

Original Article

## Intra-class Correlation among Heart Rate Variability analysis softwares across different physiological postures

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### Abstract

Heart rate variability (HRV) is a simple technique which helps to assess the alterations in cardiac autonomic activity in healthy and diseased individuals. It is performed in various research centers using different hardwares and softwares. Hence, HRV reports generated from these centers cannot be compared unless their HRV data acquisition systems and the HRV analysis softwares correlate or agree with each other. In this study, Intra-class correlation coefficient test was done to see the extent of correlation among three HRV analysis softwares used by researchers in India, namely, (1). Kubios HRV version 2.0, Department of Physics, University of Kuopio, Finland, (2). HRV soft 1.1 Version, Autonomic Function Laboratory, Department of Physiology, All India Institute Of Medical Sciences (AIIMS), New Delhi and (3). Nevrokard aHRV 12.0.0. (Medistar Inc., Slovenia). Following standard guidelines, five minutes ECG data was acquired from twenty six healthy volunteers in supine, sitting and standing positions. The R-R intervals were computed from the ECG data and was subjected to short-term HRV analysis using the above three softwares. Statistical analysis revealed a highly significantly ( $p < 0.001$ ) perfect positive correlation (ICC values  $> 0.8$ ) among the three softwares for both time domain and frequency domain parameters in the three different positions.

Hence, it may be proposed that the interpretation of short-term HRV reports generated by the three softwares, across laboratories, can be equated.

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### Introduction

Heart rate variability (HRV) also known as 'cycle length variability' or 'R-R interval variability' reflects the cardiac autonomic status of an individual. HRV analysis is a simple and non-invasive technique that

measures the beat to beat variations in the successive R-R intervals which occur due to oscillations of the cardiac sympathetic and parasympathetic supply to the sino-atrial node, the cardiac pacemaker (1, 2).

HRV is widely used in the field of medicine and cardiovascular research to test the autonomic functions of the individuals. Worldwide, many hardwares and softwares are available to help researchers assess the HRV in both healthy and diseased conditions. But not much is known regarding the extent of correlation among these different systems.

In this project, we aimed to assess the Intra-class Correlation (ICC) among three HRV analysis softwares which are commonly used in several laboratories in India to assess the short-term HRV. The softwares are as follows :

1. KUBIOS HRV VERSION 2.0, Department of Physics, University of Kuopio, Finland, a software which can be downloaded free of cost from the website of the creators with their permission.
2. HRV soft 1.1 Version, developed by the Autonomic Function Laboratory, Department of Physiology, All India Institute of Medical Sciences (AIIMS), New Delhi (3).
3. NEVROKARD aHRV ver. 12.0.0. (Medistar Inc., Slovenia), a very popular and widely used software which is available in India on payment.

Currently, HRV is used to examine the autonomic status in various clinical scenarios such as Diabetes mellitus (4, 5, 6), Hypertension (7, 8), Obesity (9, 10, 11, 12), and Psychiatric disorders (13, 14). It is also used to evaluate the effects of various drugs (15, 16) and yoga therapies (17, 18, 19) on the cardiac autonomic status of the individuals. The data acquisition system and softwares used for acquiring and analyzing HRV vary across different laboratories. If interpretation of short-term HRV reports obtained from different research labs need to be compared, it is necessary to check for correlation among these softwares and hardwares.

With this aim in mind, a prior study had been done by two of the authors of the current study to explore the correlation among three HRV analysis softwares, namely, Nevrokard aHRV ver. 12.0.0., HRV soft 1.1 Version, AIIMS, New Delhi and Advanced HRV analysis software version 1.1 from Biomedical Signal Analysis group, University of Kuopio, Finland. The last mentioned software was an earlier version of KUBIOS HRV Version 2.0. In that study, HRV indices were computed twice from 5 minute ECG recordings obtained in supine position at one month interval in normal healthy subjects. Pearson correlation coefficient test showed significant correlation among the three softwares for the time domain and frequency domain HRV indices even on repeated measurements (20).

Recently, the Advanced HRV analysis software version 1.1 from Biomedical Signal Analysis group, University of Kuopio, Finland, was upgraded to a newer version named KUBIOS HRV Version 2.0. This new version has certain advanced features and differs from its previous version with regard to its power spectrum calculation. Further, literature survey reveals that several researchers in India are using HRV soft 1.1 Version, AIIMS, New Delhi, software for studies on Heart Rate Variability (21, 22, 23). Hence, in the current study, we decided to investigate the correlation between KUBIOS HRV Version 2.0, Nevrokard aHRV ver. 12.0.0., and HRV soft 1.1 Version, AIIMS, New Delhi, by Intra-class correlation coefficient test, using the 5 minute ECG data acquired from a different group of subjects, while in three different successive physiological postures (supine, standing and sitting) and hence different cardiovascular autonomic status. This data was collected as part of another study which looked at the variations in HRV parameters under different physiological conditions in normal subjects.

The standard cardiac autonomic function tests study the variability of the heart rate brought about by perturbations of the cardiac autonomic supply, by changes in posture or by challenges such as the Valsalva maneuver (24, 25). Therefore, studying the changes in HRV parameters, produced by changes in the cardiac sympatho-vagal supply induced by postural changes, gives a superior estimate of the

cardiac autonomic control and its reactivity, compared to assessing only the supine HRV indices. Thus, when changing from supine to sitting or standing posture, the instantaneous arterial blood pressure as detected by the carotid arterial baroreceptors in the neck, falls. Unloading of the carotid baroreceptors occurs, resulting in vagal withdrawal and sympathetic stimulation of the cardiovascular autonomic supply to restore the pressure head. Consequentially, the sympatho-vagal balance of the cardiac autonomic supply to the SA node of the heart increases, reflecting in an increase in mean heart rate or decrease in mean RR interval when moving from supine to sitting or standing posture. Studies reveal that the posture induced increase in sympatho-vagal balance is associated with increased modulation of the cardiac sympathetic supply and decreased modulation of the cardiac vagal supply which is mirrored by corresponding changes in HRV indices (26). Various studies have been published reporting the changes in short-term HRV indices associated with changes in posture (27, 28, 29, 30, 31). Hence, we felt there was a need to study the correlation of HRV indices computed by the three softwares in three different postures.

## Methods

### Study group

The study was peer reviewed and approved by the Institutional review board of the Christian Medical College, Vellore. Healthy volunteers who were studying or working in the Institutional campus were recruited into the study after they had given their consent in written form. The data was collected from 26 healthy subjects which included 11 males and 15 females, with an average age of  $25.9 \pm 4.2$  yr (Mean $\pm$ SD) and an average BMI of  $22.5 \pm 4.7$  (Mean $\pm$ SD). Chronic smokers, alcoholics, pregnant women and subjects with known acute or chronic medical illness were excluded from the study.

### Data acquisition

Subjects who were included into the study were advised to abstain from smoking and consuming alcohol or caffeinated beverages twelve hours prior to the recording. They were asked to come to the

Autonomic Function Laboratory in the department of Physiology, Christian Medical College, Vellore, between 7 and 11 am, 2-3 hours after a light breakfast.

The subjects were asked to rest in supine position for 20 minutes, following which, a five minute ECG data was acquired in lead II configuration and digitized at a rate of 1000 samples per second using the data acquisition system, BIOPAC Systems, Inc., CA 93117, USA and stored in a personal computer. Then, the subjects were asked to stand up and after two minutes of quiet standing, a 5-minute ECG data was recorded in this position. Next, the subjects were asked to rest in supine posture once again, till the heart rate returned to the resting level. This was followed by asking the subjects to sit quietly on a chair and two minutes later, another 5 minute ECG data was acquired in the same position.

All the ECG data was visually screened and found to be free of ectopic beats and noise. R-R intervals computed from the ECG were fed to two softwares, Nevrokard aHRV and Kubios HRV version 2.0, Department of Physics, University of Kuopio, Finland for HRV analysis.

While the ECG recordings were done in the supine, standing and sitting positions, the ECG analogue signals were also simultaneously acquired on to another computer using the National Instruments (NI) Data Acquisition Card and the software developed by the department of Physiology, AIIMS. R-R intervals computed from this digitized ECG was analysed for HRV using HRV soft 1.1 Version, AIIMS, New Delhi.

Both time and frequency domain short-term HRV indices were analyzed using the three softwares.

### Data analysis

Short-term HRV indices were computed with the three different softwares as per the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1). Mean RR interval and time

domain indices such as standard deviation of all the normal-to-normal RR intervals (SDNN), the percentage of number of RR intervals with differences  $\geq 50$  ms (pNN50) and the root mean square of the sum of successive differences between adjacent RR intervals (RMSSD) were considered for analysis.

Similarly, the R-R interval series was transformed into the Frequency domain data using the non-parametric Fast Fourier Transformation method to obtain the power spectrum and the spectral powers. The absolute powers in  $\text{ms}^2$  in the low frequency bands (LF, 0.04-0.15 Hz) and high frequency bands (HF, 0.15-0.45 Hz), ratio of LF and HF (LF/HF) and the normalized units of LF (LF nu) and HF (HF nu) were taken for analysis. LF nu and HF nu were calculated as the ratio of the corresponding power component to the total power minus the very low frequency component (VLF) and multiplied by 100 (expressed as a percentage). Similarly, coefficient of component variance for LF and HF (CCVLF, CCVHF) were calculated as the ratio of the square root of the respective power component to the mean RR interval value and multiplied by 100. All the short-term HRV indices were computed separately for supine, sitting and standing positions.

#### Statistical analysis

The extent of correlation among the three softwares for the various time domain and frequency domain indices was tested using Intra-class correlation coefficient (ICC) or reliability coefficient, a measure of the reliability of measurements. ICC is the proportion of repeatability about the construct of interest. The measure of ICC is dependent on the homogeneity of the population of subjects being measured. Three types of variations are considered in the Intra-class Correlation test. In this study, they were the variation due to differences in the subjects, variation due to differences in the measurements with regard to positions and variation due to differences in the measurements.

Intra class correlation test assesses the reliability of the continuous measurements by comparing the variability of different measurements of the same subject to the total variation across all the

measurements and all subjects. A two way random effect with consistency was used in this study to calculate the ICC to determine the variability among the three softwares. ICC value more than 0.8 indicate almost perfect agreement, values between 0.6-0.8 indicate substantial agreement, values between 0.4-0.6 indicate moderate agreement and values less than 0.4 indicate fair to poor agreement (32). Statistical analysis was done using SPSS Version 17.0.

## Results

Five minutes ECG was recorded in 26 healthy individuals in supine, sitting and standing positions. The R-R interval data was obtained and analysed for time domain and frequency domain indices using Kubios HRV version 2.0, HRV soft 1.1 Version, AIIMS, New Delhi and Nevrokard aHRV softwares. The Mean RR showed the maximum perfect correlation (ICC = 1.000) among the 3 softwares in all three positions, indicating that the same RR interval data has been used for the HRV analysis by the three softwares. It also implies that the NI Data Acquisition Card acquired the same ECG simultaneously as the BIOPAC data acquisition system. With respect to short-term HRV measures, all the time domain and frequency domain parameters also showed a perfect positive correlation in supine, sitting and standing positions (Table II).

The short-term HRV parameters obtained with the three softwares are listed in Table I and the ICC values for the parameters are listed in Table II.

## Discussion

Heart rate variability indices serve as one of the major indicators of the autonomic control on the heart. It is now evolving as a popular tool in diagnosing the autonomic dysfunction in various cardiac and non-cardiac disorders. HRV also helps to predict the risk of sudden cardiac death or arrhythmia after acute MI (33, 34) and also serves as a prognostic indicator of diabetic autonomic neuropathy (35, 36). Despite the fact that HRV has wide clinical applications, HRV reports obtained from different labs cannot be

TABLE I: HRV indices from the three softwares in supine, sitting and standing positions.

Parameters	Supine position			Sitting position			Standing position		
	Nevrokard aHRV	HRV soft 1.1 Version, AIIMS, New Delhi	Kubios HRV version 2.0	Nevrokard aHRV	HRV soft 1.1 Version, AIIMS, New Delhi	Kubios HRV version 2.0	Nevrokard aHRV	HRV soft 1.1 Version, AIIMS, New Delhi	Kubios HRV version 2.0
Mean RR (ms)	880.58 (104.76)	880.19 (105.22)	880.6360 (105.02)	797.15 (96.95)	797.44 (96.77)	797.45 (97.13)	708.17 (84.71)	709.05 (84.66)	708.35 (85.12)
SDNN (ms)	61.27 (20.82)	62.39 (20.49)	50.78 (20.65)	56.25 (14.51)	57.24 (15.50)	41.23 (13.58)	48.27 (13.42)	48.68 (13.51)	35.99 (11.08)
pNN50 (%)	33.55 (21.76)	31.70 (21.12)	32.81 (21.70)	16.60 (15.32)	15.23 (14.80)	15.75 (15.27)	3.72 (3.15)	3.49 (3.00)	3.35 (2.90)
RMSSD (ms)	62.42 (31.76)	62.87 (31.62)	62.71 (31.77)	41.79 (21.26)	41.77 (21.19)	41.62 (21.29)	25.45 (10.81)	25.93 (10.72)	25.20 (10.71)
LF Power (ms <sup>2</sup> )	982.83 (747.04)	1068.81 (575.54)	831.52 (624.65)	1069.92 (775.61)	1449.28 (917.88)	899.96 (642.55)	1158.02 (793.70)	2052.00 (1335.88)	968.35 (662.17)
HF Power (ms <sup>2</sup> )	1710.85 (1412.60)	1722.10 (1295.73)	1478.86 (1223.02)	907.21 (815.22)	965.69 (731.27)	781.64 (700.24)	1133.98 (803.76)	2026.19 (1361.43)	946.09 (668.88)
LF nu	42.01 (16.13)	44.38 (16.06)	41.58 (15.99)	57.34 (19.49)	59.90 (18.72)	56.92 (19.19)	74.87 (12.31)	77.80 (10.77)	74.17 (12.30)
HF nu	60.09 (17.30)	57.69 (17.20)	60.54 (17.19)	45.13 (20.66)	41.65 (19.04)	45.41 (20.21)	28.16 (15.94)	24.34 (12.82)	28.90 (16.03)
LF/HF	.69 (.45)	.77 (.53)	.66 (.41)	1.51 (1.28)	1.71 (1.40)	1.46 (1.19)	3.18 (1.99)	3.67 (1.99)	3.05 (1.88)
CCVHF	4.22 (1.84)	4.2759 (1.71)	3.9184 (1.71)	3.43 (1.43)	3.64 (1.42)	3.17 (1.31)	2.54 (1.08)	2.98 (1.06)	2.37 (1.01)
CCVLF	3.30 (1.10)	3.59 (1.04)	3.03 (1.00)	3.77 (1.45)	4.44 (1.42)	3.45 (1.30)	4.52 (1.82)	6.09 (2.44)	4.14 (1.68)

Values are given as mean (SD).

Mean R-R = mean duration of R-R interval; SDNN = standard deviation of normal-to-normal intervals; pNN50% = NN50 count divided by the total number of all NN intervals; RMSSD = root mean squared standard deviation; LF power = low frequency power; HF power = high frequency power; LF nu = low frequency power, normalized units; HF nu = high frequency power, normalized units; LF:HF = the ratio of low to high frequency power; CCVHF = coefficient of component variance of HF; CCVLF = coefficient of component variance of LF.

TABLE II: Intra-class correlation coefficient values for the various HRV parameters in supine, sitting and standing positions.

Parameters	Intra-class correlation values*		
	Supine	Sitting	Standing
Mean RR	1.000	1.000	1.000
SDNN	.961	.932	.952
pNN50	.998	.998	.973
RMSSD	.999	1.000	.993
LF Power	.913	.867	.825
HF Power	.934	.960	.823
LF nu	.987	.894	.979
HF nu	.990	.900	.970
LF/HF	.959	.861	.962
CCVHF	.951	.966	.981
CCVLF	.929	.914	.929

\*P<0.001

compared if the HRV data acquisition systems and softwares used by them, fail to show a good correlation.

In this study, we assessed the extent of correlation

among three well known HRV softwares available in India (Kubios HRV version 2.0, HRV soft 1.1 Version, AIIMS, New Delhi and Nevrokard aHRV ver. 12.0.0) using Intra-class correlation coefficient. HRV analysis was done for time domain (SDNN, pNN50, RMSSD) and frequency domain indices (LF power, HF power, LF nu, HF nu, LF/HF, CCVHF, CCVLF). Among the time domain indices, SDNN, a measure of total Heart rate variability represents both sympathetic and parasympathetic activity, while, RMSSD and pNN50 represents parasympathetic activity (1).

Similarly, among the frequency domain indices, LF power represents sympathetic activity and parasympathetic activity, while HF power represents parasympathetic activity (37). LF nu and HF nu denotes the relative activity of sympathetic and parasympathetic nervous system (1) while LF/HF represents the ratio of sympathetic to the

parasympathetic tone modulations (38). CCVHF and CCVLF represent the changes in the autonomic state irrespective of the changes in heart rate (39).

Analysis of the ICC values revealed that all the time domain and frequency domain parameters showed a highly significant ( $p < 0.001$ ) perfect positive correlation in supine, sitting and standing positions. The mean R-R interval showed the maximum correlation (ICC value of 1) in all three positions. This is to be expected as the same ECG or ECG recorded simultaneously has been used to derive the Short-term HRV parameters using the three softwares. In one sense, it corroborates that the signals analysed by the three softwares were the same. In the supine position, the LF power showed the least correlation and in the sitting position both LF power and LF/HF showed a relatively lesser correlation. In the standing position, both LF power and HF power revealed a lesser correlation when compared to the ICC values of other HRV indices.

These minor differences in the ICC values for the above mentioned HRV indices could be due to the differences among the three softwares in selecting the type of window, window width, number of data points for analysis and the interpolation rate when

performing the Fast Fourier Transformation for obtaining the power spectral indices. Changes in the body position lead to alterations in HRV indices due to variations in the cardiac autonomic control (27-31). In our study, these variations in HRV that occur in supine, sitting and standing positions have been captured by the three different softwares with good correlation despite the subtle differences in their analyzing methods.

### Conclusion

Short-term heart rate variability parameters obtained on HRV analysis of 5 minute ECG signal acquired in supine, sitting and standing positions showed a highly significant positive correlation among the three softwares, namely, Kubios HRV version 2.0, Department of Physics, University of Kuopio, Finland, HRV soft 1.1 Version, Autonomic Function Laboratory, Department of Physiology, All India Institute Of Medical Sciences (AIIMS), New Delhi and Nevrokard aHRV 12.0.0.(Medistar Inc., Slovenia). Thus, the correlation among the softwares is maintained even in different physiological states. Hence, the interpretations made from the short-term HRV indices of the three softwares can be compared confidently.

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