Diagnostic performance of resting and post-exercise heart rate variability for detecting cardiac autonomic neuropathy in type 2 diabetes mellitus

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ABSTRACT

Background: Post-exercise recovery phase is associated with clustering of various cardiovascular events and, therefore, monitoring of cardiac autonomic control via heart rate variability (HRV) during this phase may allow identification of autonomic alterations that are not evident under resting conditions in type 2 diabetes mellitus (T2DM) patients.
Purpose: To investigate and compare the diagnostic performance of resting and post-exercise HRV for detecting cardiac autonomic neuropathy (CAN) in T2DM patients.
Methods: Forty-two T2DM patients were categorized as CAN-positive and CAN-negative based on standard cardiovascular autonomic reflex tests (CARTs). Short-term resting and post-exercise HRV after a graded exercise test were evaluated for each participant. Diagnostic performance of both resting and post-exercise HRV measures was computed using standard statistical procedures.
Results: Diagnostic testing yielded superior diagnostic performance of post-exercise HRV than resting HRV measures. Root mean square of successive differences (RMSSD) between adjacent R-R intervals ($p = 0.01$), percentage of consecutive N-N intervals that vary by > 50 ms (pNN50) ($p = 0.03$) and total power (TP) ($p = 0.01$) were significantly better diagnostic indicators of CAN under post-exercise conditions than at rest. Predictive ability of these post-exercise HRV measures for CAN was maintained after adjusting various clinical confounders to cardiac autonomic function.
Conclusion: Post-exercise HRV measures such as TP, RMSSD and pNN50 were found to be more accurate diagnostic tests for detecting CAN than resting HRV. Hence, monitoring of the HRV measures proposed here during exercise testing protocols may provide important diagnostic information regarding CAN in T2DM.

1. Introduction

According to a recent report of International Diabetes Federation (IDF), diabetes mellitus (DM) affects around 415 million people worldwide and, by 2040, around 642 million people are projected to be affected by it (Ogurtsova et al., 2017). Due to associated cardiovascular complications, diabetes almost doubles the mortality rate despite available therapies (Banthia et al., 2013). Cardiac autonomic neuropathy (CAN), a widely underdiagnosed complication of DM, is more prevalent in type 2 diabetes mellitus (T2DM) than type 1 diabetes mellitus (T1DM) (Fisher and Tahrani, 2017). CAN is associated with various adverse health outcomes, silent myocardial ischemia, sub-clinical left ventricular dysfunction and increased mortality (Bissinger, 2017). Being asymptomatic in nature, which is a major cause of it's underdiagnosis, CAN exists in around 20% of clinically diagnosed cases

of diabetes (Banthia et al., 2013).

Cardiovascular autonomic reflex tests (CARTs), proposed by Ewing et al. (1985), remain the reference standard for the diagnosis of CAN in T2DM patients. CARTs consist of dynamic heart rate (HR) and blood pressure (BP) tests, which identify parasympathetic and sympathetic dysfunctions by perturbing autonomic nervous system (ANS) around its operating point (Ewing et al., 1985). Heart rate variability (HRV) is a simple and non-invasive tool for investigating cardiac autonomic function in both healthy and diseased human subjects (ChuDuc et al., 2013). In a recent systematic review, da Silva et al. (2016) suggested using HRV at rest to effectively diagnose diabetic CAN. HRV at rest is being widely used because of the ease in performing it; however, analyzing it during resting conditions may not give complete insight into the signs and symptoms of CAN. Resting HRV gives information on resting cardiac autonomic activity and thus does not assesses the

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dynamic ANS control. Scientific evidence (Michael et al., 2017) suggests that ANS is more responsive to physiological perturbations and, therefore, more dynamic assessment procedures are required to be employed for an early and more accurate diagnosis of CAN.

A majority of the existing literature (Chen et al., 2015; Jelinek et al., 2017; Khandoker et al., 2009; Tank et al., 2001) on the diagnosis of CAN presents the measurement of HRV under resting conditions, whereas the significance of exercise testing has been scantily highlighted (Banthia et al., 2013; Sacre et al., 2012). Considering that coronary artery disease (CAD) is the foremost contributor of morbidity and mortality in T2DM, a referral to exercise stress testing is common in routine investigation of asymptomatic T2DM patients (Sacre et al., 2012). Moreover, "reactivity hypothesis" states that physiological autonomic response to a stressor such as exercise may be a more rigorous and beneficial diagnostic and monitoring approach as altered cardiac autonomic recovery to a stressor may be indicative of certain cardiovascular diseases (Michael et al., 2017). Consistent with the "reactivity hypothesis," recent literature (Banthia et al., 2013; Sacre et al., 2012) has adopted exercise-based investigation of CAN in DM and useful insights into autonomic stress reactivity with early identification of CAN have been gained. While the main focus of these investigations is the diagnostic performance of post-exercise heart rate recovery (HRR) (Sacre et al., 2012), areas such as ventricular repolarization, along with HRR responses under parasympathetic blockade during and after exercise, have also been examined (Banthia et al., 2013). Although exercise-based assessment provides more diagnostic information, HRV under resting condition is still considered to be a more feasible approach, mainly because of lesser involvement of patients in the entire process. Nevertheless, the potential utility of investigating post-exercise HRV is promising and requires substantial attention by researchers. However, to the best of our knowledge, no study has given a thoughtful analysis to the diagnostic utility of post-exercise HRV so far. Considering the diagnostic importance of post-exercise recovery phase from a cardiovascular perspective, the present study aimed to investigate and compare the diagnostic performance of resting and post-exercise HRV for diagnosing CAN in T2DM patients. If came out to be significant, post-exercise HRV may be acclaimed as a screening tool for CAN in those centers where exercise testing is a routine practice and there is lack of expertise in conducting standard clinical autonomic tests, considering the fact that the utility of any diagnostic test depends on the availability of instrumentation and expertise. We hypothesize that post-exercise HRV will demonstrate better diagnostic performance than resting HRV measures in the detection of CAN in T2DM patients.

2. Materials and methods

The present study is reported in accordance with the Standards for Reporting Diagnostic Accuracy Studies (STARD) guidelines (Cohen et al., 2016).

2.1. Participants

The study was performed prospectively for research purposes at Jamia Millia Islamia (a central university), New Delhi, India, between October 2017 and March 2018, after obtaining clearance from the Institutional Ethics Committee and all research procedures were carried out in accordance with the Declaration of Helsinki, 1964. Patients ($n = 42$) aged 51.1 ± 6.16 years who were diagnosed with T2DM from at least one year according to the criteria prescribed by American Diabetes Association (American Diabetes Association, 2015) were selected through referrals from medical center of Jamia Millia Islamia. Patients with a history of any cardiovascular disease, with a body mass index (BMI) > 40 kg/m^2 (morbidly obese), having severe diabetic nephropathy, suffering from uncontrolled hypertension [systolic blood pressure (SBP)/diastolic blood pressure (DBP) > 165/95 mmHg], having an electrocardiogram (ECG) with non-sinus rhythm and/or with

ectopic beats ≥10%, and with clinical orthopedic problems that limit patient's ability to participate in exercise testing without inducing pain were excluded from the study. Prior to assessment, the purpose and procedures of the study were explained to the patients and their written informed consent was obtained.

2.2. Protocol for data collection

The assessment to screen eligible participants was carried out by a medical professional at the university medical center and those not meeting the eligibility criteria were excluded at this stage. Patients found to be eligible at this stage of screening were evaluated for their glycemic and lipid profiles in the laboratory of the medical center by standard pathology lab procedures. Thereafter, eligible patients were referred to the Centre for Physiotherapy and Rehabilitation Sciences (CPRS) of Jamia Millia Islamia where further assessment was carried out. After general demographic (inclusive of variables such as age, gender, height, weight, BMI and duration of diabetes) and clinical assessment (glycemic control, lipid profile, SBP, DBP, information on drug usage, comorbidities and complications of diabetes), physical activity levels of these patients were also evaluated by International Physical Activity Questionnaire short form (IPAQ-SF). Later, their cardiac autonomic function was investigated at Human Performance Laboratory, CPRS. Following HRV assessments at rest, CARTs were performed, which was followed by post-exercise HRV measurement after a maximal exercise test protocol. Adequate rest periods were allowed before each new test and the entire cardiac autonomic testing was completed on the same day.

2.3. Standard CAN testing by CARTs

Before clinical autonomic testing, weight and height of the participants were measured using standard procedures. Resting SBP and DBP were measured by a manual sphygmomanometer (Diamond Bpdl-237 Aneroid Blood Pressure Apparatus) as per the recommended guidelines (Ogedegbe and Pickering, 2010). CARTs originally developed by Ewing et al. (1985) are still considered as the gold standard in diagnosing diabetic CAN. This test battery comprises three HR tests [deep breathing test, Valsalva maneuver and head-up tilt (HUT) test] and two BP tests [HUT and hand grip test (HGT)]. SBP fall to postural stress and R-R responses at the 15th and 30th s of the HUT test were used to compute 30/15 ratio. Deep breathing test constituted of deep inspiration–expiration for six consecutive cycles, which was later analyzed to obtain expiration to inspiration (E/I) ratio and change in HR (ΔHR) to deep breathing maneuver. ΔHR shows the integrity of the vagal afferent and efferent pathways, whereas the 30/15 ratio examines the integrity of vagal-mediated baroreflex function. Furthermore, patients were asked to perform Valsalva maneuver for 15 s to raise the mercury level to 40 mmHg on a manual manometer. Valsalva ratio was calculated by dividing the largest and the smallest R-R values at the 2nd and 4th stages of the maneuver and is considered to represent both sympathetic and parasympathetic function. Finally, DBP response to HGT was assessed during a hold of hand grip dynamometer at 30% of the patient's maximum voluntary isometric contraction (MVIC). SBP response to postural change and DBP response to HGT are considered as measures of sympathetic reactivity. Based on the findings of CARTs, patients were classified as no-CAN or with CAN (early, definite, or severe CAN) using the Ewing's criteria (Ewing et al., 1985). During CARTs assessment, R-R intervals were recorded by Lab Chart software version 7.3.7. (Power Lab 8 SP, AD Instruments, New Zealand) while BP responses were assessed using a manual sphygmomanometer.

2.4. Exercise testing

As previously stated, eligibility of the patients to participate in exercise testing was assessed initially by the medical personnel at the

university medical center. The patients undertook a symptom-limited maximal graded exercise test according to the Balke protocol (Beltz et al., 2016) on a motorized treadmill (Freemotion i7.9 Incline Trainer) with standard monitoring by lead II ECG and BP every minute. Simultaneous analysis of gas exchange was computed by a gas analyzer (ML206 gas analyzer, AD instruments, New Zealand) which gave data on the gases consumed every 10 s and yielded peak oxygen consumption (VO_{2max}) as a direct measure of exercise capacity. The termination criterion for the exercise test was either attainment of 90% of age-predicted maximal HR (MHR) or volitional fatigue or achievement of VO_{2max} (defined as VO_2 that increased < 1 mL/kg/min for ≥ 30 s despite increment in workload) (Brandenburg et al., 1999). Peak HR and HR following exercise were derived from a continuous record obtained via ECG. Heart rate recovery (HRR) was calculated as the absolute difference between the peak HR attained during exercise and the HR recorded at 1- (HRR_{1min}), 2- (HRR_{2min}) and 3-min (HRR_{3min}) post-exercise.

2.5. Resting and post-exercise HRV assessment

2.5.1. Subject preparation

Autonomic function assessment was performed between 9:00 and 12:00 am for all participants following a gap of 2 h after meals. Two days prior to the assessment, patients were familiarized with the testing procedures and were instructed to refrain from smoking and caffeine on the day of testing. Medications such as anti-hypertensive and anti-arrhythmic drugs that could affect autonomic function as well as alcohol and any vigorous physical exercise were also prohibited for at least 24 h prior to testing.

2.5.2. HRV recording and analysis

Resting HRV testing was performed in a quiet, temperature-controlled room (24 °C) after a rest of at least 15 min in supine position with spontaneous breathing. Skin preparation and electrode placement were performed as per the guidelines of American Heart Association (Drew et al., 2004). For resting HRV measurement, ECG was recorded for 10 min using the standard lead II configuration. The last 5-min segment of the 10 min record was analyzed for time and frequency domain variables of HRV by detecting R waves using the peak detection module of the software. AD instruments Lab Chart version 7.3.7 with HRV module version 1.4.2 using Hann (cosine-bell) window (Power Lab 8 SP, AD Instruments, New Zealand) was used as data acquisition software for recording ECG, which calculated R–R intervals as the measure of difference between successive beats. Resting HR was derived from the resting ECG record.

Immediately following the maximal exercise test, patients were asked to lie supine on a bed adjacent to the treadmill. ECG was recorded for next 10 min which included a 3-min transitional phase. The transitional phase provided sufficient time to the patient to dismount from the treadmill, assume supine position on the bed and ensure proper connection of the leads and surface electrodes. Post-exercise HRV was found to have a good test–retest reliability showing ICC values of 0.69–0.92 (Michael et al., 2017). HRV analysis was performed on the R-R intervals between 3 and 8 min of the ECG recorded post-exercise (Neves et al., 2011). All HRV data were stored and analyzed offline. Recorded at the sampling rate of 1000 Hz, data were visually and automatically inspected for ectopic beats using the beat classifier module of the software and were interpolated in accordance with previous suggestions (Tarkiainen et al., 2007) to provide a continuous data stream. For HRV spectral analysis, R–R interval time series was decomposed into its frequency components using Fast Fourier Transform (FFT). Data were filtered using a low pass filter with 40 Hz as the cut-off frequency. The power density in areas of low frequency (LF; 0.04 to 0.15 Hz) and high frequency (HF; 0.15 to 0.4 Hz) bands were calculated in absolute (ms^2) and normalized units (nu) at rest and after exercise. The range of HF band for post-exercise HRV analysis was extended (HF;

0.15–0.5 Hz) considering the effect of respiratory oscillations on HRV during the post-exercise period (Gąsior et al., 2016). HRV analysis was performed by an investigator who was unaware of the patient's diagnosis by the standard clinical autonomic testing. Standard time domain indices such as average of N-N intervals (Mean NN), standard deviation of N-N intervals (SDNN), square root of successive differences (RMSSD) between adjacent R–R intervals, percentage of consecutive N-N intervals that vary by > 50 ms (pNN50) and frequency domain indices such as total power (TP), LF power, HF power and the ratio of LF and HF power (LF/HF ratio) were obtained through analysis. The power spectra were calculated in absolute (ms^2) as well as normalized units (LFnu power and HFnu power) to represent the relative value of each power component as a proportion of the TP. Time domain variables such as Mean NN and SDNN reflect overall variability in HR whereas RMSSD and pNN50 indicate parasympathetic activity. Frequency domain indices such as TP denote overall variations between the R-R intervals, LF power represents combination of sympathetic and parasympathetic activity, whereas HF power indicates vagal activity. Both data acquisition and post-acquisition analysis were carried out in accordance with the standards set by the Taskforce of European Society of Cardiology and North American Society of Pacing and Electrophysiology (Taskforce, 1996). Although direct measurement of post-exercise respiratory rate was not obtained in the present study, indirect estimations were made from the post-exercise HRV spectral data. The dominant frequency within the range of 0.1–0.5 Hz represented respiratory rate in breaths per second which was later converted to breaths/min (Ng et al., 2009).

3. Statistical analysis

Prior to this study, a pilot study was carried out on 12 T2DM patients that demonstrated an area under the curve (AUC) of 0.84 for post-exercise TP for diagnosing CAN. Based on this AUC value, and at the power of 0.08 along with alpha of 0.05, a total sample size of 42 patients was found to be necessary for detecting CAN in T2DM by post-exercise HRV. Data analysis was performed using Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA) and MedCalc Statistical Software version 12.1.4 (MedCalc Software bvba, Ostend, Belgium). Normality of distribution was examined by Shapiro–Wilk test. Variables found to be non-normal were log transformed prior to further analysis. Discriminant analysis was performed using independent *t*-test or Mann–Whitney *U* test whenever required to compare the demographic and clinical autonomic test data (CARTs).The main effects of group (CAN-positive versus CAN-negative), condition (resting versus post-exercise period) and group × condition interaction were calculated for HRV parameters using 2 × 2 mixed model analysis of variance (ANOVA). The diagnostic performance of resting and post-exercise HRV indices was evaluated by receiver operating characteristic (ROC) curve analysis using non-parametric method by an evaluator blinded to the diagnosis of CAN based on CARTs. The area under ROC curve was computed by ROC curve analysis. AUC of 1.0 indicates perfect prediction, while an area of 0.5 reflects random forecasts (Hajian-Tilaki, 2013). The ROC curve analysis provided calculations for an optimal cut-off point for the HRV parameters (to correctly classify patients with or without CAN), sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio. Time with the highest Youden's Index (sensitivity + specificity1) was determined to be the optimal cut-off point for each HRV parameter. Comparative ROC curve analysis was also performed in order to compare each resting and post-exercise HRV outcome for their diagnostic robustness. True positives, true negatives, false positives, false negatives and overall test accuracy for resting and post-exercise HRV indices were manually calculated after dichotomizing each variable based on their respective cut-off values obtained in ROC curve analysis. Correlation analysis was performed to compute Pearson's *r* between various CARTs and HRV parameters. The post-exercise HRV

variables that demonstrated significant correlations with CARTs were further analyzed by logistic regression. First, predictive ability of post-exercise HRV (continuous and dichotomized, based on their respective cut-off values) for CAN was confirmed by significant univariable odds ratios. Univariable odds ratio was also calculated for age, diabetes duration, physical activity levels, VO_{2max}, SBP, number of comorbidities, number of medications and HbA1c. Multivariable logistic regression was used to calculate odds ratio adjusted for the relevant clinical data (HbA1C, VO_{2max}, number of comorbidities). Separate models were created for each post-exercise HRV variable in continuous (model 1) as well as in dichotomized form (model 2). Colinearity between independent variables in multivariable regression models was assessed by calculating the variation inflation factor (VIF) that measures inflation in the variances of the parameter estimates due to multi-colinearity potentially caused by the correlated predictors. Although there are no universal cut-off values available for VIF, roughly a value of 3–5 is considered to be a cause of concern and a value ≥10 indicates serious colinearity problems (Thompson et al., 2017). A *p*-value of < 0.05 is considered to indicate statistical significance in the present study and the data are presented as mean ± standard deviation.

4. Results

4.1. Demographic and clinical characteristics

Initially, a total of 50 T2DM patients were selected for the study; however, due to frequent ectopy in their resting ECG, the data of 8 patients (5 of them were positive for CAN) were excluded from the analysis. Of the remaining 42 patients comprising the final study population, 27 (64.2%) were found to be positive for CAN based on CARTs battery (Fig. 1). Most of the patients in the study were overweight (BMI: $27.9 \pm 4.26 \, \text{kg/m}^2$) with an average diabetes duration of 7.9 ± 5.98 years (Table 1). Significant differences were observed between CAN-positive and CAN-negative group with regard to diabetes duration, glycemic control, SBP, VO_{2max}, number of comorbidities, HDL cholesterol and physical activity levels (MET-min/week) with patients in CAN-positive group presenting more impairment in all glycemic

parameters and cardiovascular risk parameters along with reduced physical activity levels (Table 1). Hypertension was the commonest comorbidity that existed in 38% of the patients included in this study (Table 1). Among the CARTs, E/I ratio, delta HR and 30/15 ratio were significantly reduced in patients with CAN. HRV indices such as Mean NN, RMSSD, pNN50, TP, LF power and HF power, along with HRR at 2 and 3 min, were also found to be significantly impaired in patients with CAN. Majority of the HRV parameters were significantly altered from rest to post-exercise period (Table 2). Positive correlations were identified between most of the HRV parameters and indices included in the CARTs battery. However, change in DBP to HGT, as well as in SBP to HUT and VR, did not show any significant correlation with any HRV parameter (Table 3).

4.2. Diagnostic performance of resting and post-exercise HRV

ROC curve analysis indicated an AUC ranging from 0.61 to 0.85 for resting HRV parameters while the same for post-exercise HRV variables ranged from 0.63 to 0.91 (Figs. 2–3). Majority of the HRV variables both at rest and post-exercise were found to be more sensitive than specific for diagnosing CAN in T2DM (Figs. 2–3). Comparative ROC curve analysis between resting and post-exercise HRV demonstrated significant differences for TP ($p = 0.01$), pNN50 ($p = 0.03$) and RMSSD ($p = 0.01$) (Fig. 4). Diagnostic performance of resting versus post-exercise Mean NN ($p = 0.059$), SDNN ($p = 0.88$), LF power ($p = 0.48$), HF power ($p = 0.68$) and LF/HF ratio ($p = 0.39$) was not significantly different according to the findings of comparative ROC curve analysis, although majority of these demonstrated superior diagnostic performance under post-exercise conditions in individual ROC curve analysis (Figs. 2–3). AUC was found to be higher for almost all post-exercise HRV variables than resting HRV variables (except for HF and LF/HF ratio). Overall test accuracy was found to be higher for post-exercise HRV variables, and pNN50 among the time domain and TP among the frequency domain variables were able to diagnose CAN with the highest diagnostic accuracy (rest versus post-exercise; TP 85.7% versus 90.4%, $p = 0.01$; pNN50 85.7% versus 95.2%, $p = 0.03$) (Table 4). Detailed information on other diagnostic statistics is presented in Table 4. For all
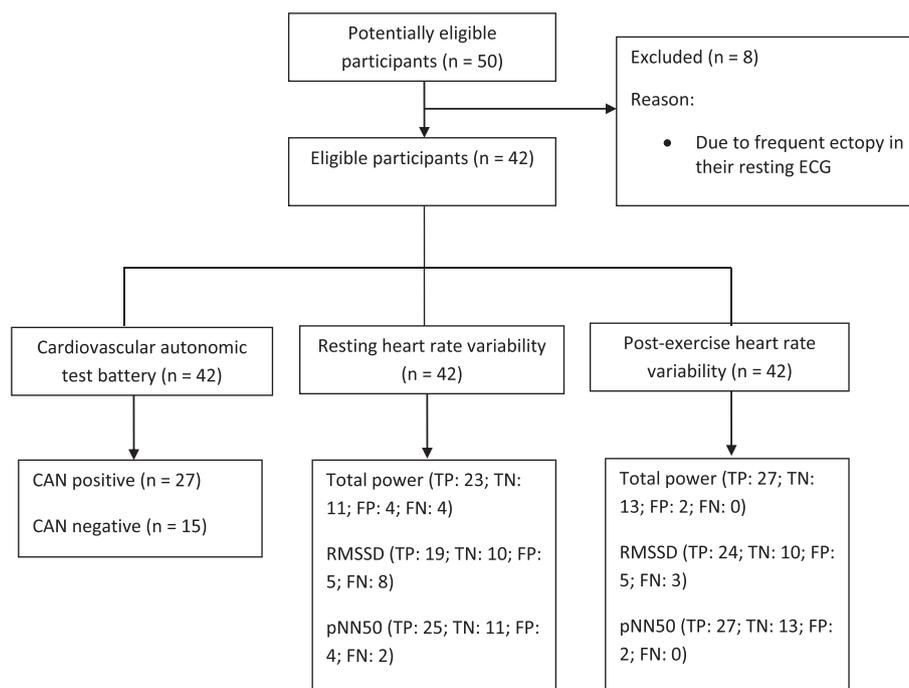

Fig. 1. Flow chart representing participant flow through the study. CAN: cardiac autonomic neuropathy; RMSSD: root mean square of the successive differences between adjacent R–R intervals; pNN50: percentage of N–N intervals that vary > 50 ms; TP: true positives; TN: true negatives; FP: false positives; FN: false negatives.

Table 1
Clinical characteristic of patients with and without cardiac autonomic neuropathy.

	Study population (N = 42)	Patients with CAN (n = 27)	Patients without CAN (n = 15)	p-Value
Demographic & clinical parameters				
Age (years)	51.1 ± 6.16	51.7 ± 6.08	50.2 ± 6.38	0.44
Gender (n) (Male/female)	31/11	18/9	13/2	
Weight (kg)	74.7 ± 11.50	73.9 ± 11.88	76.0 ± 11.05	0.58
Height (cm)	1.63 ± 0.09	1.62 ± 0.09	1.66 ± 0.09	0.13
BMI (kg/m^2)	27.9 ± 4.26	28.2 ± 4.78	27.3 ± 3.20	0.57
Duration of diabetes (years)	7.9 ± 5.98	9.26 ± 6.44	5.4 ± 4.24	0.04*
HRrest (beats/min)	79.6 ± 12.2	80.5 ± 12.4	78.0 ± 12.01	0.52
SBP (mmhg)	128.6 ± 14.95	132.1 ± 14.97	122.4 ± 13.14	0.03*
DBP(mmhg)	80.1 ± 8.75	80.5 ± 9.10	79.2 ± 8.31	0.64
MAP (mmhg)	144.4 ± 14.11	146.6 ± 14.06	140.4 ± 13.77	0.17
Physical activity levels (MET-min/week)	808.5 (324.0–10,725)	680.0 (324.00–10,725.00)	996.00 (456.30–1799.00)	0.001*
Co-morbidities, n (%)				
History of hypertension	16 (38)	13 (52)	2 (13.3)	
Thyroid dysfunction	4 (9.5)	3 (12)	1 (6.6)	
Musculoskeletal issues	6 (14.2)	5 (18.5)	1 (6.6)	
Number of co-morbidities	0.5 (0.00–3.00)	1.0 (0.00–3.00)	0.0 (0.00–2.00)	0.02*
Glycemic control				
HbA1c (%)	8.6 ± 1.82	9.0 ± 1.71	7.8 ± 1.81	0.03*
Fasting blood glucose (mmol/L)	9.2 ± 3.27	10.0 ± 3.63	7.7 ± 1.74	0.007*
Post-prandial blood glucose (mmol/L)	12.4 ± 4.34	13.4 ± 4.74	10.5 ± 2.76	0.03*
Lipid profile				
TC (mg/dl)	172.7 ± 39.38	175.9 ± 41.66	167.1 ± 35.75	0.49
LDL-C (mg/dl)	102.1 ± 38.00	107.4 ± 37.87	92.4 ± 37.53	0.2
HDL-C (mg/dl)	43.7 ± 9.26	41.2 ± 6.93	48.1 ± 11.35	0.01*
Triglycerides (mg/dl)	157.5 ± 45.67	165.7 ± 43.86	142.6 ± 46.55	0.11
Diabetes complications n (n%)				
Microalbuminuria	1 (2.3)	1 (4)	0 (0)	
Retinopathy	4 (9.5)	3 (12)	1 (6.6)	
Peripheral neuropathy	12 (28.5)	10 (37)	2 (13.3)	
Number of medication	2.5 (0–9)	2.0 (1–9)	3.0 (0–4)	0.27
Drugs, n (n%)				
Metformin	27 (64.2)	19 (70.3)	8 (53.3)	
Insulin	4 (9.5)	4 (14.8)	0 (0)	
Sulphonylureas	12 (28.5)	6 (22.2)	6 (40)	
ACE inhibitors	2 (4.7)	2 (7.4)	0 (0)	
Beta-blockers	1 (2.3)	1 (3.7)	0 (0)	
Statins	4 (9.5)	3 (11.1)	1 (6.6)	
Exercise test variables				
Peak HR (beats/min)	142.1 ± 10.15	140.4 ± 7.83	145.1 ± 13.15	0.22
HRR$_{1min}$ (beats/min)	21.5 ± 11.29	20.0 ± 12.27	24.10 ± 9.09	0.27
HRR$_{2min}$ (beats/min)	39.6 ± 12.54	36.2 ± 12.96	45.93 ± 9.17	0.01*
HRR$_{3min}$ (beats/min)	46.4 ± 14.96	42.8 ± 12.08	53.11 ± 17.6	0.03*
VO$_{2max}$	29.12 ± 8.95	26.0 ± 8.12	34.5 ± 7.92	0.002*
Post-exercise RR	15.7 ± 4.66	15.2 ± 5.16	16.5 ± 3.62	

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; MET: metabolic equivalents; VO$_{2max}$: maximum oxygen consumption; HR: heart rate; HRR: heart rate recovery; HbA1c: glycosylated hemoglobin; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TC: total cholesterol; ACE: angiotensin converting enzyme inhibitors; Ca: calcium; HR: heart rate; HRR: heart rate recovery; RR: respiratory; CAN: cardiac autonomic neuropathy; n: number of patients; *significant difference between the groups ($p < 0.05$); p-value: probability value; Data are mean ± SD, median (interquartile range), n (%) when appropriate

time domain HRV variables, AUC was found to be higher during post-exercise period. However, frequency domain variables, namely HF power, indicated similar AUC during resting and post-exercise conditions (HF power: rest; AUC: 0.85, $p < 0.0001$, sensitivity: 96.3%, specificity: 66.6%, cut-off: $\leq 322\,ms^2$ versus post-exercise; AUC: 0.85, $p < 0.0001$, sensitivity: 88.8%, specificity: 80%, cut-off: $\leq 141.12\,ms^2$) and LF/HF ratio demonstrated lower AUC values during post-exercise period as compared to resting period (LF/HF ratio: rest; AUC: 0.71, $p = 0.008$, sensitivity: 59.2%, specificity: 86.6%, cut-off: ≥ 1.11 versus post-exercise; AUC: 0.67, $p = 0.07$, sensitivity: 77.7%, specificity: 66.6%, cut-off: ≥ 1.03).

Covariates for univariable logistic regression analysis were selected on the basis of observed significant differences in outcome variables between patients with and without CAN (Table 1) except for age and

the number of medications, which are also considered as important determinants of CAN. Univariable logistic regression models demonstrated that post-exercise HRV parameters (Mean NN, RMSSD, pNN50, TP, LF, and HF), HbA1c levels, VO$_{2max}$, and the number of comorbidities were significantly associated with the presence of CAN in T2DM patients (Table 5). Multivariable logistic regression models controlling for HbA1c, VO$_{2max}$, and the number of comorbidities were used to calculate the odds ratio for the predictive ability of post-exercise HRV. Reduction in post-exercise HRV variables such as Mean NN, RMSSD, pNN50, TP, LF power and HF power was significantly and independently associated with the occurrence of CAN after adjusting for glycemic control, aerobic capacity and the number of comorbidities (Table 6). In order to examine colinearity, separate regression models were created for each of the independent variables (VO$_{2max}$, HbA1c and

Table 2

Cardiovascular autonomic reflex test parameters, resting and post-exercise heart rate variability indices in patients with and without cardiac autonomic neuropathy.

Variables	Patients with CAN (n = 27)	Patients without CAN (n = 15)	p-Value
Diagnosis of CAN			
Early CAN, n (n %)	12 (44.4)	–	
Definite CAN, n (n %)	9 (33.3)	–	
Severe CAN, n (n %)	6 (22.2)	–	
CARTs	Mean ± SD	Mean ± SD	Group
E/I ratio (DBT)	1.1 ± 0.15	1.2 ± 0.12	0.002*
ΔHR (DBT)	11.7 ± 9.31	21.4 ± 4.70	< 0.001*
Valsalva ratio (VM)	1.2 ± 0.54	1.50 ± 0.35	0.06
Delta DBP (HGT)	14.11 ± 5.96	18.1 ± 4.17	0.02*
Delta SBP (HUT)	−2.0 (−30–22)	4.0 (−18–14)	0.16
30/15 ratio (HUT)	1.2 ± 0.23	1.37 ± 0.29	0.02*

Resting and post-ex HRV			Group	Condition	Group × condition
Rest Mean NN (ms)	730.8 ± 84.37	795.7 ± 88.5	0.001*	< 0.001[†]	0.001[#]
Post-ex MeanNN(ms)	608.3 ± 65.80	724.9 ± 112.71			
Rest SDNN (ms)	37.1 ± 15.08	42.9 ± 18.79	0.08	< 0.001[†]	0.53
Post-ex SDNN (ms)	18.9 ± 7.64	27.2 ± 15.72			
Rest RMSSD (ms)	24.6 ± 11.20	30.7 ± 15.62	0.01*	< 0.001[†]	0.34
Post-ex RMSSD (ms)	10.7 ± 4.15	19.6 ± 11.30			
Rest pNN50 (%)	1.6 ± 1.20	3.9 ± 1.89	< 0.001*	< 0.001[†]	0.86
Post-ex pNN50 (%)	0.4 ± 0.35	2.8 ± 1.53			
Rest TP (ms^2)	716.8 ± 285.73	1651.6 ± 1.20	< 0.001*	< 0.001[†]	< 0.001[#]
Post-ex TP (ms^2)	358.1 ± 222.59	1277.2 ± 18.66			
Rest LF (ms^2)	207.2 ± 116.81	594.4 ± 563.73	< 0.001*	0.002[†]	0.14
Post-ex LF (ms^2)	135.8 ± 96.00	403.8 ± 253.04			
Rest LF (nu)	54.2 ± 10.28	51.6 ± 5.99	0.008*	0.85	0.03[#]
Post-ex LF (nu)	62.1 ± 21.86	44.9 ± 14.46			
Rest HF (ms^2)	170.9 ± 85.21	544.6 ± 462.99	< 0.001*	0.03[†]	0.43
Post-ex HF (ms^2)	100.0 ± 81.86	425.9 ± 370.88			
Rest HF (nu)	45.7 ± 10.28	49.5 ± 3.96	0.003*	0.77	0.29
Post-ex HF (nu)	50.30 ± 21.71	46.9 ± 19.77			
Rest LF/HF ratio	1.53 ± 0.71	1.66 ± 1.32	0.09	0.33	0.98
Post-ex LF/HF ratio	1.40 ± 0.62	1.17 ± 1.76			

CARTs: cardiovascular autonomic reflex tests; HR: heart rate; E/I ratio: expiration/inspiration ratio; DBT: deep breathing test; VM: Valsalva maneuver; HUT: head-up tilt test; HGT: hand grip test; SBP: systolic blood pressure; DBP: diastolic blood pressure; HRV: heart rate variability; Rest: resting; ex: exercise; Mean NN: average of N–N intervals; SDNN: standard deviation of N–N intervals; RMSSD: root mean square of successive differences between adjacent R-R intervals; pNN50: percentage of consecutive N–N intervals that differ by > 50 ms; TP: total power; LF: low frequency power; HF: high frequency power; LF/HF ratio: ratio of low and high frequency power; ms: milliseconds; %: percent; nu: normalized units; CAN: cardiac autonomic neuropathy; n: number of patients; *significant difference between CAN-positive and CAN-negative group; [†]significant difference between rest versus post-exercise conditions; [#]significant group (CAN-positive and CAN-negative) × condition (rest and post-exercise) interaction p-value: probability value; Data are mean ± standard deviation.

the number of comorbidities). In the regression analysis, one of these variables was considered as the dependent variable and the other two as independent variables. The values of the VIF turned out to be 1.23, 1.11 and 1.08 for other predictor variables, when VO$_{2max}$, number of comorbidities and HbA1c were considered to be dependent variables, respectively. These findings clearly indicate that the present analysis was not affected by colinearity among independent variables in the multivariable logistic regression since VIF was less than the optimal cut-off value for the presence of colinearity in the analysis.

5. Discussion

Although HRV has been considered as an important diagnostic tool for diagnosing CAN in T2DM, so far, to the best of our knowledge, no study has investigated its diagnostic performance during post-exercise recovery period. The purpose of the present study was to investigate and compare the diagnostic performance of HRV under resting conditions and during post-exercise recovery phase for diagnosing CAN in patients with T2DM. The findings of this investigation demonstrated (i) confirmation of altered HRV (more alteration in the post-exercise period) in patients with CAN; (ii) significant correlation of HRV parameters with CARTs; (iii) superior diagnostic performance of post-exercise HRV than resting HRV for diagnosing CAN in T2DM patients and (iv) predictive ability of post-exercise HRV parameters maintained for

detecting CAN after adjusting for various clinical confounders that could affect cardiac autonomic function.

5.1. Prevalence of CAN in T2DM patients

In the present study, 64.2% (n = 27) T2DM patients were found to be positive for CAN. According to a recent review (Fisher and Tahrani, 2017), the prevalence of CAN has varied greatly in previous studies ranging from 17% to 68%. This huge variation in the reported prevalence of CAN may be partially attributed to the use of different diagnostic criteria for diagnosing and staging CAN. Furthermore, previous studies on Asian Indian patients (Nanaiah et al., 2012; Sukla et al., 2016) have reported similar higher prevalence of CAN as seen in the present study owing to a genetic predisposition of CAN in these racial cohorts. Compared to other racial groups, Asian Indians have shown poorer cardiac autonomic function and these differences are largely accounted for by greater hyperglycemia found in this population. Moreover, the diabetic population of our study had been suffering from diabetes for > 5 years (Table 1) and this might be one of the reasons behind greater prevalence of CAN as longer duration of diabetes can further worsen autonomic control of the heart (Refaie, 2014).

Table 3
Correlation of cardiovascular reflex test parameters with resting and post-exercise heart rate variability indices.

	E/I ratio	Δ HR	VR	30/15 ratio	Delta SBP HUT	Delta DBP HGT
Resting HRV						
Mean NN (ms)	0.36*	0.34*	0.12	0.44**	0.16	0.20
SDNN (ms)	0.09	0.04	0.07	0.31*	− 0.03	− 0.30
RMSSD (ms)	0.09	0.07	0.10	0.44**	0.28	− 0.27
pNN50 (%)	0.10	0.21	0.28	0.20	0.14	0.26
TP (ms^2)	0.37*	0.41**	0.08	0.47**	0.04	0.14
LF (ms^2)	0.41**	0.49**	0.03	0.40**	0.06	0.20
LFnu	0.07	0.13	0.02	− 0.09	− 0.13	− 0.09
HF (ms^2)	0.39*	0.43**	0.02	0.45**	0.11	0.26
HFnu	− 0.01	− 0.06	− 0.01	0.08	0.09	0.14
LF/HF ratio	0.05	0.10	0.01	− 0.09	− 0.11	− 0.12
Post-exercise HRV						
Mean NN (ms)	0.47**	0.43**	0.10	0.44**	0.08	0.18
SDNN (ms)	0.15	0.13	− 0.04	0.13	− 0.04	0.06
RMSSD (ms)	0.30	0.24	0.06	0.45**	0.19	− 0.18
pNN50 (%)	0.61***	0.60***	0.24	0.19	0.14	0.29
TP (ms^2)	0.44**	0.51**	0.15	0.42*	0.05	0.29
LF (ms^2)	0.35*	0.42**	0.05	0.33*	0.06	0.15
LFnu	− 0.23	− 0.19	− 0.11	− 0.08	0.08	− 0.28
HF (ms^2)	0.46**	0.46*	0.26	0.47**	0.07	0.24
HFnu	− 0.03	− 0.09	0.07	0.36*	0.25	− 0.02
LF/HF ratio	− 0.12	− 0.06	− 0.13	− 0.36*	− 0.17	− 0.14

E/I ratio: expiration/inspiration ratio; HR: heart rate; HRV: heart rate variability; VR: valsalva ratio; HUT: head up tilt test; HGT: hand grip test; SBP: systolic blood pressure; DBP: diastolic blood pressure; Mean NN: Average of N–N intervals; SDNN: standard deviation of N–N intervals; RMSSD: root mean square of successive differences between adjacent R-R intervals; pNN50: percentage of consecutive N–N intervals that differ by > 50 milliseconds; TP: total power; LF: low frequency power; HF: high frequency power; nu: normalized units LF/HF ratio: ratio of low and high frequency power; ms: milliseconds.

* < 0.05 probability value.
** < 0.01 probability value.
*** < 0.001 probability value.

### 5.2. Cardiac autonomic function in T2DM patients with and without CAN

In the present investigation, patients with CAN were found to have a poorer glycemic and cardiovascular risk profile along with significantly reduced HRV parameters as compared to patients without CAN, which is in accordance with the findings of previous studies (Balcıoğlu et al., 2007; Sacre et al., 2012). Previous research (Motataianu et al., 2013) has clearly established strong associations between the presence of CAN and various other macro- and microvascular complications of diabetes. It has been further observed that the patho-physiology of CAN specifically targets vascular endothelium (Serhiyenko and Serhiyenko, 2018), which in part may play a role in worsening the cardiovascular risk profile of T2DM patients. Besides this, reduced variability in R-R intervals is the earliest manifestation of CAN, caused by an imbalance in the autonomic supply to the heart, and is found to be significantly reduced in patients with CAN as compared to patients without CAN (Lin et al., 2017) and this is in accordance with the findings of the present study. Furthermore, post-exercise HRR was also found to be delayed in patients with CAN, which again suggests a greater degree of autonomic dysfunction in T2DM in the presence of CAN. The present study also illustrated significant correlations between clinical autonomic tests of cardiac parasympathetic function (ΔHR, E/I ratio and 30/15 ratio) and measures of HRV, which may partially explain altered HRV in patients with CAN. Such associations also explain the complex relationship between HRV and the presence of CAN, and further illustrate the role of HRV in the diagnosis and monitoring of CAN. Previous studies (Sacre et al., 2012; Lin et al., 2017) have also found similar relationships between CARTs and measures of autonomic function. Despite significant

associations with most of the clinical autonomic tests, HRV measures did not demonstrate any significant correlation with the tests of sympathetic function such as delta SBP (HUT), delta DBP (HGT) and VR (which examines both sympathetic and parasympathetic baroreflex function) in the present study. Moreover, these autonomic tests were not significantly different between the CAN-positive and CAN-negative group. These findings may be partially explained by the natural progression of CAN and suggest that sympathetic function tends to be involved in the later phases of CAN (Pop-Busui, 2010). In the present study, majority of the patients (44.4%) were found to be in the early stage of CAN, which is marked by the presence of one abnormal or two borderline HR tests and, hence, most of them might have parasympathetic dysfunction. As a result, the tests of sympathetic function that were certainly normal did not correlate with the HRV parameters that were rather significantly altered in the CAN-positive group.

5.3. Diagnostic performance of resting and post-exercise HRV

The diagnostic ability of post-exercise HRV measures (TP, RMSSD, pNN50) was significantly higher than HRV under resting conditions in the present study. Furthermore, post-exercise HRV demonstrated an AUC ranging from 0.6 to 0.91 with time domain measures performing better than frequency domain measures. Majority of post-exercise HRV indices demonstrated superior diagnostic performance in the ROC curve analysis, except for post-exercise HF power and LF/HF ratio with an AUC of 0.85 ($p < 0.0001$) versus 0.85 ($p < 0.0001$) and 0.71 ($p = 0.008$) versus 0.67 ($p = 0.07$) in resting versus post-exercise conditions, respectively. These inconsistent results may be caused by an alteration in the respiratory frequencies during the post-exercise period. Although, to our knowledge, no study has been done on the diagnostic performance of post-exercise HRV for CAN, there have been studies (Chen et al., 2015; Jelinek et al., 2017) on the diagnostic ability of resting HRV, results of which are in accordance with our findings. Jelinek et al. (2017) proved resting HRV to be a sensitive diagnostic tool for diagnosing early CAN in asymptomatic subjects. However, they performed ROC curve analysis only for the frequency domain measures of HRV. Therefore, a direct comparison between the results of time domain measures of HRV in the present study and those of Jelinek et al. (2017) could not be made. Out of the power spectral components, LF power was shown to be 100% sensitive and 29.4% specific for diagnosing CAN at the cut-off value of ≤1017 ms^2, which is higher than the clinical cut-off derived from our study. This might be explained by the inclusion of patients who have been in the early stages of CAN without diagnosed diabetes in the study of Jelinek et al. (2017).

Sacre et al. (2012) investigated the diagnostic accuracy of post-exercise HRR at 1, 2, and 3 min after a maximal aerobic exercise bout in T2DM patients without any cardiovascular disease. The results of their study demonstrated HRR to be a good diagnostic tool with high sensitivity and moderate specificity [sensitivity and specificity at 1- (93% and 69%) and 2-min HRR (96% and 63%)]. Sacre et al. (2012) recommended HRR monitoring during exercise testing in order to screen patients with CAN. Moreover, in accordance with our results, post-exercise autonomic measure, that is, HRR was found to be positively associated with standard CARTs in their study. Further, Banthia et al. (2013) came up with a novel exercise-based CAN assessment method for T2DM patients. They suggested that investigating CAN during post-exercise recovery period is of utmost importance since post-exercise period allows assessment of a broader part of the dynamic range of autonomic effects. In their investigation, early post-exercise recovery after a submaximal cycle ergometer exercise protocol was observed under parasympathetic blockade. Post-exercise autonomic recovery was blunted as observed through HRR, which is in accordance with the findings of the present study and the results of Sacre et al. (2012). Diminished autonomic recovery was attributed to diminished parasympathetic effect and higher sympatho-excitation during post-exercise recovery period in T2DM patients (Banthia et al., 2013).
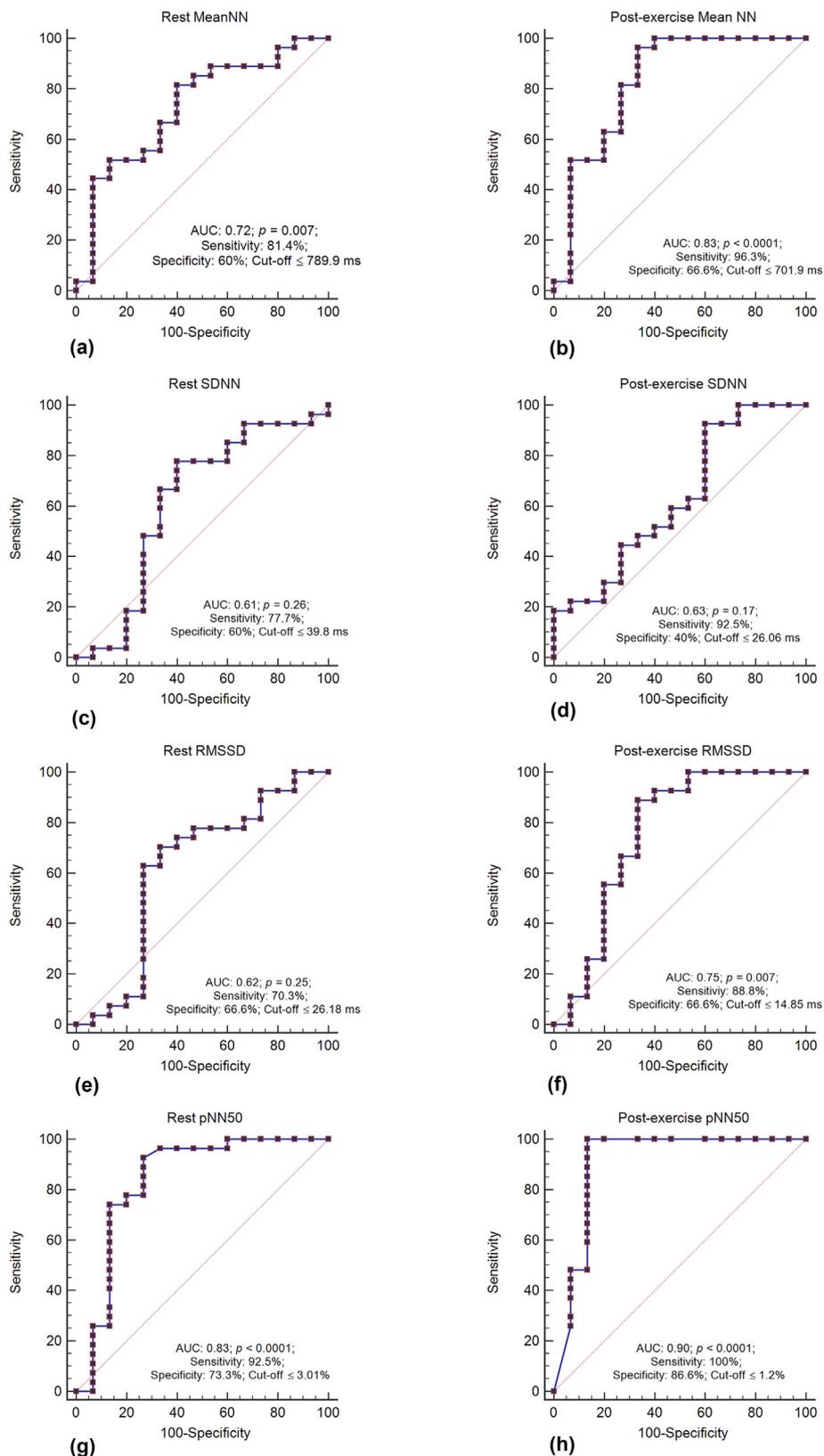
Fig. 2. ROC curve analysis of resting and post-exercise Mean NN (a and b); SDNN (c and d); RMSSD (e and f); and pNN50 (g and h) ROC: receiver operating characteristic; Mean NN: average of N–N intervals; SDNN: standard deviation of N–N intervals; RMSSD: root mean square of successive differences between adjacent R-R intervals; pNN50: percentage of consecutive N–N intervals that vary by > 50 ms.
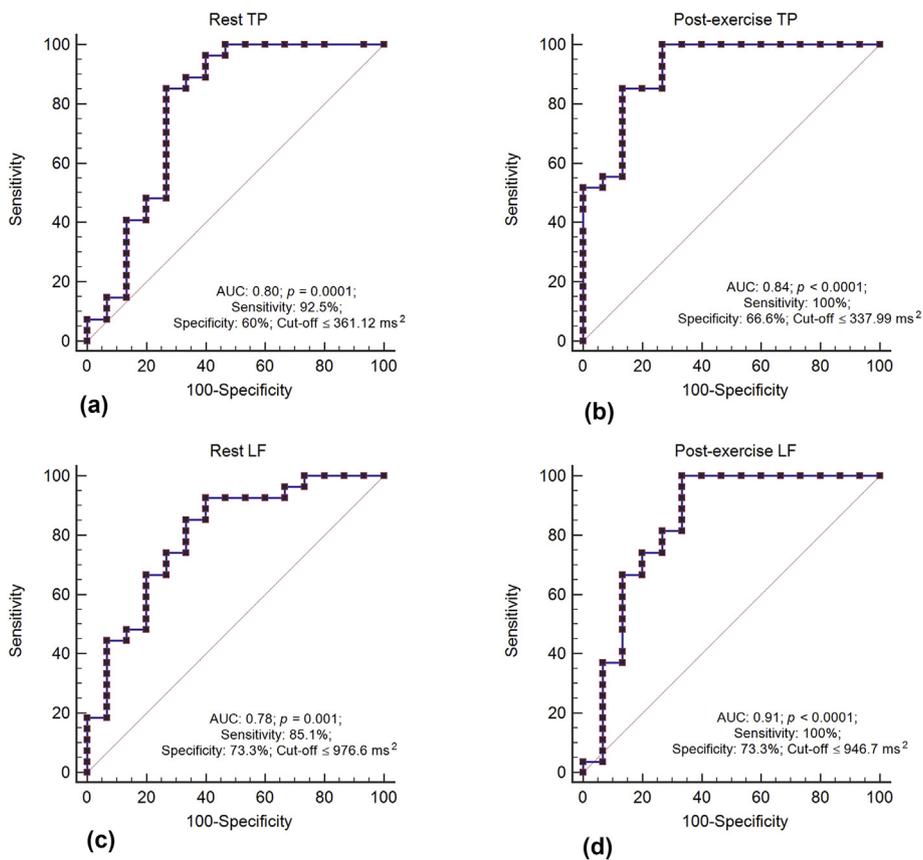
Fig. 3. ROC curve analysis of resting and post-exercise LF power (a and b) and TP (c and d); ROC: receiver operating characteristic; LF: low frequency; TP: total power.
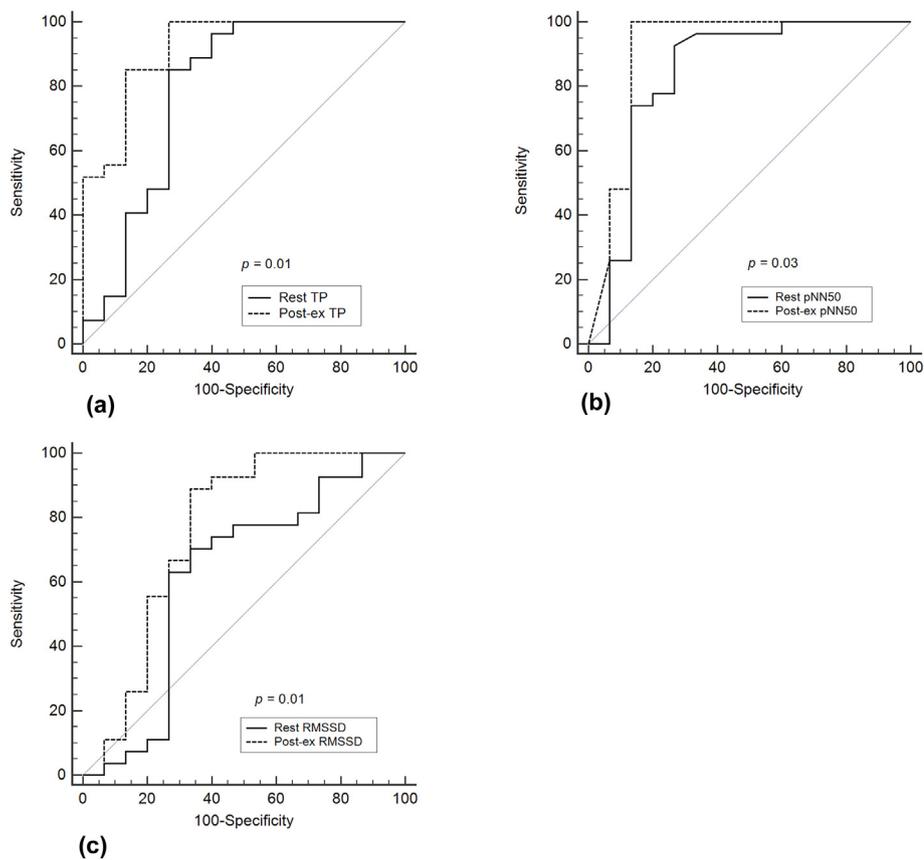
Fig. 4. Comparative ROC curve analysis demonstrating significant differences between resting and post-exercise (a) TP (b) pNN50 and (c)RMSSD; ROC: receiver operating characteristic curve; TP: total power; RMSSD: root mean square of successive differences between adjacent R-R intervals; pNN50: percentage of consecutive N–N intervals that vary by > 50 ms; Rest: resting; Post-ex: post-exercise.

Table 4

Details of diagnostic performance parameters for resting and post-exercise heart rate variability indices.

HRV variables	TP	FP	TN	FN	PPV (95% CI)	NPV (95% CI)	PLR (95% CI)	NLR (95% CI)	Accuracy
Resting HRV									
Mean NN	22	6	9	5	–	–	2.04 (1.3–3.2)	0.31 (0.1–0.8)	73.8%
SDNN	21	6	9	6	77.8 (57.7–91.4)	60.0 (31.3–84.4)	1.94 (1.2–3.1)	0.37 (0.1–0.9)	71.4%
RMSSD	19	5	10	8	79.2 (57.8–92.9)	55.6 (30–79.1)	2.11 (1.4–3.3)	0.44 (0.2–1.1)	69.0%
pNN50	25	4	11	2	86.2 (68.3–96.1)	84.6 (53–98.3)	3.47 (2.5–4.8)	0.10 (0.02–0.5)	85.7%
TP	23	4	11	4	85.2 (66.3–95.8)	73.3 (43.8–92.7)	3.19 (2.3–4.5)	0.20 (0.06–0.7)	85.7%
LF	25	6	9	2	80.6 (62.5–92.5)	81.8 (46.3–98.1)	2.31 (1.5–3.5)	0.12 (0.03–0.5)	80.9%
LFnu	18	4	11	9	85.7 (63.0–97.1)	57.1 (34.0–78.2)	3.33 (2.3–4.8)	0.42 (0.1–1.3)	69.0%
HF	26	4	11	4	83.9 (66.3–94.5)	90.9 (56.6–99.8)	2.89 (2.0–4.2)	0.05 (0.007–0.4)	85.7%
HFnu	15	1	14	12	94.1 (70.3–99.9)	56.0 (34.9–75.6)	8.89 (6.3–12.5)	0.44 (0.06–3.1)	69.0%
LF/HF ratio	16	3	12	11	88.9 (64.4–98.7)	54.2 (32.4–74.9)	4.44 (3.1–6.4)	0.47 (0.1–1.8)	66.6%
Post-exercise HRV									
Mean NN	26	5	10	1	83.9 (66.3–94.5)	90.9 (56.6–99.8)	2.89 (2.0–4.2)	0.05 (0.007–0.4)	85.7%
SDNN	25	9	6	2	73.5 (55.3–87.3)	75 (34.9–96.8)	1.48 (0.8–2.8)	0.19 (0.05–0.7)	73.8%
RMSSD	24	5	10	3	82.8 (64.2–94.2)	62.5 (34.5–85.4)	2.67 (1.8–3.9)	0.17 (0.05–0.6)	80.9%
pNN50	27	2	13	0	93.1 (77.2–99.2)	100 (73.5–100)	7.5 (6.1–9.1)	0.00	95.2%
TP	27	4	11	0	87.1 (70.2–96.4)	100 (69.2–100)	3.75	0.00	90.4%
LF	16	3	12	11	84.4 (67.2–94.7)	100 (66.4–100)	3.0 (2.1–4.3)	0.00	66.6%
LFnu	23	4	11	4	84.6 (65.1–95.6)	68.7 (41.3–89.0)	3.19 (2.3–4.5)	0.20 (0.08–0.8)	80.9%
HF	24	3	12	3	88.5 (73.4–95.3)	75 (54.0–88.5)	4.44 (1.6–12.3)	0.14 (0.05–0.5)	85.7%
HFnu	17	6	9	10	75.0 (60.4–85.5)	50.0 (33.7–66.3)	1.67 (0.8–3.3)	0.56 (0.3–1.1)	61.9%
LF/HF ratio	23	7	8	4	80.8 (66.6–89.8)	62.5 (43.0–78.6)	2.33 (1.1–4.9)	0.33 (0.2–0.7)	73.8%

HRV: heart rate variability; TP: true positives; FP: false positives; TN: true negatives; FN: false negatives; PPV: positive predictive value; NPV: negative predictive value; PLR: positive likelihood ratio; NLR: negative likelihood ratio; Mean NN: Average of N–N intervals; SDNN: standard deviation of N–N intervals; RMSSD: root mean square of successive differences between adjacent R-R intervals; pNN50: percentage of consecutive N–N intervals that differ by > 50 milliseconds; %: percent; TP: total power; LF: low frequency power; HF: high frequency power; nu: normalized units LF/HF ratio: ratio of low and high frequency power; ms: milliseconds; CI: confidence intervals.

Parasympathetic effect on R-R interval and QT-–RR slope was found to be blunted significantly ($p = 0.004$) in their study (Banthia et al., 2013).

In contrast, an earlier investigation (Tank et al., 2001) has shown HRV to be a less accurate test to diagnose CAN in comparison to Ewing's test battery. However, there are several issues in the methodology and statistical approach used by Tank et al. (2001). Previously defined cut-off values for HRV were used for discriminant analysis, and cut off values from its original data were not derived. Conclusions were reached merely on the basis of discriminant analysis and standard ROC curve analysis was not performed. Moreover, a direct comparison with the gold standard of CAN diagnosis (CARTs) would definitely lead to inferior ability of the reference test (HRV) in diagnosing diabetic CAN. Another recent study on Chinese population (Chen et al., 2015) indicated that short-term HRV under resting conditions was not inferior to standard Ewing's test battery for CAN testing based on the non-inferiority test analysis. The estimated median sensitivity and specificity of HRV test were upto 0.80 for diagnosing CAN in their study (Chen

et al., 2015). Khandoker et al. (2009) have shown that non-linear HRV measures such as sample entropy and SD1/SD2 ratio are more sensitive and specific markers of CAN than traditional linear measures in DM with an AUC of 0.79 and 0.70, respectively. In their study, the resting time and frequency domain HRV measures did not demonstrate good diagnostic ability. These results are in contrast to the findings of the present study and may be attributed to the use of only one test from the Ewing's test battery (SBP response to postural stress) in their study (Khandoker et al., 2009). The use of a single test from the entire battery might have left out many positive cases with parasympathetic dysfunction and the observed positive cases might have been detected by more sensitive complexity measures.

Regarding the diagnostic ability of post-exercise HRV, only a few studies seem to have been carried out on patients with CAD. Virtanen et al. (2007) compared the diagnostic performance of HRV measured at rest, during and immediately after exercise for detecting CAD and found better diagnostic performance by post-exercise HRV as compared with HRV taken at rest and during exercise. In their study (Virtanen et al.,

Table 5
Univariable logistic regression models predicting cardiac autonomic neuropathy.

Variable	Odds ratio (95% CI)	p-Value
Post-exercise HRV		
Mean NN (continuous)	0.98 (0.96–0.99)	0.002*
Mean NN (≤ 701.9 ms)	52.00 (5.38–502.08)	0.001*
RMSSD (continuous)	0.84 (0.74–0.95)	0.008*
RMSSD (≤ 14.85 ms)	16.0 (3.19–80.06)	0.001*
pNN50 (continuous)	0.06 (0.01–0.35)	0.001*
pNN50 ($\leq 1.20\%$)	2.18	0.99
TP (continuous)	0.99 (0.99–0.99)	0.001*
TP (≤ 946.7 ms^2)	1.09 (0.00-)	0.99
LF (continuous)	0.99 (0.98–0.99)	0.002*
LF (≤ 337.9 ms^2)	8.72	0.99
HF (continuous)	0.98 (0.98–0.99)	0.001*
HF (≤ 275.5 ms^2)	52.0 (5.38–502.08)	0.001*
Age	1.04 (0.93–1.16)	0.43
Diabetes duration	1.15 (0.99–1.33)	0.06
HbA1c	1.59 (1.00–2.52)	0.04*
Number of co morbidities	3.01 (1.10–8.20)	0.03*
SBP	1.05 (1.00–1.11)	0.051
VO$_{2max}$	0.86 (0.78–0.96)	0.009*
MET-min/week	1.00 (1.00–1.00)	0.85
Gender	0.30 (0.57–1.66)	0.17

HRV: heart rate variability; Mean NN: Average of N–N intervals; SDNN: standard deviation of N–N intervals; RMSSD: root mean square of successive differences between adjacent R-R intervals; pNN50: percentage of consecutive N–N intervals that differ by > 50 milliseconds; TP: total power; LF: low frequency power; HF: high frequency power; nu: normalized units LF/HF ratio: ratio of low and high frequency power; HbA1c: glycosylated hemoglobin; SBP: systolic blood pressure; VO$_{2max}$: maximum oxygen consumption; MET: metabolic equivalents; CI: confidence intervals; ms: milliseconds; nu: normalized units; *significant; p-value: probability value.

2007), time domain measures of HRV such as SDNN and RMSSD, showed an AUC of 0.75 and 0.74, respectively, immediately after a maximal exercise bout performed on a cycle ergometer. Later, another study (Bailon et al., 2011) suggested using HRV during and after exercise to detect CAD and reported an HRV accuracy of 76% for detecting CAD. Although, to our knowledge, no such study has been done on T2DM patients for detecting CAN and the results of the present study indicated altered HRV during post-exercise recovery period in T2DM patients with CAN, which were found to be of higher diagnostic value than resting HRV measures. Similar to the findings of Sacre et al. (2012) and Banthia et al. (2013), the results of the present study also indicate that post-exercise recovery phase provides greater diagnostic information about the presence of CAN in T2DM patients. Altered HR dynamics after exercise in T2DM patients has been noted by previous investigations (Neves et al., 2011; Sacre et al., 2012) and, according to the present findings, it is further exaggerated in the presence of CAN.

5.4. Physiology of post-exercise recovery period

The previous literature (Neves et al., 2011) has shown that the stress inflicted by exercise in order to trigger cardiovascular events is not only limited to the actual exercise bout, but also extends to the recovery phase after exercise that certainly makes this phase vulnerable for various cardiovascular events. During exercise, HR is regulated by sympathetic (SNS) and parasympathetic nervous system (PNS) in a reciprocal manner, i.e., increased sympathetic activity is accompanied by a decrease in parasympathetic activity, in which SNS works as a tone setter and PNS acts as a modulator. However, due to temporal differences in the recovery pattern of autonomic arms, this reciprocal interplay is altered in the recovery phase after exercise. There is parasympathetic reactivation for a rapid restoration of vagal activity along with a long-lasting hyperactivity of SNS due to its longer latency to return to the baseline after completion of exercise (Michael et al.,

2017). However, this recovery pattern or autonomic recovery per se assessed by HRV has been found altered in some clinical conditions including DM (Neves et al., 2011). Absence or alteration of the parasympathetic reactivation and certainly a prolonged exaggeration in sympathetic activity during the recovery phase after exercise may explain altered recovery dynamics in T2DM patients (Banthia et al., 2013).As proposed and found by the present study, this alteration in autonomic recovery could be of diagnostic importance to the clinicians and researchers working with T2DM patients. In the present study, the diagnostic ability of post-exercise HRV was maintained even after adjusting various relevant clinical parameters (such as VO$_{2max}$, HbA1C and number of comorbidities) that may modulate or alter cardiac autonomic function, which clearly indicates the diagnostic robustness of this test for CAN.

5.5. Strengths and limitations

The present study has some potential strength. All ECG recordings and HRV analyses were performed in accordance with the standard guidelines proposed by Taskforce (1996). Moreover, this study provides definitive evidence that post-exercise HRV is a superior diagnostic indicator of CAN than resting HRV measures in T2DM patients. However, there are several limitations to this study which should be taken into consideration while interpreting the results: (i) We did not exclude patients on medications that have been found to affect autonomic function (although their usage prior to testing was prohibited).Therefore, medication-induced alterations might have confounded our results, although we tried to control it by refraining the subjects from using such medications 24 h prior to autonomic function testing. (ii) Only traditional time- and frequency-domain HRV indices were analyzed due to their wider application and ease in analysis. More sensitive complexity measures might have elicited more precise results since they are found to be more reliable measures during post-exercise recovery phase. (iii) Existence of more comorbidities in the CAN-positive group could be another confounder to the alteration in autonomic function, which was nevertheless adjusted in the multivariable logistic regression. (iv) HRR values were obtained through ECG during the transitional phase and thus might have been influenced by the procedures performed during this phase. (v) Since we recorded autonomic recovery in a passive supine position, generalizations to exercise tests involving active recovery protocols could not be made.

5.6. Future recommendations

We recommend more research on the use of non-linear HRV measures monitoring in post-exercise recovery period to ascertain whether non-linear measures of HRV give more accurate diagnostic information regarding CAN owing to their superior validity and reliability on non-stationary signals in the post-exercise period. There should be more investigations to provide conclusive evidence on the role of exercise-based assessment of diabetic CAN. Also, assessment of HRV dynamics during the entire recovery period including early and late phases may give more information regarding its alteration and correction during post-exercise period. Various confounding factors that affect HRV and cardiac autonomic function should be controlled appropriately in future researches. Further, an assessment of the hormonal indicators of autonomic deterioration, such as catecholamines along with HRV, may provide additional information regarding CAN in T2DM.

6. Conclusion

The present study suggests that post-exercise HRV (TP, RMSSD and pNN50) is a superior diagnostic indicator of CAN than resting HRV in T2DM patients. Including post-exercise HRV data in the report of clinical exercise testing may provide additional information on CAN in T2DM patients. Higher sensitivity than specificity of post-exercise HRV

Table 6

Multivariable logistic regression models predicting cardiac autonomic neuropathy.

Variable	Model 1		Model 2	
Post-exercise HRV parameters	Odds ratio (CI)	*p*-Value	Odds ratio (CI)	*p*-Value
Mean NN (continuous)	0.98 (0.97–1.00)	0.04*	–	–
Mean NN	–	–	27.72	0.01*
(≤ 701.9 ms)			(2.06–372.41)	
HbA1c	1.01 (0.54–1.87)	0.96	1.26 (0.66–2.39)	0.46
VO$_{2max}$	0.94 (0.83–1.05)	0.30	0.95 (0.84–1.08)	0.45
Number of co morbidities	1.60 (0.53–4.82)	0.40	1.03 (0.30–3.49)	0.95
RMSSD (continuous)	0.87 (0.75–1.01)	0.07	–	–
RMSSD	–	–	12.10	0.01*
(≤ 14.85 ms)			(1.82–80.03)	
HbA1c	1.10 (0.61–1.98)	0.75	1.05 (0.57–1.92)	0.86
VO$_{2max}$	0.91 (0.80–1.02)	0.12	0.89 (0.79–1.02)	0.10
Number of co morbidities	1.98 (0.57–6.82)	0.27	2.27 (0.59–8.69)	0.23
pNN50 (continuous)	0.02 (0.001–0.54)	0.01*	–	–
pNN50 ($\leq 1.2\%$)	–	–	0.00	0.99
HbA1c	0.68 (0.27–1.70)	0.42	0.66 (0.24–1.80)	0.42
VO$_{2max}$	0.76 (0.56–1.04)	0.09	0.80 (0.60–1.06)	0.13
Number of co morbidities	3.42 (0.31–37.03)	0.31	3.53 (0.19–64.71)	0.39
Rest TP (continuous)	0.99 (0.99–0.99)	0.005*	–	–
Rest TP	–	–	0.00	0.99
(≤ 946.7 ms^2)				
HbA1c	1.49 (0.64–3.45)	0.35	6.15 (0.64–58.97)	0.11
VO$_{2max}$	0.98 (0.86–1.12)	0.78	0.86 (0.67–1.11)	0.26
Number of comorbidities	1.33 (0.28–6.16)	0.71	1.70 (0.14–20.27)	0.67
LF (continuous)	0.99 (0.98–0.99)	0.01*	–	–
LF (≤ 337.99 ms^2)	–	–	7.41	0.99
HbA1c	1.21 (0.67–2.20)	0.51	1.31 (0.51–2.91)	0.50
VO$_{2max}$	0.95 (0.84–1.07)	0.40	0.89 (0.75–1.05)	0.18
Number of co morbidities	1.55 (0.43–5.53)	0.49	2.22 (0.45–10.98)	0.32
HF (continuous)	0.98 (0.98–0.99)	0.006*	–	–
HF (≤ 275.53 ms^2)	–	–	24.6 (3.26–186.64)	0.002*
HbA1c	1.47 (0.71–3.04)	0.29	1.42 (0.68–2.94)	0.34
VO$_{2max}$	0.93 (0.82–1.06)	0.29	0.90 (0.79–1.03)	0.90
No of co morbidities	1.37 (0.32–5.89)	0.66	1.13 (0.28–4.57)	0.86

Continuous HRV parameters were used in Model 1 and dichotomous HRV parameters based on their respective cut-off points were used in Model 2, both controlling for HbA1c,VO$_{2max}$ and number of co morbidities; HRV: heart rate variability; Mean NN: Average of N–N intervals; RMSSD: root mean square of successive differences between adjacent R-R intervals; pNN50: percentage of consecutive N–N intervals that differ by > 50 milliseconds; TP: total power; LF: low frequency power; HF: high frequency power; VO$_{2max}$: maximum oxygen consumption; ms: milliseconds; %: percent; HbA1c: glycosylated hemoglobin

points to its utility in screening at-risk patients. The HRV measures proposed here may be incorporated in the exercise test reports by clinical exercise laboratories for at least primary screening purposes. We recommend the use of post-exercise HRV assessment as an adjunct tool in the diagnosis of CAN along with other standard clinical autonomic tests (CARTs and resting HRV).

Author contributions

PB undertook collection and analysis of the data, interpreted the results and wrote the initial draft of the manuscript. JAM contributed to the study design, statistical analysis and the final draft of the manuscript. MEH contributed to the design of the study, along with critical revisions of each draft of the manuscript. IHN helped in data collection and in preparing the final draft of the manuscript. All the authors have approved the final draft of the manuscript.

Conflict of interest

None.

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