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Original Article

Co-Existence of hypertension worsens post-exercise cardiac autonomic recovery in type 2 diabetes

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ABSTRACT

Background: Cardiac autonomic neuropathy (CAN) is a commonly overlooked complication of Type 2 Diabetes Mellitus (T2DM) characterized by imbalance between sympathetic and parasympathetic supply to the heart. The susceptibility of heart to dysrhythmias and fatal events increases during and after exercise due to a shift in autonomic regulation. Diabetes and hypertension (HTN) frequently occur concurrently and both conditions lead to impaired cardiac autonomic control. However, their impact together on post-exercise autonomic recovery remains to be explored.

Objective: The objective of the study was to investigate the effect of co-existence of HTN on cardiac autonomic recovery (assessed by heart rate recovery and heart rate variability) in patients with T2DM.

Methods: Forty eight type 2 diabetic patients (24 normotensive, 24 hypertensive), 24 non-diabetic patients with essential HTN, and 27 healthy controls, were recruited into the study and assessed for heart rate recovery (HRR) following a graded maximal test. Also, heart rate variability (HRV) was recorded before and following the bout of maximal exercise.

Results: Heart rate recovery at 1 (HRR_{1min}) and 2 (HRR_{2min}) minute(s) showed significant effects for DM ($p < 0.001$) and HTN ($p < 0.001$), while DM × HTN interaction was found to be non-significant. Resting HRV showed a significant decline in time-domain variables for the DM group ($p < 0.01$). Recovery of HRV showed a significant effect of time ($p < 0.05$) for all indices, the group effect was found significant only for time-domain measures ($p < 0.05$).

Conclusion: Both HRR and HRV recovery were impaired in DM and HTN. Moreover, the co-existence of HTN had a synergistic effect, causing further worsening of autonomic recovery in T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a growing epidemic associated with a spectrum of complications, making it a multi-faceted metabolic disorder with 2- to 4-fold higher incidence of cardiovascular disease (CVD) [1] Hypertension (HTN) is one such co-morbidity, that is twice as frequent among patients with type 2 diabetes, compared with non-diabetic population. [2] Its prevalence is around 40% among patients with newly diagnosed type 2 diabetes [3] but can be as high as 49–92% in some parts of the world. [4,5] The coexistence of DM and HTN is mainly the consequence of metabolic syndrome, abdominal obesity, diabetic nephropathy, and advanced atherosclerosis.[5] About 60%–80% people with type 2 diabetes die of cardiovascular complications, and up to 75% of specific cardiovascular complications have been attributed to high blood pressure (BP).[2,6] Moreover, hypertension is also a primary contributing factor to kidney failure and eye ailments in people with diabetes. [7,8]

A commonly overlooked complication of T2DM is cardiac autonomic neuropathy (CAN), characterized by imbalance between sympathetic and parasympathetic supply to heart. CAN is found to be associated with severe cardiovascular events such as ventricular tachyarrhythmia and sudden cardiac deaths. It has been reported to occur in 42% of patients with T2DM, with a higher incidence (54–60%) reported in the Indian population [9,10] In addition, it has been found that hypertension independently causes cardiac autonomic dysfunction. Hazari et al [11] examined cardiac autonomic control of diabetics with and without HTN, and found that the presence of HTN in T2DM patients synergistically causes progression of CAN. Pikkujamsa et al [12] proposed that essential hypertension acts synergistically with type 2 diabetes to depress cardiac reflex vagal and sympathetic function, and that

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insulin resistance may play a pathogenic role in these processes. Liao et al [13] also reported that HTN clustered with diabetes synergistically influenced cardiac autonomic control. More recently, Istenes et al [14] examining the effect of co-existence of diabetes and HTN on autonomic function, compared heart rate variability and ambulatory blood pressure monitoring (ABPM) parameters across the groups, found a similar additive effect. However, all these previous investigations have explored resting autonomic function but the impact of DM and HTN on autonomic modulation following exercise is not known.

Exercise has profound and complex effects on autonomic heart rate modulation [15] Acute physical exertion causes physiological stress affecting the cardiac autonomic modulation. [16] During transition from rest to exercise and the recovery period following exercise, the heart has been suggested to be more vulnerable to dysrhythmias and fatal events, owing to a shift in the autonomic balance from parasympathetic to sympathetic dominance. [17,18] An increased risk of death during and following exercise has been attributed to both the magnitude and subsequent restoration of normal autonomic balance. [19] Thus, exploration of the behavior of autonomic recovery following exercise is warranted. As proposed by previous investigations, [16,20] the spectral analysis of HRV can be used to delineate the autonomic disturbance after exercise cessation and during recovery. Therefore, the present study aimed to investigate the effect of co-existence of hypertension on cardiac autonomic recovery in type 2 diabetics. We hypothesized that autonomic recovery after the maximum exercise bout is compromised, and co-existence of hypertension causes further impairment of autonomic recovery in T2DM patients.

2. Methods

2.1. Study design and setting

A four-arm, cross-sectional, observational study design with comparison of variables across the groups was employed for the present study. The type 2 diabetic and hypertensive patients were recruited from the outpatient department of Ansari Health Centre, Jamia Millia Islamia, New Delhi, India, and age-matched healthy controls were recruited from the community. Assessments for heart rate recovery and variability were performed in the Human Performance Laboratory, Centre for Physiotherapy and Rehabilitation Sciences, Jamia Millia Islamia.

2.2. Participants

We recruited 48 type 2 diabetic patients (24 normotensive, 24 hypertensive), 24 non-diabetic patients with essential HTN, and 27 healthy controls into the study.

Sedentary participants (normotensive diabetics, hypertensive diabetics, hypertensives, and healthy controls) who did not engage in exercise for more than 20 min on 3 or more days a week, between 30–70 years of age, were recruited. Patients in the diabetic group were diagnosed in accordance with American Diabetes Association criteria (HbA1c level 6.5–10%) with type 2 diabetes for a minimum of 12 months and were receiving oral antidiabetic medication. The diagnosis of HTN was made when the average of 2 or more diastolic BP measurements on at least 2 subsequent visits is $\geq$90 mmHg or when the average of multiple systolic BP readings on 2 or more subsequent visits is consistently $\geq$140 mmHg [21] or the use of an anti-hypertensive medication (stage 1 and 2 hypertensives).

Exclusion criteria included participants with BMI > 40 kg/m^2, hypertensive crisis (systolic blood pressure, SBP > 180 mmHg; diastolic blood pressure, DBP > 120 mmHg), the use of exogenous insulin, any drugs that can influence heart rate kinetics, such as, beta-blockers and non-dihydropyridine type calcium channel blockers, recent onset of cardiac-origin symptoms, and orthopedic problems limiting physical activity.

2.3. Procedures

The present study was approved by the Institutional Ethical Committee, JMI and was conducted in accordance with the Helsinki Declaration. Participants were recruited based upon the inclusion and exclusion criteria, and informed about the study purpose and methodology. They were given an informed consent form explaining their rights as research subjects and a written consent was obtained. All participants were asked to refrain from the consumption of caffeine, alcohol and tobacco products at least 12 h before testing. In addition, they were also asked to abstain from any kind of exercise 48 h prior to testing. All four groups were assessed for resting heart rate variability, following which, they performed the graded maximal exercise testing on a treadmill and their peak heart rate was recorded. HR response during exercise was evaluated by the chronotropic reserve (CR), as follows: (chronotropic reserve = (peak HR-resting HR/220-age-resting HR) $\times$ 100) [22] and chronotropic incompetence (CI) was determined when subjects failed to achieve $\geq$ 80% of the chronotropic reserve. [22] Immediately after the treadmill test, HRR was recorded for 2 min and post-exercise HRV was recorded for 20 min.

2.3.1. Heart rate recovery (HRR)

Heart rate recovery is a measure of the rate of decline of heart rate during the first few minutes after peak exercise [23] Heart rate was derived from a continuous record obtained via electrocardiography (ECG) with surface electrodes placed in a Lead II arrangement. Lead site preparation and placement were standardized according to American Heart Association standards. [24] HRR was calculated as the absolute difference between heart rate at peak exercise and heart rate recorded at 1- (HRR_{1min}) and 2-minutes (HRR_{2min}) post-exercise.

2.3.2. Heart rate variability (HRV)

Electrocardiographic (ECG) signals were recorded for 20 min in supine position before graded exercise test. Participants were instructed to close their eyes, and avoid any bodily movement or conversation during recording. Since breathing rate has been found to confound the HRV results, breathing pace was controlled at 12 breaths/min with a metronome. Immediately following the maximal exercise using Balke protocol, participants were allowed a 2-minute transition from the treadmill, before comfortably assuming the supine position again. The post-exercise ECG was recorded for 20-minutes. After recording, all the data were stored and analyzed offline. Analysis of HRV was done on a time series of the last five-minutes selected from the 20-min recording [25] both at rest and post-exercise. Data were visually and automatically inspected for ectopic beats (premature, supraventricular, and ventricular) which had to be <10% for each record to be included in the analysis. Time and frequency domain variables of HRV were analyzed by the detection of R waves. AD instruments lab chart version 7.3.7 with HRV module version 1.4.2 using weltch window (Power Lab 8 SP, AD Instruments, Australia) was used as data acquisition software for recording ECG, which calculates the R-R intervals as the measure of difference between successive beats. All data acquisition and post-acquisition analyses was performed in accordance with the guidelines proposed by Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology [26]. Time-domain variables of HRV included mean of normalized R-R intervals (AvgNN), standard deviation of N-N intervals (SDNN), square root of the mean squared differences between adjacent R-R intervals (RMSSD) and

Table 1
Comparison of the demographic, clinical characteristics and heart rate during exercise test between groups.

	Healthy n = 27 Mean (SD)	DM n = 24 Mean (SD)	HTN n = 24 Mean (SD)	DMHTN n = 24 Mean (SD)	*p*-value
Age (yrs)	50.4 (4.1)	51.2 (5.9)	49 (3.6)	48.5 (3.6)	0.133
Height (cm)	160.7 (7.8)	158.6 (5.8)	161.8 (7.4)	157.9 (8.6)	0.237
Weight (kg)	77.7 (8.7)	74.2 (8.7)	72.6 (8.6)	73.5 (10.1)	0.19
BMI (kg/m^2)	29.4 (3.1)	27.8 (2.4)	29.5 (3.3)	30.1 (2.5)	0.035*
Sex (M/F)	15/12	11/13	9/15	10/14	0.605
DM duration (yrs)	–	6.9 (3.4)	–	7.2 (3.3)	0.8
HTN duration (yrs)	–	–	7.1 (3.2)	8.1 (3.4)	0.3
SBP (mmHg)	121.7 (6.4)	125.3 (8.6)	144.6 (8.9)	146.3 (9.7)	<0.001*
DBP (mmHg)	81.3 (4.3)	79.9 (5.1)	84.9 (5.4)	85.2 (5.6)	0.001*
HbA1c (%)	5.9 (0.2)	8.2 (1.2)	6.0 (0.2)	8.6 (1.4)	<0.001*
Ex HR (bpm)	157.4 (9.3)	151.5 (6.6)	150.9 (8.3)	141.3 (6.3)	<0.001*
HR_{1min} (bpm)	119.8 (9.2)	120.1 (8.6)	120.7 (8.5)	114 (8.0)	0.027*
HR_{2min} (bpm)	97 (7.4)	100.8 (7.5)	100.7 (6.8)	98.5 (6.2)	0.181
CR	88.27 (11.4)	77.9 (7.4)	79.8 (10.7)	66.7 (9.6)	<0.001*
CI n (%)	7 (25.9)	18 (75)	13 (54.2)	21 (87.5)	<0.001*
Comorbidities					
Dyslipidemia	–	14	11	10	–
Hypothyroidism	–	4	3	5	–
PCOD	–	4	–	3	–
Medications					
Metformin	–	11	–	9	–
Sulfonylureas	–	7	–	8	–
TZDs	–	1	–	2	–
DPP-4 Inhibitors	–	3	–	4	–
AGIs	–	1	–	3	–
ACE inhibitors	–	–	14	16	–
ARBs	–	–	11	13	–
Statins	–	8	6	3	–

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HbA1c: Glycosylated hemoglobin; Ex HR: Peak exercise heart; HR_{1min}: Heart rate at the end of first minute following maximal exercise; HR_{2min}: Heart rate recovery at the end of 2 min following maximal exercise; CR: Chronotropic Reserve; CI: Chronotropic Incompetence; PCOD: Polycystic Ovarian Disease; TZDs: Thiazolidinediones; DPP-4: Dipeptidyl peptidase-4; AGIs: Alpha-Glucosidase Inhibitors; ACE: Angiotensin-Converting Enzyme; ARBs: Angiotensin-II Receptor Blockers.
* significant difference.

percentage of interval differences of adjacent R-R intervals greater than 50 ms (pNN50). [26,27] Frequency-domain indices of HRV included total power, Low Frequency (LF: 0.04-0.15 Hz), High Frequency (HF: 0.15-0.40 Hz) and LF/HF ratio. Non-linear analysis included the Poincare parameters: SD1 and SD2. [28] Combination of all these variables of HRV comprehensively provides information regarding the relative contributions from sympathetic and parasympathetic branches of ANS to the heart. [29]

2.4. Sample size

The number of subjects (n = 99) was determined using Software G. Power 3.192 using the difference in heart rate variability (LF power) between 4 similar groups, from a study done by Istenes et al [14] and a total of 96 subjects (24 per group) were shown to be necessary based on the effect size of 0.348, alpha level of 0.05 and power (1-beta) of 0.80.

2.5. Statistical analyses

The normality of distribution was examined for all variables using Kolmogorov-Smirnov test, and those found to be non-normal (RMSSD, pNN50, Total power, LFms [2] HFms [2] and SD2) were log transformed for further analyses. Subject demographic characteristics were compared between the groups using One-way ANOVA and those found to be different (BMI) were adjusted. To determine the effect of Diabetes (DM), Hypertension (HTN), and their interaction (DMHTN), univariate analysis was employed with DM and HTN as fixed factors. The HRV recovery was compared between the groups using a 4×2 split plot ANCOVA, assessing the effect of group (Healthy, DM, HTN, DMHTN) and time (resting, post-exercise), with the resting values of HRV treated as covariates. Significance level was set at $p < 0.05$.

3. Results

The comparison of demographic characteristics using one-way ANOVA revealed no significant differences among the groups except for BMI ($p = 0.035$). Post-hoc analysis showed a significantly higher BMI in DMHTN than DM group. For this reason, BMI was considered as a covariate in all further analysis. In addition to expected differences in resting BP and HbA1c between the groups, a significant difference was observed for the peak exercise heart rate achieved (Ex HR) ($p < 0.001$) and the heart rate at 1 min following exercise (HR_{1min}) ($p = 0.027$) (Table 1). Post-hoc analysis for Ex HR and HR_{1min} revealed significantly lower values in DMHTN as compared to the other groups.

The HR response to exercise as determined by CR and CI were found to be significantly different between the groups (Table 1). Post hoc analysis of CR found significant reductions in DM ($p = 0.002$), HTN ($p = 0.002$) and DMHTN ($p < 0.001$) as compared to the healthy controls. Further, CR in DMHTN was also lower in comparison to both DM ($p = 0.001$) and HTN ($p = 0.001$), with CI being recorded in 87% of the DMHTN patients (Table 1).

3.1. Heart rate recovery

Heart rate recovery at 1(HRR_{1min}) and 2 (HRR_{2min}) minute(s) showed significant effects for DM ($p < 0.001$) and HTN ($p < 0.001$), while DM × HTN interaction was found to be non-significant (Table 2). Post hoc analysis revealed that HRR_{2min} in the DMHTN group was significantly lower than the other groups ($p < 0.05$) (Fig. 1).

Table 2
Effect of DM and HTN on Heart Rate Recovery.

	Healthy n = 27 Mean(SD)	DM n = 24 Mean(SD)	HTN n = 24 Mean(SD)	DMHTN n = 24 Mean(SD)	DM main effect	HTN main effect	DM × HTN interaction
HRR_{1min}	37.5 (5.6)	31.3 (5.8)	30.1 (4.8)	27.3 (5.1)	<0.001*	<0.001*	0.155
HRR_{2min}	60.3 (7.3)	50.7 (6.6)	50.2 (7.3)	42.8 (4.7)	<0.001*	<0.001*	0.487

HRR_{1min}: Heart rate recovery in the first minute following maximal exercise; HRR_{2min}: Heart rate recovery in the first 2 min following maximal exercise.
* significant difference.

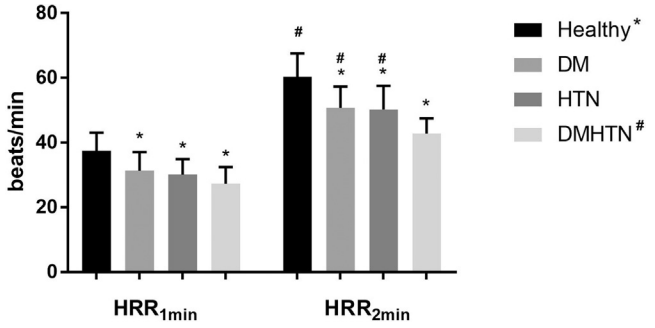

Fig. 1. HRR at 1- and 2-minutes for Healthy controls vs. patients with Type 2 diabetes Mellitus (DM), Hypertension (HTN) and both (DMHTN).
Note: * denotes significant difference from the healthy controls;
# denotes statistically significant difference when compared with DMHTN group.

3.2. Resting heart rate variability

The results of resting HRV analysis showed a significant effect of DM on all HRV indices ($p < 0.05$). Hypertension seemed to impact AvgNN ($p = 0.045$), AvgHR ($p = 0.041$), SD2 ($p = 0.001$), and the frequency-domains measures (Total power, LF, HF). The interaction effect was found to be significant for the frequency-domain indices ($p < 0.05$) (Table 3).

3.3. Post-Exercise heart rate variability

All HRV indices demonstrated a significant effect of time ($p < 0.05$). The group effect was found to be significant only for the time-domain indices of HRV ($p < 0.05$) and signified an altered recovery in the presence of DM or HTN or both (Table 4),(Fig. 2).

4. Discussion

4.1. Purpose and Main findings

The present study was the first to examine cardiac autonomic recovery in patients with T2DM and investigate the effect of co-existing HTN on the same. Our results found that post-exercise cardiac autonomic recovery as assessed by HRR and HRV was found

Table 3
Effect of DM and HTN on Resting Heart Rate Variability.

	Healthy n = 27 Mean (SD)	DM n = 24 Mean (SD)	HTN n = 24 Mean (SD)	DMHTN n = 24 Mean (SD)	DM main effect	HTN main effect	DM × HTN interac--tion
AvgNN (ms)	834.4 (82.4)	750.6 (94.9)	772.8 (93.4)	727.7 (92.3)	0.001*	0.045*	0.207
SDNN (ms)	45.1 (17.6)	21.1 (10.9)	36.1 (16.4)	27.9 (11.6)	<0.001*	0.574	0.017*
AvgHR (bpm)	73 (6.8)	81 (10.4)	79 (9.2)	84 (10.9)	0.001*	0.041*	0.291
RMSSD (ms)	37.4 (15.6)	19.9 (11.9)	37.9 (28.1)	22.0 (15.1)	<0.001*	0.896	0.984
pNN50 (%)	16.1 (15.6)	3.7 (8.5)	15.9 (18.8)	2.2 (5.7)	<0.001*	0.610	0.642
Total power (ms^2)	2377.2 (1938.5)	1008.9 (1410.2)	1041.8 (807.6)	836.9 (781.9)	0.005*	0.007*	0.043*
LF (ms^2)	647.3 (473.8)	207.6 (302.0)	197.8 (248.1)	237.6 (230.6)	0.004*	0.003*	0.001*
HF (ms^2)	655.5 (592)	400.3 (559.9)	522.9 (520.9)	207.9 (353.2)	0.008*	0.025*	0.759
LF: HF	1.15 (0.63)	0.85 (0.84)	0.75 (0.86)	1.7 (1.5)	0.095	0.219	0.002*
LFnu	48.5 (14.4)	36.7 (20.4)	31.9 (23.4)	55.6 (16.7)	0.133	0.697	<0.001*
HFnu	49.7 (14.8)	59.3 (19.2)	64.7 (21.6)	42.4 (14.9)	0.088	0.694	<0.001*
SD1	26.5 (12.1)	18.4 (14)	26.5 (20.6)	14.8 (7.6)	0.001**	0.458	0.442
SD2	32.1 (18.6)	40.3 (18.4)	36.7 (12.9)	60.5 (22.9)	<0.001*	0.001*	0.221

AvgNN: Mean of N-N intervals; SDNN: Standard deviation of N-N intervals; AvgHR: Mean heart rate; RMSSD: Square root of the mean squared differences between adjacent RR intervals; pNN50: Percentage of interval differences of adjacent RR intervals greater than 50 ms derived from differences between consecutive RR intervals; LF nu: Low frequency power normalize units; HF nu: High frequency power normalize units LF/HF ratio: Ratio of low and high frequency power.
* Significant difference, $p < 0.05$.

Table 4
Summary of Split-plot ANCOVA.

Variable	Source	df	F	*p*-value	Partial eta squared
AvgNN (ms)	Time	1	65.82	<0.001*	0.412
	Group	3	5.046	0.003*	0.139
SDNN (ms)	Time	1	7.419	0.008*	0.073
	Group	3	3.398	0.021*	0.098
AvgHR (bpm)	Time	1	34.882	<0.001*	0.271
	Group	3	3.667	0.015*	0.105
lnRMSSD	Time	1	36.46	<0.001*	0.279
	Group	3	6.509	<0.001*	0.172
lnpNN50	Time	1	93.65	<0.001*	0.639
	Group	3	2.054	0.117	0.104
lnTotal power	Time	1	51.052	<0.001*	0.352
	Group	3	1.992	0.121	0.06
lnLF	Time	1	76.583	<0.001*	0.449
	Group	3	3.389	0.021*	0.098
lnHF	Time	1	28.79	<0.001*	0.234
	Group	3	1.936	0.129	0.058
lnLF: HF	Time	1	70.962	<0.001*	0.43
	Group	3	0.477	0.699	0.015
LFnu	Time	1	64.213	<0.001*	0.406
	Group	3	0.905	0.442	0.028
HFnu	Time	1	97.926	<0.001*	0.510
	Group	3	0.696	0.557	0.022
SD1	Time	1	5.379	0.023*	0.054
	Group	3	1.660	0.181	0.05
SD2	Time	1	4.572	0.035*	0.046
	Group	3	1.198	0.315	0.037

AvgNN: Mean of N-N intervals; SDNN: Standard deviation of N-N intervals; AvgHR: Mean heart rate; RMSSD: Square root of the mean squared differences between adjacent RR intervals; pNN50: Percentage of interval differences of adjacent RR intervals greater than 50 ms derived from differences between consecutive RR intervals; LF nu: Low frequency power normalize units; HF nu: High frequency power normalize units LF/HF ratio: Ratio of low and high frequency power; ln: natural log-transformed.

* Significant difference, $p < 0.05$.

to be slower in the presence of T2DM. Moreover, the simultaneous occurrence of HTN further caused impairment in the autonomic recovery rate.

4.2. Heart rate recovery

HRR is the assessment of the rate at which HR decreases following the cessation of exercise.

HRR derived from a standard exercise stress test provides high diagnostic accuracy for CAN in patients with type 2 diabetes, and its high sensitivity points to utility in identification and preliminary screening of at-risk patients [30] Attenuated HRR after exercise testing, is indicative of decreased parasympathetic nervous system activity [31,32] and is associated with an increased risk of all-cause mortality and risk for CV events in asymptomatic patients with T2DM. [33] However, attributing the slowed HRR in CAN exclusively to vagal dysfunction may be overly simplistic. Indeed, blunting of HRR with hyperglycemia [34,35] and insulin resistance [36] may implicate additional involvement of the sympatho-vagal imbalance.

Kannankeril et al [37] proposed that HRR at 1 min is mediated by enhanced activity of parasympathetic nervous system, whereas, HRR at 2 min is primarily mediated by sympathetic withdrawal, and therefore, HRR reflects the coordinated interaction between parasympathetic re-activation and sympathetic withdrawal. In contrast, Goldberger et al [38] found that during the first 30 s of the recovery phase, most of the changes in heart rate are due to sympathetic withdrawal subsequently overlapped with the effect of parasympathetic reactivation during later part of this phase. Freeman et al [39] suggested that HRR at 1 min post exercise is more reflective of parasympathetic autonomic nervous system function,

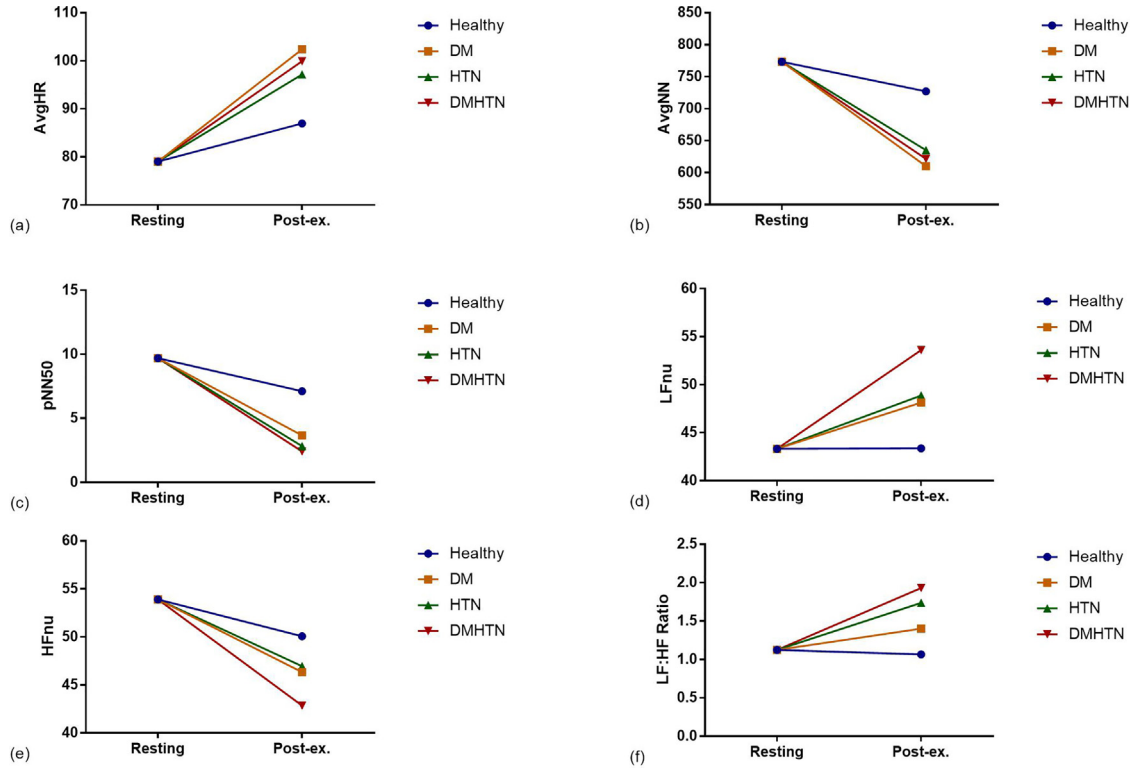

Fig. 2. Comparison of HRV recovery between the groups.
Time domain variables: 1(a) Average Heart Rate (bpm); 1(b) Average NN (ms); 1(c) pNN50 (%), and Frequency domain variables: 1(d) Low-frequency power in normalized units (LFnu); 1(e) High-frequency power in normalized units (HFnu); 1(f) Ratio of low frequency to high frequency power (LF: HF)

while HRR at 2 min reflects activity of both branches of the autonomic nervous system.

In the present study, heart rate recovery at 1 (HRR_{1min}) and 2 (HRR_{2min}) minutes showed a significant effect for both DM and HTN, denoting a slower heart rate recovery when compared to the healthy controls. Furthermore, the non-significant interaction effect implies an additive effect of the two conditions. Ng et al [40] compared HRR between diabetics and healthy controls and found HRR_{1min} to be a better discriminator than HRR_{2min}. However, we found HRR_{2min} to be more sensitive to the presence of HTN along with T2DM (Fig. 1). Moreover, in accordance with the diagnostic criteria for autonomic dysfunction proposed by Sacre et al [30] the DMHTN group in the current study demonstrated significantly altered control, with the mean HRR_{1min} and HRR_{2min} values as 27 and 43 bpm, respectively.

4.3. Resting heart rate variability

HRV has been analyzed and presented in the time domain; however, more complex assessments include power spectral (frequency domain) and non-linear analysis. These analyses of HRV provide researchers with direct information on the parasympathetic contributions, and by extension, inferred information on the sympathetic contributions to resting and post-exercise modulation of heart rate (HR). Most commonly used time domain variables of HRV are: SDNN which denotes overall variability in HR, and RMSSD & pNN50 which are indicators of vagal activity. Power spectral measures of HRV are Total power, LF power, HF power, and LF/HF ratio. Total power cumulatively indicates overall HRV, LF power in the power spectrum of HRV is an indicator of both sympathetic and parasympathetic activity, whereas, HF power has been found to be strongly associated with vagal activity, and ratio of LF and HF power (LF/HF ratio) is a measure of sympatho-vagal balance. The Poincare plot analysis is a graphical nonlinear method to assess the dynamics of HRV that provides summary information as well as detailed beat-to-beat information on the behavior of the heart. The Poincare plot parameters usually used are SD1 and SD2. SD1 is the standard deviation of projection of the Poincare plot on the line perpendicular to the line of identity, while SD2 is defined as the standard deviation of the projection of the Poincare plot on the line of identity ($y = x$). The parameter SD1 has been correlated with HF power (vagal activity), whereas SD2 has been correlated with sympathetic activity. [28]

The analysis of resting HRV in the current study revealed that both DM and HTN, independently lowered the overall HRV. Resting tachycardia (reflected in the AvgHR) and fixed heart rate (decline in the measures of overall HRV such as AvgNN, SDNN and Total Power) are characteristic late findings in diabetic patients with vagal impairment due to CAN [41] (Fig. 2). There was a depression of the resting vagal activity and an increased sympathetic outflow (indicated by LFnu and SD2) in both these diseased states. LFnu component of HRV is affected by baroreflex activity [42] which explains the significantly increased values in the DMHTN group (Fig. 2). The co-existence, moreover, showed an added effect causing a further lowering of the parasympathetic and upregulation of the sympathetic tone. These findings were similar to those reported by previous studies. An investigation by Takahashi et al [43] exploring the co-existence of hypertension with diabetes suggested that hypertensive diabetics had a lower BRS, lower myocardial uptake, and enhanced clearance of I-metaiodobenzylguanidine (I-MIBG) as compared to normotensive diabetics. Furthermore, Hazari et al [11] also revealed similar findings, and suggested that cardiovascular reflexes (Ewing's battery of tests), which exemplify cardiac autonomic function are further deteriorated in hypertensive diabetics than normotensive diabetics, and therefore, diabetes and hypertension may cause synergistic progression of CAN. More recently, Istenes et al [14] assessed resting HRV using the frequency domain measures and found an additive effect of HTN on impaired autonomic function in diabetic patients. However, Solanki et al [44,45] found non-significant difference in HRV parameters (frequency domain, time domain and non-linear measures) between diabetics with optimal vs. poor BP control. They recorded no difference in the overall HRV except for LF/HF ratio, which was found to be lower in the hypertensive diabetics. On the contrary, the present study found the LF/HF ratio to be the highest in DMHTN group. LF/HF ratio denotes sympatho-vagal balance, and an increase signifies sympathetic dominance which could result from either damage to cardiac autonomic nerves by hyperglycemia in T2DM or by the sympathetic overactivity as seen in HTN. Findings of Solanki et al [44,45] were in contrast to those of previous studies, where they reported no significant effect of HTN on autonomic function in T2DM. However, they did not include healthy controls or a euglycemic hypertensive group which makes it difficult to compare it with the results of the present study.

4.4. Post-exercise HRV

The results of present study revealed a significant difference in resting and post-exercise autonomic status for all the groups (healthy, hypertensive, normotensive diabetic, and hypertensive diabetic), suggesting a shift in cardiac autonomic modulation after exercise.

Comparison of the trend of recovery between the 4 groups suggested that while no group reached the baseline state, both DM and HTN showed a slower rate of recovery than the healthy group. Moreover, the co-existence of both HTN and DM further delayed the attaining of resting values. The time-domain variables showed a significant effect of group, with impaired recovery in diabetics (both normotensives and hypertensives) as compared to healthy controls. The frequency-domain demonstrated no significant difference between the groups. While the DMHTN group showed a slower rate of recovery in comparison to the other three groups, this difference was not statistically significant. Although, frequency domain variables are considered to be more reliable measures of HRV in short-term recordings, if the modulations are not stable, interpretation of the results of frequency analysis are less well defined [26] It has been recommended that with lower stability of heart rate modulations (during long-term recordings), time-domain methods are ideal for analysis. The post-exercise recovery period might also be speculated to possess lower heart rate stability and therefore, results of time-domain variables may be more meaningful to interpretation. The recovery of time-domain variables was found to be sensitive to the presence of both T2DM and HTN.

Acute physical exertion causes physiological stress affecting the cardiac autonomic modulation [15] During transition from rest to exercise and the recovery period following exercise, the heart has been suggested to be more vulnerable to dysrhythmias and fatal events owing to a shift in the autonomic balance from parasympathetic to sympathetic dominance. [17,18] Results of present study suggested that recovery of autonomic nervous system from sympathetic to parasympathetic was impaired in diabetics and hypertensive patients when compared to healthy controls. Present findings may be explained by the fact that the autonomic nervous system of diabetics as well as hypertensive patients is less responsive to change, which in part may be explained by baroreflex mechanism. Diminished responsiveness of the baroreflex in hypertension [46,47] and diabetes [48] may contribute to autonomic imbalance during recovery from exercise.

4.5. Possible mechanisms for impaired autonomic function in hypertensive diabetics

There exists substantial overlap in the pathophysiological pathways underlying the etiology of both T2DM and HTN. The association of T2DM and HTN has been explained by the presence of common susceptibility genes and single nucleotide polymorphisms (SNPs), oxidative stress-mediated cascades, chronic low-grade inflammation, insulin resistance and obesity [49] The pathogenesis of diabetic CAN is complex and involves a series of pathways activated by hyperglycemia resulting in neuronal ischemia and cellular death. Hyperglycemia in T2DM patients results in increased oxidative stress, which can cause direct neuronal dysfunction or endothelial dysfunction resulting in neuronal ischemia. [50] However, in HTN, owing to the fluctuations in BP, baroreflex function gets hampered and is not able to set itself to a new operating point. Diminished sensitivity of baroreflex is a leading cause of autonomic imbalance in HTN. [46,47] Although, there are some common pathways which are related to development of autonomic dysfunction in diabetes and HTN, CAN in diabetes is primarily hyperglycemia-mediated and autonomic dysfunction in HTN is in part due to changes in baroreflex mechanism. Considering different etiologies behind autonomic dysfunction in diabetes and hypertension, it may be speculated that due to the co-existence of these different etiologies together in DMHTN group, further deterioration of autonomic function at rest and impaired autonomic recovery was found when compared to normotensive diabetic group.

4.6. Limitations and recommendations for future research

The present study was the first to examine post-exercise cardiac autonomic recovery in patients with type 2 diabetes and the effect of co-existence of HTN on the same but since patients with structural abnormalities of the heart were not screened, it is likely that diabetic and hypertensive cardiomyopathies would have contributed to the blunt autonomic recovery. We assessed autonomic recovery using HRR and the change in post-exercise and resting values of HRV. Although impairments in these parameters are suggestive of CAN, the presence of CAN was not objectively assessed by standard cardiovascular autonomic reflex tests. Moreover, the unavailability of echocardiography limits the results of the study since diastolic dysfunction is very common in these patients and might significantly interfere with autonomic function. Future studies should administer definitive screening of CAN using standard tests such as the Ewing's battery and other objective markers of autonomic function such as baroreflex sensitivity, I-MIBG, chronotropic incompetence, to corroborate these findings and present a more comprehensive conclusion. A comparison of these tests could help provide a sensitive prognostic marker that occurs earlier in the disease process of T2DM leading to improved treatment guidelines and outcomes.

5. Conclusion

The present study found impaired post-exercise autonomic recovery as assessed by HRR and HRV, in patients with T2DM. The co-existence of HTN further worsened the rate of recovery. These findings have clinical implications as graded maximal tests are commonly used for cardiovascular screening, and since diabetics show compromised autonomic recovery, caution must be exercised while administration of stress tests. Moreover, hypertensive diabetics demonstrated greater autonomic dysfunction and slower recovery as compared to normotensive diabetics, making them more susceptible to exercise intolerance. Furthermore, this study gives definitive evidence on the role of post-exercise recovery period for risk stratification in T2DM, HTN and DMHTN patients. Autonomic dysfunction is a common complication in T2DM that can be reversed with strict glycemic control, however, screening in asymptomatic patients is not routinely performed and overt clinical sequelae manifest only in advanced cases. This causes unrecognized subclinical CAN to insidiously contribute to cardiovascular risk. Therefore, the early identification of at-risk patients, such as hypertensive diabetics, is imperative.

6. Conflict of interest

The authors declare no conflicts of interest.

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