	Number (%)	Number (%)	*p* value	Number (%)
1. HbA1c	15/39 (38.46%)	3/6 (50%)	0.67	18/45 (20%)
2. FBS	27/71 (38%)	7/23 (30.43%)	0.62	34/94 (37%)
3. PP2BS	23/75 (30.67%)	9/30 (30%)	1	32/105 (30%)
Blood pressure control – value	mean ± SD	mean ± SD	*p* value	mean ± SD
1. SBP (mmHg)	136.22 ± 19.2	124.24 ± 12.86	0.001*	132.52 ± 18.36
2. DBP (mmHg)	83.4 ± 9.68	78.83 ± 6.51	0.009*	82.08 ± 9.02
3. MBP (mmHg)	101.01 ± 11.95	93.81 ± 7.83	0.001*	93.87 ± 24.40
Blood pressure control – prevalence	Number (%)	Number (%)	*p* value	Number (%)
1. SBP	66/98 (67%)	40/40 (100%)	<0.0001*	106/138
2. DBP	80/98 (82%)	40/40 (100%)	0.018*	120/138
3. MBP	49/98 (50%)	40/40 (100%)	<0.0001*	89/138

* Indicates statistical significance.
Abbreviations: HT, hypertensive; NT, normotensive; BMI, body mass index; HbA1c, glycosylated haemoglobin A1c; FBS, fasting blood sugar; PP2BS, post prandial blood sugar; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure.

Table 2
Prevalence of various known risk factors for cardiovascular disease in hypertensive and normotensive type 2 diabetics (absolute number and percentage).

Risk factors	Prevalence (number)				
	Hypertensive ($n = 98$)		Normotensive ($n = 40$)		*p* value
	Present	Absent	Present	Absent	
1. Hyperlipidemia	23	75	4	36	0.09
2. Smoking	13	85	9	31	0.203
3. Alcohol	3	95	2	38	0.62
4. Known heart disease	29	69	9	31	0.52
5. Family history of DM	35	63	10	30	0.24
6. Age > 52 yaers	68	30	19	21	0.02*
7. Female gender	51	47	19	21	0.71
8. BMI > 25 kg/m^2	58	40	21	19	0.57
9. FBS > 126 mg%	44	54	16	24	0.7

* Indicates statistical significance.
Abbreviations: BMI, body mass index; FBS, fasting blood sugar.

Table 3
Quantitative comparison of HRV parameters between normotensive and hypertensive type 2 diabetics (mean ± SD).

HRV parameter	Hypertensive DM (mean ± SD)	Normotensive DM (mean ± SD)	*p* value
VLF power	407.85 ± 376.41	504.33 ± 565.78	0.81
LF power	262.1 ± 586.48	283.31 ± 313.59	0.52
HF power	262.68 ± 572.58	360.12 ± 807.49	0.7
LF(nu)	0.56 ± 0.18	0.61 ± 0.20	0.18
HF(nu)	0.43 ± 0.174	0.39 ± 0.20	0.2
Maximum LF	0.074 ± 0.081	0.26 ± 1.13	0.99
Maximum HF	0.28 ± 0.087	0.26 ± 0.086	0.13
LF/HF ratio	1.29 ± 1.29	2.61 ± 2.29	0.0002*
Heart rate	82.9 ± 14.98	88 ± 15.25	0.082
Mode value	731.09 ± 135.71	701.65 ± 138.16	0.26
Triangular HRV index	6.27 ± 6.89	5.84 ± 3.36	0.79
SDNN	26.07 ± 34.1	24.32 ± 17.42	0.83
RMSSD	21.084 ± 25.26	20.12 ± 19.91	0.98
SDSD	16.99 ± 20.5	17.46 ± 21.32	0.96
NN50 count	9.06 ± 24.71	13.87 ± 25.3	0.43
PNN50%	3.42 ± 10.82	4.21 ± 9.1	0.49
R–R interval	744.18 ± 135.58	705.8 ± 139.5	0.15
SD1	13.12 ± 13.13	14.29 ± 15.21	0.88
SD2	25.36 ± 13	26.23 ± 17.38	0.62
Scatter index	0.48 ±0.27	0.52 ± 0.33	0.68

* Indicates statistical significance.
Abbreviations: VLF, very low frequency; LF, low frequency; HF, high frequency; nu, normalized unit; SDNN, standard deviation of NN interval; RMSSD, root mean square of standard deviation; SDSD, standard deviation of standard deviation; SD1, standard deviation 1 along major axis; SD2, standard deviation 2 along minor axis.

HTN is seen in more than 75% Indians[1] and so that both should be screened and tackled simultaneously.

Diabetic autonomic neuropathy may affect many systems throughout the body, including the cardiovascular, gastrointestinal and genitourinary systems, with a variety of adverse outcomes including cardiovascular deaths due to silent myocardial ischaemia and cardiovascular autonomic neuropathy.[16] Cardiovascular autonomic neuropathy may cause abnormalities in heart rate control as well as in central and peripheral vascular dynamics that are linked to decreased heart rate variability, postural hypotension, exercise intolerance, enhanced intraoperative cardiovascular mortality, increased incidence of asymptomatic ischaemia, myocardial infarction, and decreased likelihood of survival after myocardial infarction, and thus increased overall mortality and morbidity in diabetes. Presence of hypertension further enhances the autonomic dysfunction.

We found poor glycaemic control, comparatively good blood pressure control and lack of use of life style modification interventions in our middle aged study group. We found that type 2 daibetics with or without hypertension had in general reduced total power, reduced time domain and frequency domain parameters of HRV. This is well supported by previous studies done in type 2 diabetics with[3] or without[17–20] hypertension. This can be explained by high mean age (in mid-50s),[21] mean duration of disease 5 years[8,15] and glycaemic control[7,13] which was optimum in just one third. T2DM and CAN are linked by inflammatory mediators[22] and better glycaemic control is a proven beneficial fact.[8] Yet in Indian context, strict glycaemic control is not promptly practiced and HbA1c, the gold standard of disease control is not available to the most,[1] just like our cases and our previous study in same population.[6] Five minute HRV recording can provide equal information as 24 h HRV.[23] Reduced HRV is either due to damage to cardiac autonomic nerves by hyperglycaemia of T2DM or by sympathetic over activity like one of HTN,[24] so we hypothetized better HRV profile in normotensive type 2 diabetics than hypertensive ones.

We found no difference in overall cardiac autonomic status in hypertensive diabetics as compared to normotensive diabetics. This is contradictory to few other western studies,[3] but Indian scenario of these two diseases could be different. CAN is seen even before inception of T2DM[25] and a recent study of HRV screening suggests that diabetics can be differentiated from normal controls on the basis of reduced HRV.[26] Similarly, T2DM itself could be the fore-runner of HTN wherein anti-hypertensive could cure only one affected variable–blood pressure. The same is indicated in our study, as mean age of hypertensives was 51 against that of normotensive diabetics 57 years and mean duration of T2DM was 4 years in normotensive vs 6 years in hypertensives. However, LF/HF ratio which signifies sympatho-vagal balance was significantly higher in normotensives compared to hypertensives though a recent study shows this ratio to be of less significance.[27] Result can be due to fact that hypertensive T2DM patients were offered calcium channel blockers or angiotensin converting enzyme inhibitor, both of which have anti-sympathetic effect that checks sympathetic over-activity.[28,29] Benefit of anti-hypertensive therapy can be evident if there is reduced resting heart rate[30] that was not much different in either group, being in 80s. Beta blockers have definite benefit as a drug restoring autonomic balance[30] but it is usually not given as monotherapy and was not given to any of our hypertensives. This may also explain lack of significant HRV difference between diabetics taking or not taking other anti-hypertensive monotherapy. Resting heart rate has a prognostic value as a risk factor and its higher value indicates poor control of cardiovascular risk.[31]

Autonomic functions are determined by sympathetic and parasympathetic nerves, the pituitary and pineal glands and the suprachiasmatic nucleus.[32] Western diet, exercise, tobacco and alcoholism can also cause autonomic dysfunction which may be associated with sympathetic activation predisposing atherosclerosis, end-organ damage, and hypertension.[33–36] It seems that diet and lifestyle factors can also influence autonomic function and may have an influence on HRV. Experimental studies indicate that high-fat-induced weight gain in rats elevates plasma leptin at 1–3 days after the onset of calorie-dense diets, and that diet-induced overfeeding may increase sympathetic activity within 1 week after the onset of the regimen. An unpublished part of this same work[37] has revealed no difference of HRV profile in type 2 diabetics with or without glycaemic or pressure control indicating that residual cardiac risk remains despite control of these two parameters and cardiac autonomic status must be screened and targeted for better cardiovascular outcome. CAN cannot be overlooked in type 2 diabetics, having poor glycaemic control as major risk that can be reduced by early diagnosis and strict glycaemic control and residual risk can be further reduced by life style interventions like weight reduction and exercise.[38] HRV screening is suggested to be used in all type 2 diabetics[39] and it can help in primary prevention of abnormal aftermath of CAN in T2DM. HRV can be used even by family physicians who are treating majority of type 2 diabetics and who can offer benefit of HRV screening to type 2 diabetics at the inception of disease and put appropriate preventive remarks in treatment as a whole.

5. Limitation of study

There were few limitations which are needed to be mentioned. First, the cross-sectional nature of study and moderate sample size warrants prospective vertical study with larger sample in subjects starting with baseline measurement followed serially. Present form of study cannot establish cause effect relationship. Second, we used five minute HRV that reveals only short term changes in cardiac autonomic status and 24 h HRV is required to further consolidate the results. Third, presence of confounding factors, absence of control and euglycaemic hypertensives, and reliance on manually measured blood pressure were also limiting our study. Still it underscored relatively small impact of co-existing

hypertension on cardiac autonomic status in type 2 diabetics and need of optimum control of later.

6. Conclusion

We found reduced HRV parameters in type 2 diabetics having poor glycaemic control which was not significantly affected by presence of its co-existing aftermath hypertension, though controlled and use of antihypertensive treatment excluding beta blockers. It suggests that reduced HRV can precede type 2 diabetes which itself is a fore-runner of hypertension and cardiac autonomic balance is not affected much by good pressure control but can be by good glycaemic control which along with residual cardiac risk despite it, are still left unexplored.

Conflicts of interest

The authors have none to declare.

References

1. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. *AMJ.* 2014;7(1):45–48.
2. Colosia AD, Palencia R, Khan S. Prevalence of hypertension and obesity in patients with type 2 diabetes mellitus in observational studies: a systematic literature review. *Diabetes Metab Syndr Obes.* 2013;6:327–338.
3. Mian C, Cuntai Z. Analysis of the heart rate variability in elderly patients with hypertension and type 2 diabetes mellitus. *Heart.* 2012;98(2):E1–E319.
4. Anchala R. Hypertension in India: a systematic review and meta-analysis of prevalence, awareness, and control of hypertension. *J Hypertens.* 2014;32(6):1170–1177.
5. Mohan V, Shah SN, Joshi SR, et al. Current status of management, control, complications and psychosocial aspects of patients with diabetes in India: results from the DiabCare India 2011 Study. *Indian J Endocr Metab.* 2014;18:370–378.
6. Solanki JD, Makwana AH, Mehta HB, Gokhale PA, Shah CJ. A study of prevalence and association of risk factors for diabetic vasculopathy in an urban area of Gujarat. *J Fam Med Primary Care.* 2013;2:360–364.
7. Dimitropoulos G, Tahrani AA, Stevens MJ. Cardiac autonomic neuropathy in patients with diabetes mellitus. *World J Diabetes.* 2014;5(1):17–39.
8. Pop-Busui R. Cardiac autonomic neuropathy in diabetes: a clinical perspective. *Diabetes Care.* 2010;33:434–441.
9. Thayer JF, Hansen AL, Saus-Rose E, Johnsen BH. Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann Behav Med.* 2009;37:141–153.
10. Takahashi N, Nakagawa M, Saikawa T, et al. Effect of essential hypertension on cardiac autonomic function in type 2 diabetic patients. *J Am Coll Cardiol.* 2001;38:232–237.
11. Singh RB, Ghosh S, Naiz MA, Rastogi V. Validation of physical activity and socioeconomic status questionnaire in relation to food intakes for the five city study and proposed classification for Indians. *J Assoc Phys India.* 1997;45:603–607.
12. Singh RB, Ghosh S, Niaz MA, Rastogi V, Wander GS. Validation of tobacco and alcohol intake questionnaire in relation to food intakes for the five city study and the proposed classification for Indians. *J Assoc Phys India.* 1998;46:387–391.
13. American Diabetes Association. Standards of medical care in diabetes – 2014. *Diabetes Care.* 2014;37(1):S14–S80.
14. VARIOWIN – Software for Spatial Data Analysis in 2D. *Computational Statistics & Data Analysis.* vol. 25, Issue 2. 1997;243–244.
15. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation.* 1996;93:1043–1065.
16. Neki NS, Ankur J, Bajaj R, Mohit M, Chavan V, Harsh Kumar HmrH. Cardiovascular autonomic disturbances in patients with diabetes mellitus. *World Heart J.* 2015;7:177–186.
17. Tarvainen MP, Laitinen TP, Lipponen JA, Cornforth DJ, Jelinek HF. Cardiac autonomic dysfunction in type 2 diabetes – effect of hyperglycemia and disease duration. *Front Endocrinol.* 2014;5:130.
18. Havlicekova Z, Tonhajzerova I, Jurko Jr A et al. Cardiac autonomic control in adolescents with primary hypertension. *Eur J Med Res.* 2009;14(4):101–103.
19. Fakhrzadeh H, Yamini-Sharif A, Sharifi F, et al. Cardiac autonomic neuropathy measured by heart rate variability and markers of subclinical atherosclerosis in early type 2 diabetes. *ISRN Endocrinol.* 2012;2012:1–7.
20. Faulkner MS, Quinn L, Rimmer JH, Rich BH. Cardiovascular endurance and heart rate variability in adolescents with type 1 or type 2 diabetes. *Biol Res Nurs.* 2005;7:16–29.
21. Zhang J. Effect of age and sex on heart rate variability in healthy subjects. *J Manipulative Physiol Ther.* 2007;30:374–379.
22. Vinik AI. The conductor of the autonomic orchestra. *Front Endocrinol.* 2012;3:71.
23. Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation.* 2007;115:387–397.
24. Rottman JN, Steinman RC, Albrecht P, Bigger JT, Rolnitzky LM, Fleiss JL. Efficient estimation of the heart period power spectrum suitable for physiologic or pharmacologic studies. *Am J Cardiol.* 1990;66:1522–1524.
25. Javorka K, Javorková J, Petrásková M, Tonhajzerová I, Buchanec J, Chromá O. Heart rate variability and cardiovascular tests in young patients with diabetes mellitus type 1. *J Pediatr Endocrinol Metab.* 1999;12:423–431.
26. Souza NM, Vanderlei LCM, Garner DM. Risk evaluation of diabetes mellitus by relation of chaotic globals to HRV. *Complexity.* 2015;20(3):84–92.
27. Billman GE. The LH/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physiol.* 2013;4:26.
28. Menezes Ada Jr S, Moreira HG, Daher MT. Analysis of heart rate variability in hypertensive patients before and after treatment with angiotensin II-converting enzyme inhibitors. *Arq Bras Cardiol.* 2004;83:169–172.
29. Milovanovic B, Trifunovic D, Milicevic N, Vasic K, Krotin M. The significance of amlodipine on autonomic nervous system adjustment (ANSA method): a new approach in the treatment of hypertension. *Srp Arh Celok Lek.* 2009;137(7–8):371–378.
30. Osakwe CE, Jacobs L, Anisiuba BC, et al. Heart rate variability on antihypertensive drugs in Black patients living in sub-Saharan Africa. *Blood Press.* 2014;23:174–180.
31. Pal GK. Resting heart rate is the index of cardiovascular health. *Int J Clin Exp Physiol.* 2014;1:243–244.
32. Singh RB, Hristova K, Gligorijevic T, et al. Can circadian restriction of feeding modulate autonomic nervous system dysfunction and cardiometabolic risk? *World Heart J.* 2015;7:31–42.
33. Eerola K, Rinne P, Penttinen AM, Vähätalo L, Savontaus M, Savontaus E. α-MSH overexpression in the nucleus tractus solitarius decreases fat mass and elevates heart rate. *J Endocrinol.* 2014;222(1):123–136.
34. Mischel NA, Mueller PJ. (In)activity-dependent alterations in resting and reflex control of splanchnic sympathetic nerve activity. *J Appl Physiol (1985).* 2011;111(6):1854–1862.
35. Mendonca GV, Pereira FD, Fernhall B. Effects of cigarette smoking on cardiac autonomic function during dynamic exercise. *J Sports Sci.* 2011;29(9):879–886.
36. Monforte R, Estruch R, Valls-Solé J, Nicolás J, Villalta J, Urbano-Marquez A. Autonomic and peripheral neuropathies in patients with chronic alcoholism: a dose-related toxic effect of alcohol. *Arch Neurol.* 1995;52(1):45–51.
37. Solanki JD, Basida SD, Mehta HB, Panjwani SJ, Gadhavi BP, Patel P. Impact of disease control and co-existing risk factors on heart rate variability in Gujarati type 2 diabetics -an observational study. *J Fam Med Primary Care.* 2016;5(2.). [Online Ahead of Print].
38. Gaede P, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med.* 2003;348:383–393.
39. Spallone V, Ziegler D, Freeman R, et al. Toronto Consensus Panel on Diabetic Neuropathy. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. *Diabetes Metab Res Rev.* 2011;27:639–653.