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Original Article

Assessment of autonomic functions and its association with telomerase level, oxidative stress and inflammation in complete glycemic spectrum– an exploratory study

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ABSTRACT

Aims: In the present study we intended to study autonomic functions and its association with telomerase level, oxidative stress and inflammation in complete glycemic spectrum.
Materials and methods: Age, gender and BMI matched 28 subjects in the age group of 25–50 years were recruited across complete glycemic spectrum as follows: 1) Normoglycemics (controls) 2) Prediabetics and 3) Frank diabetics. We assessed heart rate variability, cardiac autonomic function, lipid profile, adiponectin, malondialdehyde and telomerase level.
Results: Time domain parameters and frequency domain parameters were significantly lower, and LFnu and LF/HF ratio were significantly higher in prediabetes and diabetes than control. Serum Adiponectin and HDL levels were significantly lower in diabetes than prediabetes and control, and prediabetes had significantly lower HDL than controls. Other lipid profile parameters (TC, TG, VLDL, LDL, non-HDL & derived lipid parameters were significantly higher in diabetes than prediabetes and control and pre-diabetes had significantly higher values than controls. MDA levels were significantly higher and TAS was significantly lower in diabetics than prediabetics and control group. Telomerase level was significantly higher in diabetes as compared to prediabetes and control. Telomerase had significantly negative correlation with SDNN, HF, TP, HDL and adiponectin, and significant positive correlation with MDA, fasting insulin, HOMA IR, TC, and AIP.
Conclusion: Oxidative damage, inflammation and autonomic dysregulation may be involved in Telomere/Telomerase dysregulation in diabetes and telomerase levels can be used as a cardio-metabolic marker of diabetes.

1. Introduction

In 2017 ICMR-INDIAB study results have shown the overall prevalence of diabetes in India to be 7.3% and prediabetes to be 24.7% (ADA criteria) [1]. Prevalence of type 2 diabetes mellitus (T2DM) is highest in southeast Asian region including India due to higher prevalence of altered body composition, marked by higher fat mass [2]. Further, Asian Indians progress faster from prediabetes to diabetes than other ethnic groups [3] and thereby a concomitant higher propensity for cardiovascular risk. This necessitates the need to assess cardiovascular risk in the complete glycemic spectrum in the Indian Scenario.

Eukaryotic chromosomes carry tandemly repeated terminal sequences, called “Telomeres”, needed for chromosome stability. During mitosis, telomeric DNA is sacrificed in return for protecting the protein coding and regulatory elements of the genome leading

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to shorter telomeres. The enzyme "Telomerase" helps to guard against this telomere loss by copying an RNA template sequence within its RNA moiety [4]. Telomerase activity is shown to be influenced by a range of stressors like obesity and insulin resistance [7].Though several studies showing association between impaired telomerase activity and ageing are there, its causal link with T2DM is pending. Peripheral blood telomerase activity is inversely related to insulin resistance and occurrence of vascular complications in patients of T2DM and could be used as an important cardiometabolic biomarker for T2DM [4,5].

Adipose tissue secretes biomolecules such as "Adipokines" (leptin, adiponectin, etc). Adiponectin is of particular interest in T2DM as it is an important determinant of whole-body insulin sensitivity, anti-inflammatory in nature and has protective cardiovascular effects [6].

Levels of adipokines like leptin, tumor necrosis factor alpha, interleukin 6 increase in obesity and tend to function in a pro-inflammatory manner. In contrast, adiponectin level decrease in obese subjects and it functions as anti-inflammatory cytokine. Adiponectin concentration is shown to negatively correlate with increased body fat, insulin resistance and cardiovascular risk in T2DM [7].

Cardiovascular autonomic neuropathy is a major complication in patients with T2DM. Analysing the patterns of heart rate variability carries the potential for the detection of autonomic imbalance in the subclinical and symptomatic stages of T2DM [8]. Previous studies have shown that there is autonomic imbalance characterized by increased sympathetic activity and/or decreased parasympathetic activity in patients of T2DM which is also a major risk factor for developing cardio metabolic diseases [9].

The objective of the present study was to assess telomerase level in the complete glycemic spectrum (controls, prediabetes and diabetes) along with adiponectin level and cardiac autonomic function and to identify the association between these parameters. To the best of our knowledge, this is the first study in which association of these parameters has been studied in Indian population.

2. Materials and methods

Study design: This is an observational case-control study, conducted between February 2016 - June 2018 by Department of Physiology, JIPMER, Puducherry, India in collaboration with Department of Medicine and Biochemistry of the same institution. The study was commenced after obtaining approval from the institute ethics committee for human studies (No. JIP/IEC/2015/22/774).

Participants: Based on the study objective, to include subjects of the glycemic spectrum (Normoglycemic, prediabetes and diabetes) we considered individuals of either gender in the age group of 25–50 years. In India, obesity, age, family history of diabetes and higher socio economic status were the main factors that drives the diabetes epidemic [1] and we have taken steps to match age, BMI and socioeconomic status in this study. A ten year follow up study in south India has shown smoking and alcohol to be an independent risk to develop diabetes [3]. Hence, we have selected subjects without smoking and alcohol intake (>3 drinks per day).

Subjects who are diagnosed with T2DM within last two years, who are visiting JIPMER diabetic clinic were considered for diabetic group (n = 70) and explained the procedure to volunteers (n = 60). We excluded subjects with any diabetic complication or under insulin treatment and those with hypertension, any known organic disorders, taking any medication for acute or chronic disease (n = 32) and 28 (male = 12; Female = 16) subjects were recruited for diabetic group after obtaining written informed consent.

Subject were asked to report to Department of Physiology, between 7 and 8 am in fasting condition (at least 8 h of caloric restriction was recommended) [10] and 5 ml of venous blood was withdrawn from the anterior cubital vein for biochemical parameters. Followed by which their anthropometric measurements and body fat percentage was recorded and was asked to report the next day for recording cardiovascular parameters and cardiac autonomic function.

For normoglycemic and prediabetic groups we considered apparently normal patients' relatives attending JIPMER outpatient department and staffs of JIPMER (n = 200). Volunteers were recruited after getting written informed consent and then matched for BMI (±2) with the diabetic subjects (n = 125). Subjects were then asked to report to Department of Physiology, between 7 and 8 am in fasting condition and 5 ml of venous blood was withdrawn from the anterior cubital vein for biochemical parameters. Following 2 h of oral glucose tolerance test (OGTT - 75 g of glucose in 200 ml of water), another 2 ml of venous blood was withdrawn. As per the OGTT report, they were divided into Normoglycemic (2hr plasma glucose <140 mg/dl during an OGTT (n = 93), Prediabetics (2hr plasma glucose >140 - <199 mg/dl, (n = 32)) and diabetics (2 h plasma glucose >200 mg/dl – excluded (n = 3)). Matching for age (±5), gender socioeconomic status [11] and by excluding those with family history of diabetes from normoglycemic control group by convenience sampling we choose 28 subjects each for normoglycemic group and prediabetic group. They were then oriented to research lab and explained about the procedures that will be done on subsequent day.

Participants (including diabetic group) were requested to have sound sleep before the day of recording and to refrain from caffeinated beverages, exercise, alcohol and nicotine 24 h prior to recording. Participants then reported to the Cardiac autonomic function testing lab 2 h after light breakfast with comfortable clothing (suitable for exercise), empty bowel and bladder between 9 AM and 11 AM. The temperature of the lab was maintained between 24 and 26 °C. The room was lit with dim light. Their cardiovascular parameters, short term Heart rate variability and autonomic reactivity tests were done.

2.1. Parameters recorded

1. **Anthropometric measurements:** Measurements were made by International Society for the Advancement of Kinanthropometry certified investigator. A wall mounted stadiometer (V M Electronics Hardware Ltd) accurate to the nearest 0.1 cm was used to measure height. Weight was measured using digital weighing scale (Charder Electronic Co. Ltd, Taiwan) accurate to the nearest 0.1 kg. BMI was calculated using Quetelet's index [12].
2. **Body fat percentage (BF%):** After removing any metals, participants were made to lie supine on the couch. Body fat electrodes were placed in a tetrapolar manner and body fat percentage (BF%) was measured using bio impedance method (Quadscan 4000 R, UK). After removing BF% electrodes, ECG electrodes were placed.
3. **Cardiovascular parameters**: Blood pressure (BP) and heart rate (HR) were measured after 10 min of rest in sitting position [13]. HR was assessed manually from the radial artery. The BP (mm hg) was recorded from right arm using mercury sphygmomanometer (Model: Diamond, Industrial Electronic & allied product, India). BP recordings were taken thrice with 2 min rest intervals and the average was taken [14,15]. All measurements were taken by the same investigator.
4. **Short-term heart rate variability:** We followed guidelines formulated by Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [16]. After 5 min of supine rest, lead II electrocardiography (ECG)

was recorded for 5 min. The conversion of analog to digital signal was done using 16 bit, 16- channel data acquisition system with Acqknowledge 3.8.2 software (Biopac MP100, USA). The sampling rate was 500 Hz and Band pass filter of 2 Hz–40 Hz was used. From the RR tachogram both frequency and time domain measures were computed using Kubios

1.0 software (Bio-signal analysis Group, Finland) using Fast Fourier Transformation (FFT) and RR trend respectively. The frequency domain indices - Very Low Frequency (VLF; 0.003 Hz - 0.04 Hz), Low frequency (LF; 0.04–0.15 Hz) and High Frequency (HF; 0.15 Hz- 0.4 Hz) both in absolute powers (ms^2) and in normalized unit (nu), Total power (TP) ms^2 and LF/HF ratio was calculated. Time domain measures - Standard deviation of all NN intervals (SDNN), sum of the squares of differences between adjacent RR intervals (RMSSD), adjacent RR interval differing more than 50 ms (NN50) and its percentage (pNN50).

5. Autonomic reactivity tests:
   a. **Forced timed breathing:** Subjects in supine position were asked to breath at the rate of 6 breaths per minute comprising inspiratory and expiratory cycles for 5 s [17] avoiding abrupt inhalation/exhalation/holding breath during the procedure. Deep breathing was synchronized to a paced voice metronome and if necessary guided by hand movement. The ratio between maximal RR interval during expiration (E) and minimal RR interval during inspiration (I) is considered as E: I ratio.
   b. **Orthostatic stress test (OST):** Participants were instructed to stand within 3 s from supine position [23]. The ratio of longest RR interval around 30^{th} beat and shortest around 15th beat (30:15 ratio) was calculated to obtain HR response to OST. During the procedure BP was monitored continuously (every 40 s) using automatic BP monitor for 5 min.
6. **Biochemical parameters:** The biochemical parameters were analyzed according to the manufacturer guidelines.
   a. **Blood glucose, Insulin resistance and sensitivity:** Fasting blood glucose - glucose oxidase-peroxidase method (Genuine Biosystem). Fasting Insulin - ELISA (Enzyme linked Immuno Sorbent Assay) (DIASOURCE, Germany). Insulin resistance was calculated using HOMA2: the updated HOMA model (i.e., the computer model) [18], HOMA- AD [fasting glucose (mmol/L) x fasting insulin (mU/L)]/[22.5 x fasting adiponectin (μg/ml)] [19], and HOMA %S was used to calculate insulin sensitivity [20].
   b. **Oxidative stress markers:** Oxidant-antioxidant status was measured by quantification of malondialdehyde (Cayman) and total antioxidant status (BT-Lab) using ELISA.
   c. **Lipid profile:** Total cholesterol (TC) was estimated by Colorimetric, enzymatic methods with cholesterol oxidase peroxidase by using Diagnostic kit from Agappe diagnostics. High density Lipoprotein (HDL) was estimated by direct enzymatic, colorimetric method with cholesterol oxidase esterase by using Diagnostic kit from Accucare, Lab care diagnostics. Triglycerides (TG) was estimated by Colorimetric, enzymatic method with GPO – PAP – ESPAS by using Diagnostic Kit from Agappe diagnostics using fully automated clinical chemistry analyzer (AU400, Olympus, USA). VLDL was calculated using Friedwalds's formula [21]. Other lipid profile derived parameters were calculated (TC/HDL, TG/HDL, LDL/HDL, atherogenic index).
   d. **Telomerase measurement:** Human TE (telomerase) ELISA kit (Elabscience boitech Inc, USA) was used for this analysis. We followed Sandwich-ELISA principle as recommended by manufacturer guidelines. ELISA well plates were pre-coated with an antibody specific to Human Telomerase. 100 μL standard or samples were added to each well and incubated for 90 min at 37^{O}C. The liquid was removed, following which 100 μL biotynylated dectection Antibody was added. We again incubated for 1 h at room temperature (37 °C). After five washes with PBS-Tween, 90 μL of substrate solution was added to each well, and incubated for 15 min, 50 μL of stop solution was added to each well to stop the reaction. The absorbances were read in the microplate reader in dual-wavelength mode (450 nm).

Statistical analysis: Physiological and biochemical data were tested for normality. Normally distributed data such as age, height, weight, BMI, Heart rate, Systolic and diastolic blood pressure and all biochemical parameters are expressed as mean ± standard deviation and comparison between groups were done using One-way ANOVA followed by Posthoc test using least significant difference (LSD) analysis. Non-normally distributed data such as short-term Heart rate variability and autonomic reactivity parameters were expressed in median with interquartile range and comparison between groups were done using Kruskal Wallis test followed by posthoc test using Mann-Whitney *U* test. Correlation between these measures were studied using Pearson's correlation or Spearman's rank correlation coefficient as appropriate. All analyses were two-tailed and a significance level of $p < 0.05$ was be used in the study.

3. Results

Table 1: Groups were comparable based on age, gender, height, weight, and BMI. Body fat percentage was significantly different among the groups. On post hoc analysis BF% was higher in prediabetes group ($p = 0.024$) and diabetes group ($p < 0.001$) than control group. Further BF% of diabetes group was significantly more than prediabetes group ($p = 0.02$).

Table 2: Heart rate was significantly higher in diabetic group as compared to control and prediabetes group. Systolic blood pressure was higher in prediabetic and diabetic group as compared to control group and diabetic group had higher than prediabetic group. Diastolic blood pressure was higher in diabetic group as compared to control and prediabetes group.

Table 3: Time domain parameters (SDNN, RMSSD, NN50, pNN50) were significantly lower in prediabetes and diabetes as compared to controls, while prediabetes and diabetes were comparable in these parameters. Groups were significantly different in all the frequency domain parameters based on Kruskal-Wallis test. On post hoc analysis TP, VLF, LF, HF, and HFnu were significantly less in prediabetes and diabetes as compared to control and LFnu and LF/HF ratio were significantly higher in prediabetes and diabetes as compared to control. Further, diabetes had significantly lower HF and TP as compared to prediabetes while other parameters of the frequency domain parameters were comparable. Groups were significantly different in autonomic reactivity test based on Kruskal-Wallis test. On post hot analysis we observed that the prediabetes and diabetes has significantly lesser autonomic reactivity as compared to control, while the prediabetes and diabetes groups were comparable in these parameters.

Table 4: Groups were significantly different in their biochemical profiles based on one-way ANOVA. Fasting insulin and glucose, HOMA IR, and HOMA AD were significantly higher and HOMA %S significantly lower in diabetes as compared to control and prediabetes. Fasting insulin and glucose, HOMA IR and HOMA AD were higher and HOMA %S lower prediabetes as compared to controls however statistical significance was seen only for HOMA IR, and HOMA %S. Adiponectin and HDL was significantly lower in diabetes

Table 1
Comparison of demographic profile.

Parameters	Control (n = 28)	Prediabetes (n = 28)	Diabetes (n = 28)	ANOVA
	Mean ± SD	Mean ± SD	Mean ± SD	
Male/Female	12/16	12/16	12/16	
Age (years)	37.82 ± 7.29	37.79 ± 7.76	38.04 ± 6.92	.991
Height (cm)	159.64 ± 6.08	158.54 ± 5.94	161.04 ± 5.56	.284
Weight (kg)	60.75 ± 5.56	59.68 ± 6.74	60.61 ± 5.49	.766
Body mass index (kg/m^2)	23.93 ± 2.80	23.80 ± 2.90	23.45 ± 2.68	.807
Body fat percentage	26.20 ± 4.25	30.02 ± 4.65	33.91 ± 6.54	<.001

Comparison was done using One- Way Anova and post-hoc analysis using LSD test. The significance was set at P value < 0.05.

Table 2
Comparison of cardiovascular parameters.

Parameters	Control (n = 28)	Prediabetes (n = 28)	Diabetes (n = 28)	P value			
	Mean ± SD	Mean ± SD	Mean ± SD	ANOVA	control vs prediabetes	control vs diabetes	prediabetes vs diabetes
Heart rate (beats/min)	74.43 ± 6.02	77.21 ± 7.51	82.61 ± 6.43	<.001	.123	<.001	.003
Systolic blood pressure (mmHg)	114.14 ± 5.73	118.79 ± 4.71	121.25 ± 6.12	<.001	.002	<.001	.101
Diastolic blood pressure (mmHg)	76.14 ± 8.24	75.86 ± 6.14	84.93 ± 6.39	<.001	.879	<.001	<.001

Table 3
Comparison of cardiac autonomic function parameters.

Parameters	Control (n = 28)	Prediabetes (n = 28)	Diabetes (n = 28)	P value			
	Median (IQR)	Median (IQR)	Median (IQR)	Kruskal- Walis	control vs prediabetes	control vs diabetes	prediabetes vs diabetes
Time domain parameters							
SDNN (ms)	54.35 (39.33)	29.35 (24.98)	23.85 (14.30)	<.001	.001	<.001	.100
RMSSD (ms)	32.45 (28.33)	18.65 (16.00)	19.05 (14.33)	<.001	.002	<.001	.611
NN50 (counts)	41.50 (108.50)	3.00 (22.25)	2.50 (8.50)	<.001	<.001	<.001	.482
pNN50	9.50 (26.53)	0.75 (6.00)	0.60 (1.95)	<.001	<.001	<.001	.442
Frequency domain parameters							
VLF (ms^2)	703.00 (1150.25)	267.00 (481.50)	67.00 (242.00)	<.001	.001	<.001	.008
LF (ms^2)	816.00 (888.00)	205.00 (284.50)	166.50 (272.50)	<.001	<.001	<.001	.118
HF(ms^2)	1186.50 (924.25)	191.50 (105.75)	126.00 (106.50)	<.001	<.001	<.001	<.001
TP (ms^2)	2794.50 (2462.50)	714.00 (1028.50)	466.50 (527.25)	<.001	<.001	<.001	.019
LF/HF	0.73 (0.43)	0.92 (0.63)	1.33 (1.75)	.006	.011	.006	.174
LFnu	42.05 (14.36)	47.85 (16.53)	56.89 (30.62)	.006	.011	.006	.169
HFnu	57.96 (14.36)	52.16 (16.53)	43.12 (30.62)	.006	.011	.006	.169
Reactivity tests							
3015 ratio	1.34 (0.32)	1.24 (0.29)	1.20 (0.19)	.034	.052	.014	.611
EI ratio	1.30 (0.23)	1.18 (0.16)	1.22 (0.18	.039	.019	.041	.909

Comparison was done using Kruskal-Wallis test and post-hoc analysis using Mann- Whtiney *U* test. The significance was set at P value < 0.05. SDNN: Standard deviation of all NN intervals; RMSSD: Square root of mean of the sum of the squares of differences between adjacent NN intervals; NN50 count: Number of pairs of adjacent NN intervals differing by more than 50 ms in entire recording. Total power: The variance of NN intervals over the temporal segment; LF: Power in low frequency range (0.04–0.15 Hz); HF: Power in high frequency range (0.15–0.4 Hz); LF norm: LF power in normalized units(LF/(TP-VLF)*100); HF norm: HF power in normalized units(HF/(TP-VLF)*100); LF/HF ratio: Ratio LF (ms2)/HF (ms2). 30 15 ratio: ratio of the longest RR interval after standing to the shortest RR interval. EI ratio: ratio of longest RR interval during expiration to shortest RR interval during inspiration.

than prediabetes and control, and prediabetes has significantly lower HDL than controls. Other lipid profile parameters (TC, TG, VLDL, LDL, non-HDL, TC/HDL, TG/HDL, LDL/HDL, AIP were significantly higher in diabetes than prediabetes and control and prediabetes has significantly higher values than controls. Oxidative stress was significantly higher in diabetes than prediabetes and control group and prediabetes has higher oxidative stress than controls. Total antioxidant status was significantly lower in diabetes as compared to prediabetes and control group and prediabetes has significantly lower TAS than controls. Telomerase level was significantly higher in diabetes as compared to prediabetes and control. While the values were comparable between prediabetes and control group.

Table 5: Telomerase has significantly negative correlation with SDNN, LF, HF, TP, TAS, HDL and adiponectin, and significant positive correlation with MDA, fasting insulin, HOMA IR, TC, and AIP and no correlation with fasting glucose. Adiponectin has significant positive correlation with SDNN, LF, HF, TP, TAS, HDL and significant negative correlation with MDA, fasting insulin and glucose, HOMA IR, TC, AIP and telomerase.

4. Discussion

The present study was aimed to evaluate the telomerase activity via telomerase level in complete glycemic spectrum in Indian population. Further, the telomerase level and associated variables causing cardiovascular risks were studied to identify the relationship between these parameters.

Higher BMI/body fat percentage is associated with increased insulin resistance and diabetes [22]. However, the location of the fat would play a pivot role as visceral fat has greater implications for T2DM, than other fat depots (subcutaneous, ectopic), in the form of insulin resistance, metabolic derangements and glucose intolerance [23]. Our study demonstrated that although along with age

Table 4
Comparison of biochemical profile.

Parameters	Control (n = 28)	Prediabetes (n = 28)	Diabetes (n = 28)	P value			
	Mean ± SD	Mean ± SD	Mean ± SD	ANOVA	control vs prediabetes	control vs diabetes	prediabetes vs diabetes
Insulin and glucose profile							
Fating Insulin (μIU/ml)	2.58 ± 1.10	6.51 ± 1.56	23.75 ± 13.12	<.001	.175	<.001	<.001
Fasting glucose (mg/dl)	97.54 ± 6.34	104.46 ± 10.61	125.71 ± 26.85	<.001	.398	<.001	<.001
HOMA IR	37.94 ± 10.59	64.14 ± 14.96	119.72 ± 63.99	<.001	.013	<.001	<.001
HOMA %S	356.57 ± 173.24	120.31 ± 28.20	40.19 ± 18.75	<.001	<.001	<.001	.004
HOMA AD	0.05 ± 0.03	0.24 ± 0.16	2.82 ± 1.85	<.001	.510	<.001	<.001
Lipid profile							
TC (mg/dl)	141.21 ± 17.37	171.96 ± 6.32	206.50 ± 10.75	<.001	<.001	<.001	<.001
TG (mg/dl)	60.68 ± 17.68	103.14 ± 15.06	151.36 ± 19.09	<.001	<.001	<.001	<.001
HDL (mg/dl)	50.71 ± 7.31	37.00 ± 2.11	23.68 ± 6.68	<.001	<.001	<.001	<.001
VLDL (mg/dl)	12.14 ± 3.54	20.63 ± 3.01	30.27 ± 3.82	<.001	<.001	<.001	<.001
LDL (mg/dl)	84.08 ± 12.73	112.29 ± 8.64	176.19 ± 39.46	<.001	<.001	<.001	<.001
non-HDL	156.89 ± 33.59	236.06 ± 26.59	357.81 ± 61.22	<.001	<.001	<.001	<.001
TC/HDL	2.88 ± 0.68	4.67 ± 0.44	9.64 ± 3.48	<.001	.002	<.001	<.001
TG/HDL	1.26 ± 0.50	2.82 ± 0.58	7.04 ± 2.52	<.001	<.001	<.001	<.001
LDL/HDL	1.72 ± 0.46	3.06 ± 0.41	8.25 ± 3.47	<.001	.016	<.001	<.001
AIP	0.14 ± 0.47	1.02 ± 0.20	1.89 ± 0.35	<.001	<.001	<.001	<.001
Adiponectin (mg/mL)	23.94 ± 19.03	12.41 ± 10.96	2.95 ± 1.15	<.001	.003	<.001	.020
Oxidative stress and anti-oxidant status							
MDA (μmol/L)	0.18 ± 0.03	0.82 ± 0.99	1.36 ± 0.84	<.001	.006	<.001	.025
TAS (mmol/L)	812.34 ± 159.90	695.39 ± 225.92	495.90 ± 118.71	<.001	.014	<.001	<.001
Telomerase							
Telomerase level	7.67 ± 2.63	6.10 ± 2.78	10.54 ± 6.51	.001	.184	.016	<.001

Comparison was done using One- Way Anova and post-hoc analysis using LSD test. The significance was set at P value < 0.05.
HOMA-IR - Homeostasis model assessment-Insulin resistance; HOMA-AD: Homeostasis model assessment- Adiponectin; HOMA % S– Insulin sensitivity in percentage. TC- Total cholesterol; TG- Triglycerides; HDL-High density lipoprotein; VLDL- Very low density lipoprotein; LDL- Low density lipoprotein. AIP – Atherogenic index of plasma; MDA-malondialdehyde; TAS- total antioxidant status.

Table 5
Correlation between telomerase, adiponectin, cardiac autonomic function parameters, oxidative stress and anti-oxidant parameters, and lipid profile.

Parameters	Adiponectin	telomerase level
Adiponectin (mg/mL)	1	-.219*
SDNN	.608**	-.492**
LF	.679**	-.365**
HF	.528**	-.284**
TP	.561**	-.321**
MDA (μmol/L)	-.417**	.376**
TAS (mmol/L)	.634**	-.370**
Fating Insulin (μIU/ml)	-.428**	.260*
Fasting glucose (mg/dl)	-.425**	.213
HOMAIR	-.441**	.272*
TC (mg/dl)	-.728**	.304**
HDL (mg/dl)	.715**	-.286**
AIP	-.727**	.295**
telomerase level	-.219*	1

Pearson's correlation was done. The significance was set at P value < 0.05. *p < 0.05; **p < 0.01. SDNN: Standard deviation of all NN intervals; Total power: The variance of NN intervals over the temporal segment; LF: Power in low frequency range (0.04–0.15 Hz); HF: Power in high frequency range (0.15–0.4 Hz); MDA-malondialdehyde; TAS- total antioxidant status. TC- Total cholesterol; HDL-High density lipoprotein; AIP – Atherogenic index of plasma.

and gender distribution matching, subjects were matched for BMI (±2), however, there was significantly higher body fat percentage in prediabetes than controls and further diabetes had higher body fat percentage than prediabetes. Our study finding corroborates with previous studies which also found that prediabetes and diabetes have lesser lean mass and higher adiposity especially centripetal in distribution [23,24].

Previous study demonstrated that visceral fat accumulation occurs early in pathogenesis of diabetes due to environmental and genetic factors which leads to higher levels of NEFA (non-esterified fatty acids) or inflammatory adipokines to liver that alters reactivity in the autonomic nervous system marked by higher sympathetic nervous system (SNS) reactivity and/or reduced parasympathetic nervous system (PNS) reactivity and leads to higher insulin resistance [25]. Other mechanism proposed that diabetes progression primarily causes autonomic alteration that leads to altered body fat distribution leading to Insulin resistance [26]. Our study also found significantly higher body fat percentage and altered autonomic balance, however, cause and effect relation cannot be determined from the present study.

Further, the adipose tissue present could be dysfunctional too [27] as indicated by deranged lipid profile and decreased adiponectin levels which was observed even in prediabetic group. Adiponectin is endogenous insulin sensitizer and decreased levels of adiponectin could in turn induce insulin resistance, oxidative stress and increase cardiovascular risk [28]. In support, we observed that adiponectin correlated negatively with MDA, total cholesterol and atherogenic index of plasma. Body fat dysfunction is also known to be associated with sympathetic overactivity, decreased parasympathetic activity [29,30]. We also observed higher sympathetic activity (LFnu and LF:HF ratio) and decreased parasympathetic activity (total power, HFnu, LF power, HF power, SDNN, RMSSD, NN50) in prediabetes and diabetes group. Further, hyperinsulinemia alone is shown to increase sympathetic activity and hyperglycaemia induced stress damages long fibres of the autonomic nervous system (parasympathetic fibres) mainly [31].

Research studies have reported that hyperinsulinemia or insulin resistance contributes to the onset of hypertension in diabetes subjects by inappropriate sympathetic activity [32]. This goes hand in hand with our observation as evident by increased HOMA IR and HOMA AD in prediabetic and diabetic subjects. Increased sympathetic activity increases circulating nor epinephrine and total peripheral resistance causing increase in systolic and diastolic blood pressure. However, the increase in diastolic blood pressure was seen only in diabetes which denotes the progression of sympathetic over activity from prediabetes to diabetes. Sympathetic overactivity through renal mechanism (not studied) could also contribute to hypertension by increasing tubular reabsorption of urinary sodium

and water, reducing renal blood flow and Glomerular filtration rate and by increasing renin release [33].

Hyperglycemia and insulin resistant states increases the lipid peroxidation causing alterations in oxidant and antioxidant balance [34]. Higher oxidative stress and inflammation observed in diabetes could lead to decreased telomere length in diabetic individuals. Telomeres cap chromosome ends and prevent DNA damage during replication. They are essential for chromosomal stability and thereby delays cell cycle arrest (senescence) or apoptosis. Telomere length shortening in diabetes is first reported by Jeanclos et al., in 1998 [35]. We observed that plasma telomerase level was increased in diabetes. Telomerase enzyme counteracts telomere shortening and their levels rise to maintain the telomere length. We observed a positive correlation between oxidative stress and telomerase level in our study. We hypothesize that telomerase level increase as oxidative damage to the cells increase as a protective mechanism.

Earlier studies have shown that decreased telomerase activity impairs replicative capacity of pancreatic beta cells to cause impaired insulin secretion and glucose intolerance [36]. However, we have not measured the local telomerase activity in the pancreatic cells. The telomerase level in the plasma indicates mainly the telomerase activity in the endothelial cells lining the vascular bed. Oxidative stress by hyperglycemia in diabetes and higher physical stress given by high blood pressure to the vascular endothelium puts them at higher risk for damage and higher plasma levels of telomerase could indicate the declining vascular bed health. Our study findings corroborate with previous studies which demonstrated that serum telomerase level and oxidative stress levels were significantly higher in COPD patients during the attack period and in subjects with COPD exacerbation when compared with asymptomatic smokers [37]. It was proposed that telomerase could be used as the beneficial primary marker of inflammation-related diseases and oxidative stress [38].

Decreased HRV is by itself a harbinger of cardiovascular disease [16]. Telomerase level has significant negative correlation with total power, indicating that telomerase level could be used as a prognostic indicator of cardiovascular health. Further telomerase also correlates significantly with insulin (positive) total cholesterol (positive), HDL (negative), adiponectin (negative) and MDA (positive). Hence, telomerase level assessment could emerge as a single most important indicator of overall health in diabetes. However, we have not observed marked change in telomerase level in prediabetes. We hypothesize that increase in telomerase level could indicate a break point where the compensatory mechanisms of the body are overcome by pathological mechanisms and the disease progress from prediabetes to diabetes. This claim requires further study.

Based on our study results, we propose following mechanism for the association of parameters and pathophysiology of diabetes progression:

Diabetes is considered a state of chronic inflammation and accelerated age condition [39]. There is increased synthesis of pro-inflammatory cytokines (IL-1b, TNF-alfa, CRP) and Tol like receptors (TLR) that can also be activated by endogenous ligands that can promote inflammation even in the absence of infection [40]. This stimulates many intermediates (MAP kinases) that activate pro-inflammatory genes transcribing to produce overactivation of NF-κB which regulates inflammation and immunity [41,42]. Simultaneously, there is decreased production of anti-inflammatory mediators like IL-10 and adiponectin [43,44]. Chronic inflammation leads to the generation of higher level of reactive oxygen species (ROS) that results in telomeric attrition, triggering of DNA damage response pathways (DDR), mitochondrial destruction and finally causing cellular senescence. This results in insulin resistance and higher occurrence of atherosclerosis and CAD events in diabetics [45]. Previous studies also demonstrate association between higher oxidative stress, chronic inflammation and serum telomerase level [38,39].

Many previous studies have linked Telomere/Telomerase imbalances with chronic inflammation and vice-versa. Since diabetes is also considered age-accelerated disease, it is marked by higher telomere attrition rate leading to occurrence of state of replicative senescence in dividing cells like endothelial cells, germ cells and fibroblasts [46]. Senescent cells are fully functional, but have no telomerase activity and regenerative capacity, and release proinflammatory cytokines that contribute to the low-level grade inflammation [39]. Also, higher visceral fat contributes to the altered autonomic activity [29] that leads to insulin resistance and chronic inflammation progression. It has been found that PBMC telomeric length is associated with the duration of disease and good glycemic control [35,47].

Overall, our study showed that prediabetes and diabetes pathophysiology show association amongst higher telomerase, altered autonomic nervous system reactivity, oxidative stress, higher inflammation (decreased adiponectin level) and higher body fat percentage.

5. Conclusion

In the present study, increased telomerase level was observed in prediabetes and diabetes which was associated with oxidative stress and hyperinsulinemia and insulin resistance. Vagal tone reduction was manifested along with sympathetic overactivity in FDR of T2DM, prediabetes and diabetes subjects. Magnitude of TP was inversely correlated with telomerase level. Telomerase level per se indicates cardiovascular risk. It can be concluded that increased telomerase level could be a major predictor of cardiovascular risk in high-risk population of metabolic syndrome. Overall, adopting healthy lifestyles such as increased physical activity and antioxidant intake may curb the progression of reduction in telomerase activity.

6. Limitations

As this is an exploratory study, convenience sampling was done on the basis of funds availability. Also, we could not measure inflammatory mediators and telomere length of PBMCs due to the lack of funds. In future, we will plan to study these parameters in larger sample size and would like to include first degree relatives of diabetes mellitus patients in order to further understand genetic component of diabetes mellitus.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2019.01.011.

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