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ORIGINAL ARTICLE

Evaluation of Stability, Repeatability, and Agreement of Short term Heart rate Variability in Healthy Male Subject

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ABSTRACT
Measuring short term ECG derived heart rate variability (HRV) has been used for many years as a non-invasive technique for evaluation of cardiac autonomic function. The aim of this study was to evaluate stability and repeatability of short term HRV measures in four sessions of ECG recordings during four weeks. In each session, 5-minute lead II ECG with spontaneous breathing, and 1-minute lead II ECG with controlled deep breathing were recorded. There was no significant difference between four session recordings in any of the HRV measures (P>0.05). Intra-class correlation coefficient and Kendall's W coefficient of concordance revealed the least repeatability and agreement in frequency domain, and the most repeatability and agreement in time domain as well as in geometric measures. Moreover, frequency domain measures showed the lowest correlation of variation compared to time domain and geometric measures. The results indicated that, use of HRV measures, especially time domain and geometric measures is a suitable tool for evaluating autonomic performance of pharmacological and non-pharmacological interventions in a four-week interval.
***Key words**: Heart rate variability, Reliability, Geometric index, Time domain index, Frequency domain index*

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INTRODUCTION

Although regular heart rate is superficially accepted as a sign of a healthy heart, the reality is different. In fact, fluctuation in heart rhythm indicates healthy cardiac autonomic nervous system. Measuring HRV has been used for many years as a simple and non-invasive technique for evaluating cardiac autonomic performance [1] Attenuation of the HRV values has been observed in a number of pathological conditions including: coronary artery disease [2], diabetes mellitus [3], anemia [4]. It has been found that attenuation of HRV in these clinical settings [5] and even in the general population [6] is a predictor of mortality. Yet, using of HRV for determination of cardiac autonomic balance is controversial, since heart rate modulation is strongly influenced by factors such as temperature [7], mental and physical stress [8, 9], and position of the body [10]. Thus, determination of HRV measures repeatability is necessary in these applications in clinical settings and in epidemiological and pharmacological studies, where a person's ECG recordings are taken at various times.

In terms of the duration of ECG recording for assessment of HRV measures, two main approaches have so far existed that include use of long-term recordings (mainly 24 hours), and short-term recordings (mainly 5 minutes)[1]. Time-domain, frequency-domain and geometric parameters are commonly produced from ECG-derived RR intervals (RRi). Another method for assessing short-term HRV is simultaneous ECG recording with controlled deep breathing for one minute. In this method, two quantitative time-domain indices that are indicative of breathing-induced HRV, known as Respiratory Sinus Arrhythmia (RSA) can be calculated, which often depict cardiac vagal modulation [11]. Although, in the short-term recordings are not quantifiable very low frequency oscillations, instead, do have advantages that make its use possible as an applicable tool in clinical conditions. These advantages include fast data acquisition and analysis, and possibility of performing experiments in controlled settings under supervision of a doctor or a researcher in terms of compliance with the required standards [12]. Despite of the importance of HRV measures derived from the short-term ECG recordings, only a few studies have dealt with their repeatability in healthy [13] and unhealthy subjects [14]. In addition, in relation to repeatability of HRV geometric measures, there are no published reports to the best of our knowledge. Furthermore, the common defect in all HRV repeatability studies has been twice ECG recordings with a few hours[13] to a few weeks' [15] intervals, which means route of change of these measures in a time interval has not been examined. Therefore, the aim of this study was to investigate repeatability of time domain, frequency domain and geometric measures of HRV derived from 5-minute recording of ECG and also RSA amplitude during one minute controlled deep breathing in healthy young male people in supine position over 4 consecutive weeks.

MATERIAL AND METHODS

Subjects

Eight male subjects in the age range 19-22 years, after being informed of the study stages, completed written consent, and voluntarily participated in this study. None of the subjects had history of cardiovascular or neurological disorders, smoked, or was receiving medication. The protocol was approved by ethics committee of Mazandaran University of Medical Sciences.

Experimental protocol

Prior to the experiment, at the first attendance of subjects, the following data were collected from each subject: age, body mass index, systolic blood pressure, and diastolic blood pressure. Data were collected in a laboratory with controlled temperature (22±2 °C), dim light, and away from noise. Subjects were requested to avoid alcohol and caffeine (coffee, cocoa, and tea), and heavy physical activity 24 hours before experiment. In order to minimize circadian rhythm, experiments were conducted between 9 and 11 in the morning. All data collection procedures were explained to the subject, and it was emphasized that during ECG recording they should avoid any extra movement and talking. Every subject attended 4 times in a month with one week interval for experiments. In every experimental session, electrodes of lead II ECG were attached to the limbs of the subject, and after 15 minutes of rest in supine position, 5 minutes of recording ECG with spontaneous breathing was performed. Also, after 5 minutes of rest, simultaneous with ECG recording, the subjects were requested to perform 1 minute controlled deep breathing (with attention to metronome). Analog ECG signals, after amplification by (Harvard Isolated preamplifier, USA) amplifier at 1000 Hz sampling rate, using digitalized sound card (Creative LABS, Malaysia) were saved in the computer in the form of two files: ECG trace file, and text file of successive RR intervals . After visual reviewing of ECG and RRi, noises or ectopic beats were removed and normal-to normal (NN) time series were created. Then, using the HRV analysis software (http://kubios.uef.fi/) time domain, frequency domain and geometric measures of HRV were calculated from NN time series for used in the statistical analysis. In this study, four time domain measures were calculated: Mean of NN intervals (MNN), standard deviation of the NN intervals (SDNN), root mean squared differences of successive NN intervals (RMSSD), percentage of NN intervals with a difference of duration greater than 50 ms (PNN50%). In calculation of HRV frequency domain, measures based on NN time series, fast Fourier transformation algorithm were used. In this power spectrum analysis, the same as previous studies[1], high frequency (HF) band (0.4 to 0.15 Hz) and low frequency (LF) bands (0.15 to 0.04 Hz) were calculated and by dividing them by total power, they were presented in normalized form (LFn, HFn). The LF band is indicative of both sympathetic and parasympathetic activities, and HF band expresses cardiac parasympathetic (vagal) activity [16]. Additionally, the LF/HF ratio, as an indicator of sympathovagal balance was calculated for statistical analysis. The third group of calculated measures, given the NN time series derived from the 5-minute recordings, were the geometric measures*,* triangular interpolation of NN interval histogram (TINN*)* and HRV triangular index (RRtri) [1] . Finally, in the controlled deep breathing test, two HRV time domain measures were calculated, each expressing RSA amplitude; RSAI1 calculated as subtracting six mean maximum RRi (with 6 deep expirations) from six mean minimum RRi (with 6 deep inspirations), and RSAI2 calculated as subtracting maximum RRi from minimum RRi during 1 minute deep breathing [17].

Statistical analysis

After performing Kolmogorov-Smirnov test to determine distribution of data for all variables, decision had to be made whether to use parametric or non-parametric test for comparison of groups. Given that in most study measures, distribution of data was not normal, and given small sample size, and also, to homogenize analysis of data, it was decided to use non-parametric tests. To compare all HRV measures in four experimental sessions, non-parametric Friedman test was used. To evaluate absolute repeatability of HRV measures, coefficient of variation (CV), and for relative repeatability Kendall's W coefficient of concordance (KWC) and intraclass coefficient correlation (ICC) were used. KWC is a non-parametric test that is used to assess level of agreement between several measurements of one variable.

RESULTS

Anthropometric and hemodynamic characteristics of subjects are presented in table 1. It can be seen that, the above measures, not only in terms of central tendency (Mean) and variability (SD) are in the normal range, but also are in a very narrow range, which shows that subjects have high homogeneity in terms of HRV influencing factors, especially age and body mass index.

Table 1, The demographic and hemodynamic characteristics of the study subjects

	Subject (n=8)	
	Mean ±SD	**Range**
Age (years)	20.62± 0.92	19- 22
Mass (kg)	69.6± 7.7	58- 80
Height (cm)	1.8 ± 0.1	1.68- 1.91
BMI (kg/m2)	22.01 ± 1.9	19.8- 24.4
Basal HR (bpm)	69.2± 7.7	58- 84
SBP (mm Hg)	123.6± 5.3	118- 130
DBP (mm Hg)	76.4 ± 7.7	68- 88

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure

Table 2 presents values of time domain, frequency domain and geometric measures of HRV, and also amplitude of RSA-induced controlled deep breathing calculated from 4 sessions of ECG recordings (week 1-4). Comparison of all HRV measures between experimental sessions using Friedman test (a one-way repeated measures ANOVA) did not show significant differences.

Table 2. Differences in heart rate variability indexes among four sessions of recording by nonparametric Friedman test

	Session of recording				RM-ANOVA P-value	Friedman Statistic
	Weak1	**Weak2**	**Weak3**	**Weak4**		
Controlled deep breathing indexes						
MRRmax-MRRmin	251 (220- 409)	382 (171- 425)	382 (163- 500)	371 (322- 465)	0.36	3.23
RRmax-RRmin	373 (295- 505)	475 (233- 555)	475 (309- 588)	479 (411- 607)	0.42	2.85
Time domain indexes						
MNN	0.869 (0.791- 0.913)	0.820 (0.733- 0.892)	0.833 (0.790- 0.940)	0.830 (0.809- 0.855)	0.7	1.4
SDNN	0.052 (0.039- 0.084)	0.069 (0.042- 0.099)	0.075 (0.045- 0.103)	0.050 (0.041- 0.070)	0.33	3.46
rMSSD	83 (55.5- 86.6)	62.7 (47.8- 81.1)	68.1 (45.7- 113.5)	62.8 (46- 102.9)	0.65	1.63
PNN50%	40.6 (26- 50.4)	33.9 (26.7- 38.7)	41.1 (30- 59.8)	41.2 (27.3- 51.5)	0.32	3.53
Geometric indexes						
RRtri	0.093 (0.076- 0.130)	0.109 (0.079- 0.126)	0.104 (0.083- 0.170)	0.087 (0.073- 0.110)	0.7	1.4
TINN	240 (200- 405)	372 (185- 496)	382 (237- 495)	332 (221- 421)	0.35	3.3
Frequency domain indexes						
LFnu	61.6 (59.6- 64.7)	59.6 (54.9- 63.2)	53.2 (48.8- 62.6)	56 (50.2- 60.5)	0.25	4.14
HFnu	39.3 (37.9- 41.2)	39.1 (33.7- 46.2)	41.6 (38.2- 44.2)	43.8 (34.4- 49.8)	0.63	1.7
LF/HF ratio	1.54 (1.40- 1.70)	1.48 (1.40- 1.60)	1.52 (1.30- 1.70)	1.46 (1.30- 1.80)	0.68	1.5

MNN: Mean of NN intervals (NN= normal-to-normal, i.e., interval between two R-peaks); SDNN: standard deviation of the NN intervals; rM root mean squared differences of successive NN intervals; PNN50%: percentage of NN intervals with a difference of duration greater than RRtri: triangular index; TINN: triangular interpolation of NN interval histogram; LFnu: low frequency normalized unit; HFnu: high frequen normalized unit; LF/HF ratio: low frequency/high frequency ratio. The values are expressed as median (quartile1- quartile3).

ICC as a measure of agreement between HRV measures in 4 sessions of recording is presented in table 3. ICC range was found in time domain measures (0.54-0.94), frequency domain (0.58-0.68), and geometric (0.83-0.84). ICC in both RSA measures induced by controlled deep breathing was 0.91. It can be seen from table 3 that the least reliability and repeatability was observed in frequency domain measures and the most repeatability, in time domain measures of: rMSSD, PNN50%, RSAI1, RSAI2 respectively and in geometric RRtri and TINN.

Table 3. Reliability of time-domain, frequency domain and geometric measures of HRV and RSA amplitude of co deep breathing among four sessions of recording regarded to ICC.

	ICC
Controlled deep breathing indexes	
MRRmax-MRRmin	0.91
RRmax-RRmin	0.91
Time domain indexes	
MNN	0.85
SDNN	0.54
rMSSD	0.94
PNN50%	0.92
Geometric indexes	
RRtri	0.84
TINN	0.83
Frequency domain indexes	
LFnu	0.58
HFnu	0.68
LF/HF ratio	0.59

MNN: Mean of NN intervals (NN= normal-to-normal, i.e., interval between two R-peaks); SDNN: standard deviati the NN intervals; rMSSD: root mean squared differences of successive NN intervals; PNN50%: percentage of NN intervals with a difference of duration greater than 50 ms; RRtri: triangular index; TINN: triangular interpolatior interval histogram; LFnu: low frequency normalized unit; HFnu: high frequency normalized unit; LF/HF ratio: lo frequency/high frequency ratio. The values are expressed as median (quartile1- quartile3).

Table 4 presents results of analysis of the 4 session HRV measures using KWC. Correlation and agreement between the 4 session recorded HRV measures, except for LFnu and LF/HF ratio, were significant. The most agreement and reliability, given KWC, were observed in time domain measures: rMSSD, RSAI2, PNN50%, RSAI1 and geometric: TINN, RRtri, respectively. The CV of each of HRV measures (as a relative repeatability marker) for each of the 4 experimental sessions was calculated and presented in table 5. As can be seen, the least CV was in frequency domain and geometric measures, except for MNNi that showed the lowest CV (9-15%). Also, the highest CV was observed in time domain measure SDNN (46-87%).

DISCUSSION

The main findings of the study were: 1) all HRV measures investigated in this study among 4 experimental sessions were the same in terms of median and interquartile range, 2) time domain measures of: rMSSD, PNN50%, and also two measures of RSAI1 and RSAI2 in controlled deep breathing showed very high relative repeatability (ICC≥0.9); MNNi and two geometric measures RRtri and TINN also had high relative repeatability (0.7≤ICC≤0.85); time domain SDNN and frequency domain HF and LF and LF/HF measures had moderate relative repeatability (0.5≤ICC≤0.69), 3) agreement and reliability of all HRV measures, except for LF and LF/HF ratio, given KWC were significant. However, the best agreement between 4 experimental sessions was observed in time domain measures: rMSSD, PNN50% and also two measures of RSAI1 and RSAI2, 4) absolute repeatability of HRV measures from 4 experimental sessions, given CV, was in the range of 87-89%. The lowest CV in MNNi, LF, and HF, and the highest CV in SDNN were observed. In the remaining measures, CV was in the range of 31-60%.

Table 4. Reliability of time-domain, frequency domain and geometric measures of HRV and RSA amplitud controlled deep breathing among four sessions of recording regarded to Kendall's W coefficient.

	Kendall W	ChiSq	P-value
Controlled deep breathing indexes			
MRRmax-MRRmin	0.76	21.33	= 0.003
RRmax-RRmin	0.82	22.83	= 0.002
Time domain indexes			
MNN	0.73	20.42	= 0.005

SDNN	0.67	18.67	= 0.009
rMSSD	0.9	25.33	= 0.0007
PNN50%	0.8	22.42	= 0.002
Geometric indexes			
RRtri	0.59	16.49	= 0.02
TINN	0.63	17.78	= 0.01
Frequency domain indexes			
LFnu	0.47	13.08	= 0.07 NS
HFnu	0.53	14.83	= 0.04
LF/HF ratio	0.49	13.67	= 0.06 NS

MNN: Mean of NN intervals (NN= normal-to-normal, i.e., interval between two R-peaks); SDNN: standard deviation of the NN intervals; rMSSD: root mean squared differences of successive NN intervals; PNN50% percentage of NN intervals with a difference of duration greater than 50 ms; RRtri: triangular index; TINN triangular interpolation of NN interval histogram; LFnu: low frequency normalized unit; HFnu: high frequ normalized unit; LF/HF ratio: low frequency/high frequency ratio. The values are expressed as median (quartile1- quartile3).

A developing tendency in evaluation of autonomic nervous system activity has been created in various clinical conditions, especially in coronary artery disease [2] and other cardiac diseases [3, 5], and also in non-cardiac patients with changing autonomic nervous system [4]. Investigating HRV over 24 hours using Holter monitor device has an extensive application among electrocardialogists and electrophysiologists. Of course, their main goal is assessment of cardiac arrhythmia during 24 hours ordinary daily life. But, short term ECG (usually 5 minutes) recording and calculation of HRV measures, since it is easily performed and well tolerated by patients (subjects), has gained huge interest in pharmacological studies [18, 19].

Table 5. Coefficient of variation of time-domain, frequency domain and geometric measures of HF RSA amplitude of controlled deep breathing in four sessions of recording.

	Coefficient of variation (%)			
	Weak1	**Weak2**	**Weak3**	**Weak4**
Controlled deep breathing indexes				
MRRmax-MRRmin	44	60	58	34
RRmax-RRmin	35	48	42	33
Time domain indexes				
MNN	12	12	15	9
SDNN	47	87	46	59
rMSSD	31	40	47	48
PNN50%	43	32	40	44
Geometric indexes				
RRtri	48	36	49	47
TINN	44	52	40	47
Frequency domain indexes				
LFnu	11	15	18	18
HFnu	14	28	29	24
LF/HF ratio	26	13	22	18

MNN: Mean of NN intervals (NN= normal-to-normal, i.e., interval between two R-peaks); SDNN: standard deviation of the NN intervals; rMSSD: root mean squared differences of successive NN intervals; PNN50%: percentage of NN intervals with a difference of duration greater than 50 ms; R triangular index; TINN: triangular interpolation of NN interval histogram; LFnu: low frequency normalized unit; HFnu: high frequency normalized unit; LF/HF ratio: low frequency/high frequen ratio. The values are expressed as median (quartile1- quartile3).

It is frequently observed in literatures that pharmacological [18, 19] and non-pharmacological [20] interventions are applied with several hour or several week intervals, and before and after intervention, measure short term HRV as cardiac autonomic modulation, so that effects of various drugs on cardiac sympathovagal balance can be evaluated. As opposed to long-term HRV measures [21, 22], few studies have investigated repeatability and agreement in short term HRV measures [13].

Leicht and Allen [23] measured repeatability of time domain and frequency domain of HRV during rest in supine position and moderate exercise in 10 children of 7 to 12 years old (6 male and 4 female) in two experimental sessions with 8-week interval, and data analysis with calculation of CV and ICC revealed poor repeatability between HRV variables [23]. Perhaps, changes in autonomic nervous system performance in 8-week interval could be associated with developing trend in child subjects.

Dantas et al [13] investigated repeatability of HRV measures in short terms recordings in supine and standing positions in 30 healthy 20- to 49-year-old (14 male and 16 female) in two experimental sessions with 2-3 hour interval and found that PNN50%, rMSSD, LFnu, HFnu, and LF/HF ratio measures, irrespective of body position, had good repeatability [13]. But SDNN measure in supine position was not repeatable. In the present study, repeatability of frequency domain measures, especially LF and LF/HF, as opposed to the above study, was poor. It appears the short interval between two recording sessions (2-3 hours) was one of the causes of high repeatability of HRV in the above study.

Schroder et al [24] aiming to investigate repeatability of HRV measures derived from ECG recordings of 10 seconds, 2 minutes and 6 minutes (with one week interval) in 63 healthy male and female subjects aged 45 to 64 years, showed that, in 6 and 2 minutes recordings, ICC was over 0.7. But in the 10 second recording, it was less and nearly 0.5 [24]. Generally, HRV measures in the study showed high repeatability and reliability compared to the above study. In the our study, three characteristics of the subjects: being young (19 to 22 years), being male, and with normal body mass index, and also two methodological characteristics: ECG recording between 9 and 11 in the morning, and after 15 minutes rest in supine position, could probably explain high repeatability of HRV measures.

Findings in this study partly showed that frequency domain measures: LFnu and LF/HF ratio, represent a specific pattern of absolute and relative repeatability indices, so that, compared to other measures, low relative repeatability (low KWC and ICC) and high absolute repeatability (poor CV) were found. Perhaps, changeability between subjects, given the small sample size in the present study, could explain the disagreement between relative and absolute repeatability. Thus, it could be suggested that frequency domain measures of LFnu and LF/HF ratio derived from short term ECG recording in terms of repeatability, are not suitable for longitudinal studies with small sample size.

SDNN is one of the most common time domain measures that is calculated and used in both short term and long term recordings of HRV. Results of the present study showed that this measure, compared to the remaining measures, is associated with low absolute repeatability and not so high a relative repeatability. SDNN is not a specific measure in evaluation of cardiac sympathovagal performance in isolation, since fluctuation factors due to the change in both sympathetic and vagal activities are involved in determination of SDNN value [1]. This could perhaps explain negligible repeatability of SDNN compared to other measures. Given the high reliability and repeatability of respiratory sinus arrhythmia in controlled deep breathing (i.e. RSAI1, and RSAI2) that are purely due to cardiac vagal activity, could be confirmation of the above justification.

Findings of the present study showed that any HRV measures investigated in four sessions of measurement with one week interval did differ. This stability of HRV measures, together with high correlation, based on KWC and ICC shows acceptability and suitability of level of agreement and repeatability of short term HRV measures. Given that time domain and geometric measures had ICC≥0.8, it is recommended that use of these measures is appropriate for investigating pharmacological and non-pharmacological interventions in a one-month interval. In this study, subjects were male in a narrow age range 19 to 22 years with normal body mass index. Given that the differences in gender, age, and body mass index can affect cardiac autonomic activity [25], thus, care must be taken in generalizing it to clinical conditions.

CONCLUSION

Generally, HRV variables, especially rMSSD and PNN50% derived from 5-minute ECG recording with spontaneous breathing, and also RSAI1 and RSAI2 derived from one-minute controlled deep breathing during one month period, display significant stability and repeatability. Thus, use of these measures in investigation of cardiac autonomic performance in various pharmacological and non-pharmacological interventions in healthy young people is recommended.

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