Effect of glycemic control and disease duration on cardiac autonomic function and oxidative stress in type 2 diabetes mellitus

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Abstract

Purpose Cardiac autonomic neuropathy (CAN) is a commonly overlooked complication of type 2 diabetes mellitus (T2DM), with a complex pathogenesis involving hyperglycemia-induced oxidative stress which results in neuronal ischemia and cellular death. The level of hyperglycemia as well as disease duration might be significant determinants of the prognosis of T2DM, but limited studies have explored their relationship with these diabetic complications. Therefore, the purpose of this study was to examine the effect of glycemic control and disease duration on cardiac autonomic function and oxidative stress in patients with T2DM.

Methods 60 T2DM patients along with 63 healthy controls were recruited for the study. Diabetic patients were further classified based on glycemic control (HbA1c levels <8% vs. ≥8%) and disease duration (<5 vs. 5–10 vs. >10 years). All participants were assessed for cardiac autonomic function (HRR: heart rate recovery; HRV: heart rate variability), levels of antioxidant enzymes (CAT: catalase; SOD: superoxide dismutase), serum nitric oxide (NO) and other cardiometabolic risk factors (resting blood pressure, glycemic and lipid profile).

Results T2DM patients showed a significant reduction in HRR, HRV, CAT, SOD and an increase in LFNu, LF: HF ratio and NO. These impairments were significantly greater for the group with poor glycemic control ($p < 0.05$). However, no difference for these parameters was observed with respect to different disease durations.

Conclusion Cardiac autonomic regulation and endogenous antioxidant defense were compromised and levels of nitric oxide found to be raised in patients with Type 2 diabetes. These findings were more pronounced in subjects with poor glycemic control.

Keywords Heart rate variability · Heart rate recovery · Nitric oxide · Antioxidant defense

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Introduction

Diabetes currently affects 424.9 million people worldwide, of which 82 million reside in South-East Asia [1] and Type 2 diabetes mellitus (T2DM) accounts for at least 90% of the cases [2]. 1.1 million deaths in South-east Asia this year, were attributed to diabetes, with 51.5% occurring in people less than 60 years of age [1]. The economic burden of the disease in this region was 9.5 billion USD in 2017 [1]. India ranks second globally with 72.9 million diabetics and is projected to become the world diabetic capital with a diabetic population of 134.3 million in 2045 [1]. With 1 million estimated diabetes-related deaths in 2017, India is also the largest contributor to the regional mortality.

T2DM is associated with dysfunction of various organs especially the heart and peripheral blood vessels, making it a multi-faceted metabolic disorder, with 2- to 4-fold higher incidence of cardiovascular disease compared with patients

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without diabetes [3]. A commonly overlooked complication of T2DM, cardiac autonomic neuropathy (CAN), is characterized by an imbalance between sympathetic and parasympathetic supply to heart and contributes to cardiovascular morbidity and mortality [4]. CAN has been reported to occur in 42% of patients with T2DM [5] with a higher incidence (54–60%) reported in the Indian population [6, 7]. The pathogenesis of diabetic CAN is complex and involves a series of pathways activated by hyperglycemia resulting in increased oxidative stress, which can cause direct neuronal dysfunction or endothelial dysfunction resulting in neuronal ischemia [8]. Growing evidence indicates that mechanisms contributing to the formation of free radicals in diabetes mellitus may include not only increased non-enzymatic and auto-oxidative glycosylation, but also metabolic stress resulting from changes in energy metabolism, the levels of inflammatory mediators, and the status of antioxidant defence systems [9, 10]. Additionally, nitric oxide (NO) is another molecular determinant of cardiac autonomic regulation whose effects on autonomic function are altered by its isoform (constitutive vs. inducible), concentration and the presence of diseased states. Moreover, NO bioavailability might also influence levels of oxidative stress bearing a close relationship with reactive oxygen species (ROS) as well as antioxidant enzymes.

The level of hyperglycemia as well as disease duration are significant determinants of the prognosis of T2DM. The link between good glycemic control and the low incidence of microvascular and macrovascular complications in patients with type 2 diabetes is well established [11]. Kalyani et al. [12] reported poor glycemic control as one of the major causes of comorbidities in patients with type 2 diabetes. Stolar et al. [13] suggested that macrovascular complications may develop early, and not correlate linearly with HbA1c and thus, managing hyperglycemia in the later stages of type 2 diabetes does not appear to be associated with improved cardiovascular outcomes. Other studies have also confirmed that while tight glycemic control prevented microvascular complications of T2DM, prevention of cardiovascular complications and mortality might be limited to those newly diagnosed with this condition [14, 15]. These findings emphasize that not only the maintenance of glucose levels but also the timing of intervention from disease onset is crucial for improved outcomes. Recently, Jelinek et al. [16] also observed that disease duration was the most significant predictor for the development of type 2 diabetes complications but there is a dearth of relevant data in the Indian population. Considering the fact that Indians demonstrate differences in phenotypic distribution of fat, are genetically predisposed to coronary artery disease due to dyslipidemia and low HDL levels [17], and susceptible to developing complications at an early age (20–40 yrs) compared with Caucasians (>50 yrs) [17], it becomes imperative to explore the role of disease severity and history in this high-risk population. Therefore, the purpose of this study was to

examine the effect of glycemic control and disease duration on cardiac autonomic function and oxidative stress in Indian patients with T2DM.

Methods

Subjects

60 patients with Type 2 Diabetes Mellitus were recruited through referrals from the university health center and nearby hospitals. Sedentary subjects between 30 and 70 years of age, diagnosed with type 2 diabetes for a minimum of 12 months, receiving oral anti-diabetic medication but having HbA1c level 6.5–10%, able to walk continuously for at least 20 min and climb one flight of stairs unaided without stopping, were recruited. Subjects were defined as sedentary if they did not engage in exercising more than 20 min on 3 or more days a week. Exclusion criteria included subjects with BMI > 40 kg/m², autoimmune diseases, retinopathy, nephropathy, peripheral neuropathy, inflammatory and other conditions potentially associated with altered cytokine regulation, liver impairment, renal insufficiency (creatinine levels >2.0 mg/dl), diagnosed cardiovascular disease, prescription of a very low caloric diet (less than 1000 kcal/day) or drugs for the treatment of obesity, recent onset of cardiac-origin symptoms and orthopedic problems limiting physical activity. In addition, patients on exogenous insulin or already engaging in intensive physical activity >2 h/week, were also excluded.

Sixty-three age-matched healthy controls were enrolled in addition to the sample population and assessed for oxidative stress and cardiac autonomic function to establish a reference value for these variables and quantify the effect of T2DM on the same. The study was approved by the Institutional Ethical Committee, Jamia Millia Islamia, New Delhi, India. All participants were given an information sheet explaining the study purpose, methodology as well as their rights as research subjects and a written consent was obtained.

Cardiac autonomic function

- a) *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. 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. HRR was calculated as the absolute difference between heart rate at peak exercise and heart rate recorded at 1- (HRR_{1min}) and 2- min (HRR_{2min}) post-exercise [18].
- b) *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. After recording, all the data were stored and analysed offline. Analysis of HRV was done on a time series of the last five-minutes selected from the 20-min recording. 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 analysed by the detection of R waves. AD instruments lab chart version 7.3.7 with HRV module version 1.4.2 using welch 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 [18].

Oxidative stress

Blood samples were collected after a 12-h fast and a 48-h period of no exercise from the antecubital vein. All samples were taken in the morning to avoid the confounding effect of diurnal variation of oxidative stress parameters. Blood samples were collected in EDTA, sodium heparin, and serum separator vacuum tubes (Vacutainer). All samples were allowed to clot, and then serum and plasma separated by centrifugation for 15 min at 2000 rpm. The samples were stored at -80°C until analysis. All samples were analyzed in duplicate and then averaged. Catalase (CAT) and Superoxide dismutase (SOD) activity in plasma was assessed using commercial kits (Item no. 707002 and 703,102 respectively, Cayman Chemicals, Ann Arbor, MI, USA) at wavelengths 540 nm and 450 nm respectively (BIO-RAD, iMark Microplate Reader). NO levels were measured as nitrites, using the Griess reaction and the absorbance read at 540 nm (Item no. 780001, Cayman Chemicals, Ann Arbor, MI, USA) [18].

Glycemic and lipid profile HbA1c was measured using 2–3 ml of blood drawn from participants who fasted at least 10 h from the night before and analyzed. HbA1c was measured using high performance liquid chromatography (HPLC). Fasting blood glucose and lipid profile (comprising total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol were estimated from serum samples (Span Cogent Diagnostics, Surat, India). Total

cholesterol and triglycerides were measured using enzymatic colorimetric methods with cholesterol oxidase-peroxidase amino phenazone phenol and glycerol-3-phosphate oxidase-peroxidase amino phenazone phenol. HDL-C was measured using homogenous enzymatic colorimetric assay. LDL-C was calculated using the Friedewald formula [18].

Resting BP SBP and DBP were measured by standard sphygmomanometer. The participants were strictly prohibited to consume any caffeinated products 30 min prior to measuring, also exercising and smoking were prohibited for the same duration before measuring. The patient remained in relaxed seated state for a duration of 15 min. The BP cuff was placed on the left arm of the patient and the BP was recorded with a manual sphygmomanometer in accordance with standard guidelines. A second recording of the patient was taken after 2 min. If the measurements displayed a difference of ≥ 5 mmHg of BP, further readings were obtained until there were 2 consecutive stable measurements. Final reading recorded was considered as the average of the 2 stable measurements [18].

Sample size

To compare the differences between Type 2 Diabetics and healthy controls, a power analysis was conducted for the sample size of 123 (60 subjects; 63 controls) using the software G. Power 3.1. Based on the mean, between-groups comparison, the effect size observed in the present study ($d = 0.62$) revealed a power of 0.93, at an alpha level = 0.05.

Statistical analyses

The normality of distribution was examined for all variables using Kolmogorov-Smirnov test. Variables that were found to be non-normal (DBP, Total power, SDNN, RMSSD and pNN50) were log transformed for further analyses. The effect of diabetes on oxidative stress and cardiac autonomic function was explored by comparing these variables between the patients with T2DM and age-matched non-diabetic participants, using independent *t*-test. Independent *t*-test was also used to assess the effect of glycemic control (HbA1c levels <8% vs. $\geq 8\%$) on patient characteristics. The effect of disease duration (<5 vs. 5–10 vs. >10 years) however, was examined using one-way ANOVA. Level of significance was set at $p < 0.05$.

Results

Effect of DM

Patients with T2DM and age-matched controls were found to be comparable in terms of demographic characteristics and

cardiometabolic risk factors except for systolic blood pressure which was found to be significantly higher in the non-diabetic participants ($p = 0.032$) (Table 1).

The cardiac autonomic function between the two groups was assessed using heart rate recovery and heart rate variability. The resting heart rate for the diabetic group was found to be significantly elevated while the exercise heart rate achieved during maximal exercise was reduced ($p < 0.001$). The heart rate recovery at both 1st and 2nd minute showed a significant decline (HRR_{1min} and HRR_{2min} , $p < 0.001$). Heart rate variability was assessed using both time-domain (AvgNN, SDNN, RMSSD, pRR50) and frequency-domain (Total power, LFnu, HFnu and LF/HF ratio) variables. AvgNN, SDNN and total power reflect overall heart rate variability, of which AvgNN was found to be significantly reduced ($p < 0.001$). The indices of vagal activity such as RMSSD, pRR50 and HFnu were also diminished. On the contrary, the markers of sympathetic activity (LFnu and LF/HF ratio) were shown to be significantly elevated implying an autonomic imbalance with an overall dominance of sympathetic outflow and depressed vagal tone, in patients with T2DM (Table 2).

The markers of antioxidant defense namely serum catalase and superoxide dismutase were found to be significantly reduced in patients with T2DM ($p = 0.001$), while serum levels of NO were elevated ($p < 0.001$) (Table 2).

Effect of disease duration and glycemic control

The patient characteristics when compared for disease severity and chronicity revealed that glycemic control rather than disease duration affected the manifestations in terms of lipid

profile, oxidative stress and autonomic function. The results of one-way ANOVA comparing variables across patients with different disease duration showed no differences except for HRR1 which was found to be significantly reduced in patients diagnosed with Type 2 diabetes for over 10 years ($p = 0.019$) (Table 3).

The HbA1c levels were used as an index of blood glucose control and patients classified as either having fair control ($HbA1c < 8\%$) or poor control ($HbA1c \geq 8\%$). Those with poor glycemic control revealed higher NO levels, fasting blood glucose, total cholesterol and low-density lipoprotein and lower levels of antioxidant enzymes. The indices of autonomic function, i.e. heart rate recovery (both during first and second minute) and heart rate variability (both time and frequency domain indices) were also significantly reduced (Table 4).

Discussion

The results of the present study revealed that while the heart rate recovery, resting heart rate variability and antioxidant enzymes were significantly reduced in patients with poor glycemic control as compared to fair control, there was no difference among patients with different disease duration.

Heart rate recovery

Heart rate recovery (HRR) derived from a standard exercise stress test, provides high diagnostic accuracy for CAN in patients with Type 2 Diabetes [19] and a low HRR is proven to

Table 1 Comparison of demographics and cardiometabolic risk factors

Variable	Non-diabetic $n = 63$ Mean (SD)	T2DM $n = 60$ Mean (SD)	<i>t</i> -value	<i>p</i> value
Gender (M/F)	27/36	28/32	—	0.672
Age (years)	49.9 (4.7)	51.5 (6.3)	1.612	0.110
Weight (kg)	73.8 (8.2)	77.6 (9.2)	2.437	0.016*
Height (cm)	159.9 (6.8)	161.4 (8.7)	1.289	0.200
BMI (kg/m^2)	28.9 (2.6)	29.7 (3.4)	1.615	0.109
Duration (years)	—	6.7 (3.6)	—	—
HbA1c (%)	5.4 (0.7)	8.1 (1.0)	15.96	<0.001*
SBP (mmHg)	130.6 (13.7)	135.5 (11.8)	2.169	0.032*
DBP (mmHg)	85.9 (8.1)	84.2 (5.7)	1.421	0.158
TC (mg/dl)	185.3 (53.1)	192.0 (54.2)	0.696	0.488
HDL (mg/dl)	48.5 (10.4)	49.3 (12.5)	0.178	0.859
LDL (mg/dl)	114.2 (49.9)	118.2 (50.9)	0.433	0.665
TG (mg/dl)	155.8 (65.5)	143.3 (66.3)	1.054	0.294

BMI Body mass index; HbA1c Glycosylated hemoglobin; SBP Systolic blood pressure; DBP Diastolic blood pressure; TC: Total Cholesterol; HDL High density Lipoprotein; LDL Low Density lipoprotein; TG Triglycerides. Significance level: $p < 0.05$

*significant difference

Table 2 Comparison of cardiac autonomic function and oxidative stress between Type 2 diabetics and non-diabetic controls

Variable	Non-diabetic n = 63 Mean (SD)	T2DM n = 60 Mean (SD)	t-value	p value
RHR (bpm)	76.0 (9.2)	82.6 (9.9)	3.799	<0.001*
Ex. HR (bpm)	153.9 (9.3)	146.3 (8.4)	4.803	<0.001*
HRR1 (bpm)	33.9 (6.7)	29.2 (5.8)	4.097	<0.001*
HRR2 (bpm)	55.6 (9.1)	46.6 (7.1)	6.127	<0.001*
AvgNN (ms)	802.3 (95.8)	738.6 (87.3)	3.855	<0.001*
SDNN (ms)	35.5 (18.3)	31.7 (15.8)	1.252	0.213
RMSSD (ms)	32.8 (19.2)	26.9 (24.4)	2.579	0.011*
pNN50 (%)	12.8 (16.7)	6.9 (13.7)	2.274	0.025*
TP (ms ²)	1559.5 (1732.8)	1019.9 (897.9)	1.540	0.126
LFnu	36.8 (18.0)	51.4 (22.7)	3.961	<0.001*
HFnu	60.6 (17.3)	45.6 (19.8)	4.489	<0.001*
LF/HF ratio	0.8 (0.6)	1.7 (1.6)	3.717	<0.001*
CAT(nmol/L/min)	33.1 (15.9)	24.3 (13.0)	3.360	0.001*
SOD (U/ml)	3.9 (2.0)	2.8 (1.5)	3.634	<0.001*
NO (μmol/L)	73.0 (24.1)	122.9 (29.9)	10.15	<0.001*

RHR Resting heart rate; *Ex HR* Peak exercise heart; *HRR1* Heart rate recovery in the first minute following maximal exercise; *HRR2* Heart rate recovery in the first 2 min following maximal exercise; *AvgNN* Mean of N-N intervals; *SDNN* Standard deviation of N-N intervals; *RMSSD* Square root of the mean squared differences between adjacent RR intervals; *pNN50* Percentage of interval differences of adjacent RR intervals greater than 50 milliseconds derived from differences between consecutive RR intervals; *TP* Total power; *LF nu* Low frequency power normalize units; *HF nu* High frequency power normalize units; *LF/HF ratio* Ratio of low and high frequency power; *CAT* serum catalase activity; *SOD* serum superoxide dismutase activity; *NO* serum nitric oxide

*Significant difference at $p < 0.05$

be an independent predictor of CVD and all-cause mortality in diabetic patients [20]. Sacre et al. [19] confirmed the association of HRR with clinical autonomic tests and the diagnostic performance of blunted HRR_{1min} and HRR_{2min} in cardiac dysautonomia with optimal cut-off being ≤ 28 and ≤ 50 bpm (beats per minute), respectively, and sensitivity 93 and 96%, respectively. The present study recorded a decline in both HRR_{1min} and HRR_{2min} in the diabetic patients as compared to their healthy counterparts. Moreover, in accordance with diagnostic criteria by Sacre et al. [19], patients with poor glycaemic control demonstrated cardiac dysautonomia, as did those with over 5 yrs. of disease diagnosis.

Heart rate variability

Impaired HRV in diabetic autonomic neuropathy is not only a strong indicator of prognosis but also precedes the clinical manifestations [21] making it a sensitive marker of subclinical autonomic dysfunction. Previously, T2DM patients have shown a reduction of LF and HF power even in the absence of overt evidence of autonomic neuropathy [21]. However, the LF/HF ratio was not found to be significantly different from non-diabetics, implying that the initial manifestation of this neuropathy is likely to involve both efferent limbs of the autonomic nervous system [21, 22]. The results of the present study demonstrated a significant reduction in the vagal indices

of HRV (SDNN, RMSSD, pNN50, HFnu) in T2DM, while the sympathetic marker, LFnu was found to be increased. LF/HF ratio was also found to be higher in the diabetic group.

There exists sufficient literature examining the association of blood glucose levels with HRV parameters [23–26]. The studies have consistently shown a reduction in HF [23] and LF components [24] with hyperglycemia while, LF/HF ratio was found to be increased [25]. Increased blood glucose was also correlated negatively with the total power, an index of overall HRV [26]. Tarvainen et al. [27], found a strong correlation of HRV indices with both hyperglycemia and diabetes duration. However, in the present study, while blood glucose level was found to have a significant effect on HRV, no significant difference in HRV measures as a function of disease duration was found. The difference in results could be explained by the parameters used for HRV analysis. While Tarvainen et al. [27], demonstrated a strong association of disease duration with Renyi entropy, recording most significant changes around 10 yrs. of disease duration; this non-linear measure was not assessed in the current study.

Oxidative stress

Oxidative stress in DM is a result of both increased free radical production and diminished antioxidant defence [28, 29]. Increased levels of the products of oxidative damage to lipids

Table 3 Comparison of patient characteristics with respect to disease duration

Variables	<5 years <i>n</i> = 21	5–9 years <i>n</i> = 30	≥ 10 years <i>n</i> = 9	<i>p</i> value
HbA1c (%)	8.2 (1.2)	7.8 (0.9)	8.3 (1.1)	0.310
FBG(mg/dl)	164.5 (30.4)	171.9 (42.2)	183.6 (42.4)	0.462
SBP (mmHg)	138.2 (12.0)	134.9 (12.2)	131.6 (8.9)	0.340
DBP (mmHg)	85.9 (5.7)	82.9 (5.5)	84.4 (6.2)	0.198
TC (mg/dl)	193.7 (48.7)	189.4 (63.1)	196.7 (35.7)	0.928
HDL (mg/dl)	47.0 (12.3)	51.4 (14.1)	47.6 (5.6)	0.546
LDL (mg/dl)	123.2 (44.4)	115.2 (60.4)	116.3 (30.2)	0.858
TG (mg/dl)	145.0 (64.2)	128.9 (65.8)	186.8 (58.7)	0.069
RHR (bpm)	83.7 (10.1)	82.9 (10.5)	78.9 (7.6)	0.473
Ex. HR (bpm)	142.8 (6.7)	147.8 (8.7)	149.1 (9.2)	0.062
HRR1 (bpm)	27.5 (4.2)	31.3 (6.1)	26.4 (6.2)	0.019*
HRR2 (bpm)	44.6 (6.5)	48.5 (7.0)	44.9 (7.8)	0.122
AvgNN (ms)	728.4 (87.5)	736.3 (91.5)	768.6 (77.5)	0.517
SDNN (ms)	35.3 (20.2)	28.4 (10.6)	34.1 (18.1)	0.555
RMSSD (ms)	31.0 (33.0)	23.6 (15.7)	28.7 (25.9)	0.873
pNN50 (%)	8.5 (18.9)	5.4 (9.7)	7.9 (11.6)	0.739
TP (ms ²)	1287.5 (1153.1)	807.7 (706.0)	1102.9 (671.6)	0.369
LFnu	54.7 (23.8)	47.3 (22.5)	57.3 (20.4)	0.374
HFnu	40.3 (17.5)	50.3 (21.1)	41.9 (18.6)	0.173
LF/HF ratio	1.9 (1.4)	1.5 (1.6)	2.1 (2.2)	0.424
CAT (nmol/L/min)	26.4 (11.9)	23.3 (12.8)	22.7 (16.9)	0.654
SOD (U/ml)	2.9 (1.8)	2.7 (1.3)	2.7 (1.5)	0.828
NO (μmol/L)	121.6 (29.9)	123.4 (29.7)	124.1 (33.8)	0.971

HbA1c Glycosylated hemoglobin; *FBG* Fasting blood glucose; *SBP* Systolic blood pressure; *DBP* Diastolic blood pressure; *TC* Total Cholesterol; *HDL* High density Lipoprotein; *LDL* Low Density lipoprotein; *TG* Triglycerides; *RHR* Resting heart rate; *Ex HR* Peak exercise heart; *HRR1* Heart rate recovery in the first minute following maximal exercise; *HRR2* Heart rate recovery in the first 2 min following maximal exercise; *AvgNN* Mean of N-N intervals; *SDNN* Standard deviation of N-N intervals; *RMSSD* Square root of the mean squared differences between adjacent RR intervals; *pNN50* Percentage of interval differences of adjacent RR intervals greater than 50 milliseconds derived from differences between consecutive RR intervals; *TP* Total power; *LF nu* Low frequency power normalize units; *HF nu* High frequency power normalize units *LF/HF ratio* Ratio of low and high frequency power; *CAT* serum catalase activity; *SOD* serum superoxide dismutase activity; *NO* serum nitric oxide.

*Significant difference at $p < 0.05$

and protein have been detected in the serum of diabetic patients and their presence correlates with the development of complications [30]. Chronic exposure to hyperglycemia and insulin resistance has been implicated in altered oxidative metabolism. The initial event resulting in the increase in ROS formation is the depletion of adenosine triphosphate (ATP) due to its increased conversion to adenosine monophosphate (AMP), adenosine, inosine, and hypoxanthine, which is accompanied by superoxide formation. In addition, glycolytic intermediates can themselves generate oxidative reactants compounding hyperglycemia-induced oxidative stress. Excessive plasma and tissue glucose can exert pathological effects through non-enzymatic glycosylation, which leads to the production of superoxide and hydrogen peroxide [31], depleting SOD and CAT that quench these reactive species.

Different studies have provided evidences of increased oxidative stress with depleted antioxidant enzymes and vitamins in both type 1 and 2 diabetes [32, 33]. Hyperglycemia, a hallmark of diabetic condition, depletes natural antioxidants and facilitates the production of ROS, which can react with all biological molecules like lipids, proteins, carbohydrates, DNA and exert cytotoxic effects on cellular components [34]. There have been conflicting results regarding the antioxidant enzyme activities in T2DM. While high glucose concentration has been shown to increase the levels of oxygen radical scavenging enzymes [35, 36], several studies have reported lower concentration of non-enzymatic as well as enzymatic antioxidants in type 2 diabetes [32, 37, 38].

Our results showed a significant reduction in the activity of the both SOD and CAT in T2DM when compared with non-diabetics. When assessed for the effect of glycemic control,

Table 4 Comparison of patient characteristics with respect to glycemic control

Variable	Fair control (<i>n</i> = 28) Mean (SD)	Poor control (<i>n</i> = 32) Mean (SD)	<i>p</i> value
Duration (years)	6.6 (3.2)	6.8 (3.9)	0.772
FBG(mg/dl)	155.9 (25.3)	184.4 (43.1)	0.003*
SBP (mmHg)	133.3 (10.4)	137.5 (12.7)	0.169
DBP (mmHg)	83.1 (5.7)	85.2 (5.6)	0.144
TC (mg/dl)	170.7 (44.6)	210.6 (55.6)	0.004*
HDL (mg/dl)	47.8 (12.9)	50.6 (12.2)	0.358
LDL (mg/dl)	99.1 (44.8)	134.9 (50.8)	0.006*
TG (mg/dl)	148.7 (58.2)	138.5 (73.2)	0.553
RHR (bpm)	78.5 (7.8)	86.1 (10.4)	0.002*
Ex. HR (bpm)	149.9 (8.4)	143.0 (7.0)	0.001*
HRR1 (bpm)	31.1 (6.3)	27.6 (4.7)	0.016*
HRR2 (bpm)	50.1 (6.6)	43.5 (6.1)	<0.001*
AvgNN (ms)	773.5 (77.4)	707.7 (85.7)	0.003*
SDNN (ms)	35.4 (17.5)	28.4 (13.5)	0.094
RMSSD (ms)	34.5 (27.6)	20.4 (19.3)	0.011*
pNN50 (%)	10.8 (15.6)	3.5 (10.9)	0.001*
TP (ms ²)	1117.4 (899.8)	934.6 (901.9)	0.267
LFnu	42.1 (23.3)	59.5 (18.9)	0.002*
HFnu	54.9 (20.7)	37.4 (14.9)	<0.001*
LF/HF ratio	1.2 (1.5)	2.1 (1.7)	0.003*
CAT (nmol/L/min)	32.8 (11.2)	16.8 (9.5)	<0.001*
SOD (U/ml)	3.9 (1.5)	1.8 (0.5)	<0.001*
NO (μmol/L)	106.0 (25.2)	137.6 (25.8)	<0.001*

FBG Fasting blood glucose; SBP Systolic blood pressure; DBP Diastolic blood pressure; TC Total Cholesterol; HDL High density Lipoprotein; LDL Low Density lipoprotein; TG Triglycerides; RHR Resting heart rate; Ex HR Peak exercise heart; HRR1 Heart rate recovery in the first minute following maximal exercise; HRR2 Heart rate recovery in the first 2 min following maximal exercise; AvgNN Mean of N-N intervals; SDNN Standard deviation of N-N intervals; RMSSD Square root of the mean squared differences between adjacent RR intervals; pNN50 Percentage of interval differences of adjacent RR intervals greater than 50 milliseconds derived from differences between consecutive RR intervals; TP Total power; LF nu Low frequency power normalize units; HF nu High frequency power normalize units; LF/HF ratio Ratio of low and high frequency power; CAT serum catalase activity; SOD serum superoxide dismutase activity; NO serum nitric oxide. *Significant difference at *p* < 0.05

our findings were in agreement with those of Kumawat et al. [39] who reported lower levels of SOD and GPX in diabetic patients with nephropathy as compared to patients without nephropathy and non-diabetics. Madi et al. [40] also reported decreased serum SOD in T2DM and proposed that in addition to the increased production of oxidants caused by hyperglycemia, the glycosylation of SOD might result in the inactivation of this enzyme.

Nitric oxide

Scientific literature presents conflicting data on serum NO levels in T2DM patients. Adela et al. [41] reported significantly higher serum NO levels in Type 2 diabetic patients. Their findings were in agreement with the observations of [42, 43]. More recently, Ozcelik and Algul [44] also found higher NO levels in not only diabetics but prediabetics as well.

On the contrary, Ghosh et al. [45] and Tessari et al., [46] observed low serum NO in diabetics in comparison with healthy controls.

The present study observed significantly higher levels of NO in T2DM that varied with glycemic control. These findings concurred with Adela et al. [41] that demonstrated NO levels to be positively correlated with FBG and HbA1c. Unlike Adela et al. [41], we did not observe a significant effect of disease duration on NO levels. They reported a decline in NO concentration with disease duration as patients less than 5 yrs. of diabetes demonstrated higher NO levels than those greater than 5 yrs. The increased NO production in T2DM has been attributed to macrophages that also play a significant role in β -cell destruction [47]. The macrophage activity might also explain the linear relation of NO with hyperglycemia observed in the current study. Although, we have not measured the iNOS and eNOS activity, most of the scientific literature

showed that hyperglycemia mostly increased NO level through activation of iNOS [48, 49].

NO levels seem to be correlated with both cardiac autonomic modulation and antioxidant status. While there is strong evidence for the presence of nitric oxide synthase (NOS) throughout cardiac autonomic neurons, studies have reported equivocal results of its action as a sympatholytic agent or hampering vagal control or an overall depressor of autonomic activity. Regarding oxidative status, superoxide reacts rapidly with NO, reducing NO bioactivity and producing the oxidative peroxynitrite radical [50]. SOD on the other hand, catalyses the conversion of superoxide to hydrogen peroxide, sparing NO. It also potentiates the exercise-induced eNOS expression through hydrogen peroxide [51]. This suggests a close linear relationship of NO bioavailability with antioxidant enzymes. Paradoxically, due to its labile state, NO at high concentrations is also a source of oxidative stress.

Limitations and future perspective

The small sample size limits the generalizability of our results. Also, since glucose fluctuations exhibit a more specific triggering effect on oxidative stress than chronic sustained hyperglycemia, it would be interesting to also explore intraday glycaemic variability with continuous glucose monitoring systems. We speculated that the increased NO levels in the diabetic subjects were through the activation of iNOS but the different isoforms of NOS were not separately measured. Future studies must explore the response of the specific NOS isoforms (eNOS, nNOS and iNOS) not only to disease variables but also management interventions such as exercise training.

Conclusion

The results of the current study found that cardiac autonomic regulation as assessed by heart rate recovery and heart rate variability, was impaired in patients with T2DM. Also, endogenous antioxidant defense measured by levels of catalase and superoxide dismutase, was compromised while levels of nitric oxide were found to be raised in comparison to non-diabetics. These findings were more pronounced in subjects with poor glycaemic control. Therefore, pharmacological and non-pharmacological interventions should be designed for rigorous control of hyperglycemia in order to prevent various devastating complications in T2DM patients.

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Authors' contributions SV and MEH contributed to the creation and design of the study. The co-authors provided expertise as follows: SV and

IA were involved in enrolling the patients and assessments. SV and MEH drafted the global study methodology and statistical methods. SV and RA performed the biochemical analyses. DS and KA were involved in manuscript revision and critical review. All authors helped to write the article and have proofread and approved the final manuscript.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethical approval and consent to participate The study was approved by the Institutional Ethical Committee, Jamia Millia Islamia. (Date: 23 February 2016; IEC reference No: 17/9/43/JMI/IEC/2015). All procedures followed were in accordance with the institutional ethical standards for human experimentation and with the Helsinki Declaration. All participants were given an information sheet explaining the study purpose, methodology as well as their rights as research subjects and written consent was obtained.

Data sharing statement No additional data available.

Abbreviations T2DM, Type 2 Diabetes Mellitus; CAN, Cardiac Autonomic Neuropathy; NO, Nitric Oxide; ROS, Reactive Oxygen Species; HRR, Heart Rate Recovery; HRV, Heart Rate Variability; CAT, Catalase; SOD, Superoxide Dismutase; HDL-c, High-density lipoprotein cholesterol; LDL-c, Low-density lipoprotein cholesterol; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; AvgNN, Mean of N-N intervals; SDNN, Standard deviation of N-N intervals; RMSSD, Square root of the mean squared differences between adjacent RR intervals; pNN50, Percentage of interval differences of adjacent RR intervals greater than 50 milliseconds derived from differences between consecutive RR intervals; TP, Total power; LF nu, Low frequency power normalize units; HF nu, High frequency power normalize units LF/HF ratio: Ratio of low and high frequency power; eNOS, endothelial Nitric Oxide Synthase; nNOS, neuronal Nitric Oxide Synthase; iNOS, inducible Nitric Oxide Synthase.

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