

Comparative study on cardiac autonomic modulation during deep breathing test and diaphragmatic breathing in type 2 diabetes and healthy subjects

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ABSTRACT

Aims/Introduction: Diaphragmatic breathing is known to have a beneficial effect on the cardiopulmonary system, and enhances parasympathetic activation. We evaluated the influence of diaphragmatic breathing on time domain measures of heart rate variability in diabetics and healthy subjects.

Materials and Methods: A total of 122 type 2 diabetics and 94 healthy subjects (controls) were randomly allocated to a deep breathing test and diaphragmatic breathing (61 diabetics and 47 controls in each group). Heart rate variability parameters; namely, expiratory:inspiratory ratio (E:I ratio), root mean square of successive N–N interval difference (r-MSSD) and standard deviation of all the N–N intervals (SDNN), were quantified from 1-min supine electrocardiogram obtained while subjects carried out the deep breathing test/diaphragmatic breathing at six respiratory cycles per min. Data analysis was carried out by Student's unpaired *t*-test. A *P*-value <0.05 was taken as significant.

Results: E:I ratio, SDNN and r-MSSD of type 2 diabetics was significantly lower compared with controls in the diaphragmatic group (*P* < 0.001). E:I ratio and SDNN were significantly lower in type 2 diabetics compared with controls in the deep breathing group (*P* < 0.0001, *P* < 0.019, respectively). In controls, E:I ratio, r-MSSD and SDNN of the diaphragmatic breathing group were significantly higher compared with the deep breathing group (*P* < 0.01). In diabetics, none of the measured heart rate variability parameters varied between diaphragmatic breathing and deep breathing.

Conclusions: Subclinical cardiac autonomic neuropathy persists in type 2 diabetics. In type 2 diabetics, diaphragmatic breathing quantifies certain aspects of parasympathetic dysfunction, which is not shown by the deep breathing test. Diaphragmatic breathing induces greater cardiac autonomic modulation in healthy subjects.

INTRODUCTION

The human heart beat in a healthy individual is neither absolutely regular nor completely random. This subtle fluctuation in sinus rhythm is known as heart rate variability (HRV). Indices of HRV provide an insight into the autonomic modulation of the heart¹. One important clinical application of HRV is in the assessment of diabetic cardiac autonomic neuropathy². In the

intact heart, parasympathetic fibers are inhibitory and sympathetic fibers are excitatory. Inhibitory actions of cardiac parasympathetic nerves are reported to provide electrical stability to the heart, thus preventing ventricular tachycardia in humans³. Vagal nerve traffic cannot be measured directly in humans. The assessment of HRV has thus become the most widely-used indirect measure of cardiac vagal function.

Measures of HRV in response to cyclic deep breathing at six respiratory cycles per min are among the simplest to record and the most sensitive indicator of parasympathetic function.

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Both afferent and efferent pathways are vagally mediated⁴. Diaphragmatic breathing or slow abdominal breathing is also a technique of deep breathing. This is a form of chest physical therapy program. They are designed to improve the efficiency of ventilation, decrease the work of breathing, increasing the excursion of the diaphragm, and improve gas exchange and oxygenation⁵. Srinivasa *et al.*⁶ have reported improvements in measures of HRV with slow abdominal breathing. Diaphragmatic breathing is also an integral part of yogic breathing exercises known as “pranayama”. Pranayama breathing has been shown to alter autonomic activity. A study by Udupa *et al.*⁷ showed that pranayama training produces a decrease in basal sympathetic tone. Raghuraj *et al.*⁸ reported that Nadi-shodhana pranayama increases parasympathetic activity. Slow and deep breathing itself has a calming effect on the mind, and helps an individual to de-stress⁹. This calming effect could also exert profound physiological effects on pulmonary, cardiovascular and mental functions of the brain¹⁰.

Thus, analyzing the cardiac parasympathetic activity in these two modes of deep breathing; namely, a conventional deep breathing test and diaphragmatic breathing, might provide better insight into cardiac autonomic modulation in a healthy and diseased state. Thus, the present study was undertaken to investigate the influence of diaphragmatic breathing on cardiac autonomic modulation in type 2 diabetes mellitus patients and non-diabetic healthy subjects.

MATERIALS AND METHODS

The present study was carried out at the outpatient department of medicine of Kasturba Medical College Hospital, Mangalore, India. This study was undertaken after the approval by the institutional ethical committee in accordance with the ethical standards laid down in the Declaration of Helsinki, and obtaining consent from the study participants.

A total of 122 patients with type 2 diabetes mellitus and 94 non-diabetic healthy subjects who were willing to participate were enrolled into the present study. Patients with diabetes mellitus were selected based on established diabetes mellitus, according to American Diabetes Association criteria. Exclusion criteria were diabetic patients with: (i) any comorbid state or medication known to affect HRV; (ii) heart disease in which regular sinus arrhythmia was lost; (iii) known neuropathy of other etiology; and (iv) smokers and alcohol consumers.

All the participants were underwent a clinical examination. The height and weight of all the participants were measured. The body mass index was calculated using the formula: weight in kilograms (kg) divided by height in meters (m) squared. A 12-lead electrocardiogram was carried out in all the participants.

The San Antonio Consensus report states that at least one measurement should be carried out in five different diagnostic categories for diagnosing somatic neuropathy (PNP). The use of all categories together leads to a large degree of overdiagno-

sis. Manageability in the outpatient clinic is difficult because of the large number and complexity of the tests that have to be carried out¹¹. Therefore, PNP was diagnosed based on neurological examination of the peripheral somatic nervous system. The diverse pattern of clinical manifestations of PNP compelled us to adopt the neurological scoring system to define PNP based on neurological examination findings and symptoms of PNP. However, to diagnose PNP, a minimum of four scores on neurological examination was a prerequisite. Cases with isolated signs or symptoms were excluded from the study (as a case of doubt).

Neurological examination included the following. Reflexes: biceps, quadriceps, Achilles. Reflexes were graded as normal = 0, sluggish = 1 and absent = 2. Sensation: tested both in lower and upper limbs. Sensation included vibration, pain, temperature and touch. Sensory test response was scored as: normal = 0, impaired = 1 and severely impaired = 2. Muscle power: examined both in upper and lower limbs. From normal to severely abnormal were graded as grade V = 0, grade IV = 1, grade III = 2, grade II = 3, grade I = 4, grade 0 = 5. Each PNP symptom was given one score.

Fasting and postprandial blood sugars were measured in all the participants. Glycated hemoglobin (HbA1c) could be measured in 37 diabetics in the diaphragmatic breathing group and 37 diabetics in the conventional deep breathing group (a total of 74 diabetics) owing to financial constraints.

Blood pressure was recorded in all the participants. Two readings were taken 5 min apart in the sitting position. The mean of the two was recorded as blood pressure. Systolic and diastolic blood pressure were also recorded in the supine position, and then in the standing position with an interval of at least 2 min between positions. A sustained drop in systolic (>20 mmHg) or diastolic (>10 mmHg) blood pressure after standing for at least 2 min was considered as having orthostatic hypotension¹².

The diabetic patients were divided into groups of 61 for diaphragmatic breathing (DPB) and 61 for the deep breathing test (DBT). Non-diabetic healthy participants were divided into groups of 47 for DPB and 47 for DBT. Consecutive alternate eligible diabetic patients and non-diabetic healthy participants were allocated to the DPB and DBT groups at the time of entry into the study.

Furthermore, type 2 diabetics in the DPB and DBT groups were divided into three subgroups. Group A were essentially with hypertension, but essentially free from PNP. Group B were essentially with PNP, but essentially free from cardiovascular disease including hypertension. Group C were free from clinical evidence of any diabetes-related complications.

The National Glycohemoglobin Standardization Program (NGSP) and Japanese Diabetes Society (JDS) allow grading of glycemic control based on HbA1c values¹³. Owing to a relatively smaller sample size, a correlation between HRV parameters and HbA1c was considered (instead of dividing them into four subgroups: excellent, good, fair and poor).

Study Protocol

The following HRV parameters were quantified during DPB and the DBT from lead II electrocardiogram tracing of 60 s:

1. Expiratory:inspiratory ratio (E:I ratio).
2. Standard deviation of all the N–N intervals (SDNN).
3. Root mean square of successive N–N interval difference (r-MSSD).

Procedure Followed for DBT

This test was carried out in the morning when the participants were completely relaxed. Before beginning the test, the participants were taught to breathe, at six breaths per min: 5 s for each inhalation and 5 s for each exhalation. The examiner raised his hand to signal the start of each inhalation and lowered to signal the start of each exhalation. Lead II electrocardiogram was then recorded continuously at a speed of 25 mm/s for 60 s while the participants breathed as instructed (Cardiart 108T/MK-VII; BPL Ltd. Bangalore, Karnataka, India).

Procedure Followed for DPB

This was carried out in the morning when the participants were completely relaxed. This method was adopted from Kisner *et al.*⁵ with certain modifications. The modification being, in addition to the breathing pattern, relaxation of the mind and concentration on the act of breathing were emphasized. The participants were taught to carry out this act of breathing at six respiratory cycles per min: 5 s for each inhalation and 5 s for each exhalation. In the beginning, the participants were asked to rest for 5 min in the supine position. Next, the participants were asked to place their right hand on their chest and left hand below the anterior costal margin. Participants were asked to breathe in slowly and deeply through the nose, with the shoulders relaxed and upper chest still, allowing the abdomen to rise. The participants were told to slowly let all the air out using controlled expiration. The participants' left hand would rise during inspiration and fall during expiration, whereas the right hand remained still. Precautions were taken to avoid hyperventilation. Lead I electrocardiogram was then recorded continuously at a speed of 25 mm/s for 60 s while the participants breathed as instructed (Cardiart 108T/MK-VII; BPL Ltd. Bangalore).

Assessment of E:I Ratio

The R–R intervals were measured accurately from a lead II electrocardiogram recorded during the deep breathing test and diaphragmatic breathing separately. The longest interval during expiration and the shortest R–R interval during inspiration was expressed as E:I ratio¹⁴.

Assessment of SDNN

All the R–R intervals recorded during DPB and the DBT from the lead II electrocardiogram were measured accurately and fed into a computer separately. SDNN was then estimated with appropriate statistical functions using Microsoft

Windows XP Professional (Microsoft Corporation, Redmond, WA, USA).

The steps followed in computing SDNN were: (i) the mean of the entire set of R–R intervals was calculated; (ii) from each of the duration of R–R interval mean R–R interval was deducted; (iii) each R–R interval was squared; (iv) all the squared R–R intervals were summed; (v) squared R–R intervals were divided by 1–sample size; and (vi) the square root of the number obtained in step (v) was estimated.

Assessment of r-MSSD

All the R–R intervals recorded during diaphragmatic breathing and conventional deep breathing from lead II electrocardiogram were measured accurately and fed into a computer separately. r-MSSD was then estimated with appropriate statistical functions using Microsoft Windows XP Professional (Microsoft Corporation).

Steps followed in computing r-MSSD.

Step #1: The difference between the RR waveform and the delayed waveform was obtained. Step #2: The differences between the R–R intervals were squared. Step #3: sum of the squared differences were calculated. Step #4: The mean of the sum squared differences between the adjacent normal R–R intervals were derived. Step # 5: The square root of the mean of the sum squared differences between adjacent normal R–R intervals was derived. Step # 6: The square root of the mean of the sum squared differences between the adjacent normal R–R intervals in the record was divided by the number of R–R intervals within a given time minus one R–R interval.

Standard deviation/r-MSSD Estimation

From Standard deviation and r-MSSD, Standard deviation/r-MSSD was calculated.

Statistical Analyses

Statistical analysis was carried out suitably by using Student's unpaired t-test and Pearson's correlation coefficient test. The level of significance was determined by two-tailed test. Statistical significance was taken to be as a *P*-value <0.05.

RESULTS

Baseline Clinical Characteristics of Type 2 Diabetics in the DPB and DBT Groups

The data on baseline characteristics of study participants in the DPB and DBT groups are presented in Table 1. Type 2 diabetics in the DBP group were comparable with regard to age, body mass index, male-to-female ratio, blood pressure, heart rate, fasting blood sugar, postprandial blood sugar, HbA1c, duration of diabetes, diabetes-related complications and therapy (Table 1). Typical symptoms/signs of autonomic neuropathy observed were impotence and orthostatic hypotension in one participant each from the DPB group.

In many of the diabetics, more than one diabetes-related complication were present. The prevalence of hypertension was

Table 1 | Characteristics of type 2 diabetics in the diaphragmatic breathing and deep breathing groups

Variables	Diaphragmatic breathing group (n = 61)	Deep breathing group (n = 61)
Age (years)	55.75 ± 10.91	55.63 ± 11.60 (NS)
Male/female ratio	26/35	26/35 (NS)
Body mass index (kg/m ²)	23.29 ± 3.14	23.27 ± 3.30 (NS)
Systolic blood pressure (mmHg)	141.10 ± 20.18	137.68 ± 20.18 (NS)
Diastolic blood pressure (mmHg)	85.44 ± 9.57	85.22 ± 8.21 (NS)
Resting heart rate (b.p.m.)	83.09 ± 12.98	85.97 ± 14.73 (NS)
Fasting blood sugar (mg/dL)	173.08 ± 60.77	184.70 ± 76.59 (NS)
Post prandial blood sugar (mg/dL)	232.99 ± 104.36	239.24 ± 93.57 (NS)
HbA1c (%)	8.67 ± 2.42	8.56 ± 3.11 (NS)
Duration of diabetes (years)	8.16 ± 6.42	7.36 ± 6.99 (NS)
Free from complications	12 (19.67%)	17 (27.86%) (NS)
Hypertension	34 (55.73%)	26 (42.62%) (NS)
Stable angina	8 (13.11%)	11 (18.03%) (NS)
Myocardial infarction	10 (16.39%)	5 (8.19%) (NS)
Diabetic retinopathy	18 (29.50%)	12 (19.67%) (NS)
Foot ulcer	9 (14.75%)	7 (11.47%) (NS)
Somatic neuropathy	34 (55.73%)	31 (50.81%) (NS)
Microalbuminuria	11 (18.03%)	12 (19.67%) (NS)
Oral hypoglycemic agents	50 (81.96%)	52 (85.24%) (NS)
Insulin	15 (24.59%)	14 (22.95%) (NS)
Beta blockers	12 (19.67%)	11 (18.03%) (NS)
ACE inhibitors	12 (19.67%)	10 (16.39%) (NS)
Diuretics	6 (9.83%)	3 (4.91%) (NS)
Calcium channel blockers	6 (9.83%)	5 (8.19%) (NS)

Complications and drug therapy given in frequency,% is in parentheses; continues variables are presented as mean ± standard deviation. ACE, angiotensin converting enzyme; HbA1c, glycated hemoglobin; NS, non-significant compared with the diaphragmatic breathing group.

55.73% (*n* = 34) and 42.62% (*n* = 26) in the DPB and DBT groups, respectively (Table 1). Among these hypertensive diabetics, hypertension alone was observed in 14 participants in each group. In the rest of the diabetics, hypertension was coexisting with PNP.

The prevalence of PNP was 55.73% (*n* = 34) and 50.81% (*n* = 31) in the DPB and DBT groups, respectively (Table 1). Among them, PNP alone was observed in 14 diabetics in the DPB group and 16 diabetics in the DBT group. A total of 21 participants in the DBT group, and 24 participants in the DPB group showed symptoms of PNP. The symptoms observed were tingling and numbness. The remaining participants were asymptomatic. In these asymptomatic diabetics, more than one deficit was found on neurological examination.

Foot ulcer, microalbuminuria, diabetic retinopathy, stable angina and myocardial infarction complications were mainly coexisting with PNP. There were 17 participants in the DBT

group and 12 participants in the DPB group without any clinical evidence of diabetes-related complications.

HRV Parameters in Type 2 Diabetics and their Subgroups Compared With Healthy Participants in the DPB and DBT Groups

Data comparing the mean HRV parameters of type 2 diabetics and their subgroups with age- and sex-matched healthy participants in the DPB group and DBT group are presented in Table 2.

In the DPB group, the mean E:I ratio, r-MSSD and STD were significantly lower in type 2 diabetics compared with healthy participants (Table 2).

In the DBT group, the mean E:I ratio and STD were significantly lower in type 2 diabetics compared with healthy participants. In the DBT group, the mean r-MSSD did not differ significantly between the type 2 diabetics and the healthy participants (Table 2).

In the DPB group, type 2 diabetics with hypertension had significantly lower E:I ratios compared with healthy participants; type 2 diabetics with PNP had significantly lower E:I ratios, r-MSSD and STD compared with healthy participants; type 2 diabetics free from complications had significantly lower E:I ratios compared with healthy participants; r-MSSD and STD of type 2 diabetics free from complications were not significantly different compared with healthy participants (Table 2).

In the DBT group, type 2 diabetics with hypertension and PNP had significantly lower E:I ratios compared with healthy participants; r-MSSD and STD were not significantly different compared with healthy participants; mean E:I ratios, r-MSSD and STD of type 2 diabetics free from complications were not significantly different compared with healthy participants (Table 2).

Comparison of Baseline Characteristics and HRV Parameters of Healthy Participants in the DPB and DBT Groups

Participants in the DPB group were comparable with the DBT group with respect to age, sex distribution, body mass index, blood pressure and fasting blood sugar. The mean E:I ratio, r-MSSD and STD of the DPB group were significantly higher compared with the DBT group (Table 3).

Correlative Studies in Type 2 Diabetics

Correlative studies were carried out in type 2 diabetics in the DPB and DBT groups separately in relation to HbA1c level.

In the DPB group, a significant negative correlation was observed between STD/r-MSSD and HbA1c (Figure 1). There was no significant correlation between HbA1c and E:I ratio, r-MSSD and STD (*r* = 0.05, 0.21 and 0.018, respectively).

In the DBT group, a significant negative correlation was observed between r-MSSD and HbA1c values (*r* = -0.3267, *P* = 0.048). There was no significant correlation between HbA1c and E:I ratio, STD and STD/r-MSSD (*r* = -0.1985, -0.2972 and 0.1293, respectively).

Table 2 | Comparison of heart rate variability parameters of type 2 diabetics and their subgroups with age- and sex-matched non-diabetic healthy participants in the deep breathing test and diaphragmatic breathing groups

Type of breathing	Type 2 diabetics/subgroups of type 2 diabetics		Non-diabetic healthy participants	t-value	P-value	
Deep breathing test	Type 2 diabetics (<i>n</i> = 61); (Control: <i>n</i> = 47)	E:I ratio	1.30 ± 0.13	1.42 ± 0.10	5.24	0.0001
		r-MSSD	36.92 ± 23.78	39.25 ± 15.42	0.58	0.56
		STD	45.87 ± 24.38	56.14 ± 19.02	2.38	0.019
	With Hypertension (<i>n</i> = 14)	E:I ratio	1.26 ± 0.12	1.35 ± 0.07	2.42	0.02
		r-MSSD	31.23 ± 14.16	35.10 ± 15.16	0.69	0.49
		STD	38.45 ± 17.12	51.14 ± 18.43	1.88	0.07
	With somatic neuropathy (<i>n</i> = 16)	E:I ratio	1.29 ± 0.07	1.39 ± 0.07	4.04	0.0003
		r-MSSD	37.41 ± 24.08	34.47 ± 15.30	0.41	0.68
		STD	44.62 ± 18.22	50.93 ± 14.94	1.07	0.29
	Free from complications (<i>n</i> = 17)	E:I ratio	1.42 ± 0.14	1.47 ± 0.12	1.11	0.27
		r-MSSD	48.29 ± 27.05	43.40 ± 17.92	0.62	0.53
		STD	62.07 ± 29.11	60.16 ± 22.70	0.21	0.83
Diaphragmatic breathing	Type 2 diabetics (<i>n</i> = 61) (Control: <i>n</i> = 47)	E:I ratio	1.28 ± 0.13	1.48 ± 0.12	8.19	0.0001
		r-MSSD	35.19 ± 23.14	50.59 ± 21.29	3.54	0.0006
		STD	43.24 ± 25.33	67.85 ± 22.01	5.29	0.0001
	With Hypertension (<i>n</i> = 14)	E:I ratio	1.34 ± 0.16	1.46 ± 0.13	2.17	0.03
		r-MSSD	45.59 ± 36.16	54.50 ± 24.81	0.76	0.45
		STD	54.57 ± 35.58	76.91 ± 27.08	1.86	0.07
	With somatic neuropathy (<i>n</i> = 14)	E:I ratio	1.23 ± 0.11	1.46 ± 0.13	5.05	0.0001
		r-MSSD	27.04 ± 14.65	48.03 ± 20.49	3.11	0.0004
		STD	33.50 ± 19.94	67.30 ± 24.42	4.01	0.0005
	Free from complications (<i>n</i> = 12)	E:I ratio	1.37 ± 0.13	1.54 ± 0.10	3.59	0.001
		r-MSSD	41.28 ± 23.81	53.27 ± 19.71	1.34	0.19
		STD	57.80 ± 22.18	75.53 ± 24.56	1.85	0.07

E:I ratio, expiratory:inspiratory ratio; r-MSSD, root mean square of successive N-N interval difference; STD, standard deviation.

Table 3 | Comparison of baseline characteristics and heart rate variability parameters of non-diabetic healthy participants in deep breathing test and diaphragmatic breathing groups

Variables	Deep breathing (<i>n</i> = 47)	Diaphragmatic breathing (<i>n</i> = 47)	t-value	P-value
Age (years)	52.10 ± 8.52	52.34 ± 8.24	0.13	0.88
Male/female ratio	28/19	28/19	–	–
Body mass index (kg/m ²)	22.23 ± 3.19	22.45 ± 2.51	0.37	0.71
Systolic blood pressure (mmHg)	124.46 ± 11.37	122 ± 9.8	1.12	0.26
Diastolic blood pressure (mmHg)	81.21 ± 4.76	79.85 ± 4.20	1.46	0.14
Fasting blood sugar (mg%)	83.06 ± 4.85	83.08 ± 5.96	0.01	0.98
E:I ratio	1.42 ± 0.10	1.48 ± 0.12	2.63	0.009
r-MSSD (ms)	39.25 ± 15.42	50.59 ± 21.29	2.95	0.004
STD (ms)	56.14 ± 19.02	67.85 ± 22.01	2.75	0.007

Data presented as are mean ± standard deviation. E:I ratio, expiratory:inspiratory ratio; r-MSSD, root mean square of successive N-N interval difference; STD, standard deviation.

Parameters of Type 2 Diabetics and Their Subgroups in the DPB Group Compared With the DBT Group

Data on comparison of HRV parameters between the DPB and DBT groups in type 2 diabetics and their subgroups are presented in Table 4. The mean E:I ratio, mean r-MSSD, and mean STD of the DPB group in type 2 diabetics and their subgroups did not differ significantly compared with the DBT group in type 2 diabetics and their subgroups (Table 4).

DISCUSSION

In the present study, we compared the influence of two distinct patterns of deep breathing (namely diaphragmatic breathing and conventional deep breathing bedside diagnostic test of diabetic cardiac autonomic neuropathy) on certain time domain measures of HRV; namely, E:I ratio, r-MSSD, and STD in type 2 diabetics and healthy participants.

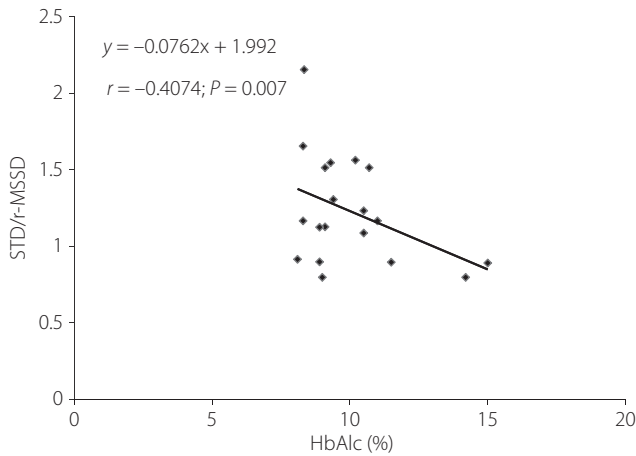


Figure 1 | The correlation between Glycosylated hemoglobin A1c and STD/r-MSSD with diaphragmatic breathing in type 2 diabetics. $y = -0.0762x + 1.992$; $r = -0.4074$, $P = 0.007$, where $y = \text{STD/r-MSSD}$ and $X = \text{HbA1c (\%)}$.

In the present study, at baseline we compared the HRV parameters in response to DPB and DBT between patients with type 2 diabetes mellitus and non-diabetic healthy participants separately. The E:I ratio in response to DPB and DBT was significantly lower in type 2 diabetics compared with non-diabetic healthy participants (Table 2). The E:I ratio was not only significantly lower in type 2 diabetics with hypertension and PNP, but also in diabetics devoid of any known type 2 diabetic complications (Table 2). Numerous methods of analyzing the magnitude of the resultant enhanced sinus arrhythmia have been described^{4,15}. The simplest and widely carried out measure is the E:I ratio – a ratio of the longest R–R interval in expiration to the shortest R–R interval in inspiration. The E:I ratio is a widely used indicator of parasympathetic function¹⁶. Thus,

the present study finding suggests that cardiac parasympathetic dysfunction might either be isolated or might precede other complications of diabetes mellitus. However, the E:I ratio was significantly lower in response to DPB, but not in response to DBT in type 2 diabetics free from complications compared with healthy subjects (Table 2). This finding suggests that the diaphragmatic mode of breathing might aid in subclinical cardiac autonomic dysfunction detection where DBT could fail to detect it. r-MSSD is also an indicator of cardiac parasympathetic function^{17,18}. However there was no significant difference in mean r-MSSD between type 2 diabetics and healthy participants with DBT (Table 2). Thus, it appears that although r-MSSD and E:I ratio quantify the cardiac parasympathetic function, the components of cardiac parasympathetic functions that are quantified by these two parameters might not be the same. However, the mean r-MSSD of type 2 diabetics in response to DPB was significantly lower compared with the DPB group of healthy participants (Table 2). Thus, comparing the parasympathetic function of type 2 diabetics with normal data derived from diaphragmatic breathing could provide insight into those aspects of parasympathetic function that are not reflected by the E:I ratio.

In non-diabetic healthy subjects, DPB induced higher time domain measures of HRV compared with DBT (Table 3). The participants in the DPB group were comparable with the participants in the DBT group with regard to age, sex, body mass index, fasting blood sugar and blood pressure (Table 3). Respiratory sinus arrhythmia increases when respiratory frequency approaches the frequency of the intrinsic baroreflex-related heart rate fluctuations. Therefore, respiratory sinus arrhythmia in adults is maximal at a breathing rate of six per min¹⁹. However, in the present study, both the techniques of deep breathing were carried out at six respiratory cycles per min in the supine position for 1 min. Thus, it could be said that the diaphragmatic mode of breathing followed in the present study

Table 4 | Comparison of heart rate variability parameters of type 2 diabetics and their subgroups between diaphragmatic breathing and deep breathing test groups

Group/subgroups	Variables	Deep breathing	Diaphragmatic breathing
Type 2 diabetics (n = 61)	E:I ratio	1.30 ± 0.13	1.28 ± 0.13 (NS)
	r-MSSD (ms)	36.92 ± 23.78	35.19 ± 23.14 (NS)
	STD (ms)	45.87 ± 24.38	43.24 ± 25.33 (NS)
Without complications	E:I ratio	1.42 ± 0.14 (n = 17)†	1.37 ± 0.13 (n = 12)† (NS)
	r-MSSD (ms)	48.29 ± 27.05	41.28 ± 23.81 (NS)
	STD (ms)	62.07 ± 29.11	57.80 ± 22.18 (NS)
With hypertension	E:I ratio	1.26 ± 0.12 (n = 14)‡	1.34 ± 0.16 (n = 14) (NS)
	r-MSSD (ms)	31.23 ± 14.62	45.59 ± 36.16 (NS)
	STD (ms)	38.45 ± 17.12	54.57 ± 35.58 (NS)
With somatic neuropathy	E:I ratio	1.29 ± 0.07 (n = 16)§	1.23 ± 0.09 (n = 14)§ (NS)
	r-MSSD (ms)	37.41 ± 24.08	27.04 ± 14.65 (NS)
	STD (ms)	44.62 ± 18.22	33.50 ± 19.94 (NS)

Values are mean ± standard deviation. †n = sample size of without complications group; ‡sample size of with hypertension group; §sample size of with somatic neuropathy group. E:I ratio, expiratory:inspiratory ratio; NS, non-significant compared with deep breathing (deep breathing test); r-MSSD, root mean square of successive N–N interval difference; STD, standard deviation.

induces higher vagal modulation in healthy subjects. Pranayama is the art of controlling breathing. A practitioner of pranayama not only tries to breathe, but at the same time, also tries to keep his/her attention on the act of breathing, leading to concentration. These acts of concentration remove his/her attention from worldly worries and de-stresses him/her. This stress-free state of mind evokes relaxed responses^{9,10}. In this relaxed state, parasympathetic nerve activity overrides sympathetic nerve activity²⁰. Therefore, the higher amplitude of HRV parameters could be largely due to diaphragmatic breathing-induced cardiac autonomic modulation of the heart.

In the present study, we explored the possible utility of STD/r-MSSD in assessing the cardiac autonomic function in relation to glycemic control in type 2 diabetics. STD/r-MSSD derived during DPB showed a significant negative correlation with HbA1c (Figure 1). This significant negative correlation observed between STD/r-MSSD and HbA1c in the DPB group suggests that STD/r-MSSD quantified during diaphragmatic breathing could be a suitable marker in monitoring cardiac autonomic dysfunction in relation to glycemic control.

In type 2 diabetics and their subgroups, there was no significant difference in time domain measures of HRV between the DBT and DPB (Table 4). At the same time, the DBT and DPB groups of type 2 diabetics had significantly lower HRV parameters compared with the DBT and DPB groups of non-diabetic healthy subjects (Table 2). The E:I ratio has proven to be a sensitive and reliable parameter for the early detection of cardiovascular dysfunction in a wide spectrum of autonomic disorders, including diabetic autonomic neuropathy²¹. Wheeler and Watkins have shown that heart rate response to deep breathing is a reflex, and the efferent component of reflex is vagally mediated²². Therefore, with damage to efferent fibers in type 2 diabetics, the beneficial effect of diaphragmatic breathing could fail to reflect any improvement in cardiac autonomic modulation. Sandeep *et al.*⁹ have reported an improvement in oxidative status with yogic breathing in young healthy males. Shreelaxmi *et al.*²³ have reported that regular practice of diaphragmatic breathing for 3 months reduces oxidative stress and improves anti-oxidant status in type 2 diabetes mellitus²³. Reduced oxidative stress has been reported to improve cardiac parasympathetic function in type 2 diabetes mellitus²⁴. Therefore, long-term studies might be required to explore the beneficial effect of diaphragmatic breathing on cardiac autonomic control in type 2 diabetes mellitus.

Based on the present study findings, it could be concluded that the DPB pattern induces higher cardiac autonomic modulation compared with the DBT in healthy subjects. In type 2 diabetics, DPB and the DBT induce cardiac autonomic modulation equally. STD/r-MSSD estimated during diaphragmatic breathing could aid in assessing cardiac autonomic dysfunction in relation to glycemic control.

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