Original Research Article

Variations in time domain measures of HRV and analysis of SDNN/RMSSD ratio as a surrogate for LF/HF in obese women with PCOD

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Abstract

Background: Polycystic ovary syndrome is a common heterogeneous endocrine disorder in women of reproductive age where cardiovascular disease is considered as long term health risks. Heart rate variability is a non-invasive test to assess the cardiac autonomic dysfunction in women with PCOS. Estimation of Heart rate variability test could help us to identify women who are at increased risk of developing cardiovascular complications. Inspite of results from other studies, we intended to substantiate a new entity RMSSD/SDNN ratio as a surrogate for LF/HF in women with PCOD. **Aims:** 1.To analyse and compare the variations in time domain measures of HRV analysis between women (obese and non-obese) with PCOD and non-obese women without PCOD. 2. To assess the correlation and reliability analysis between SDNN/RMSSD ratio and LF/HF ratio. **Methodology:** A sample size of 90 women of age group 15-39 years were recruited for this study. Based on the BMI and the clinical diagnosis of PCOD, Women who were diagnosed as PCOS with BMI>25 as Group I (n=30); Women who were diagnosed as PCOS with BMI<25 as Group II (n=30); and age-sex matched clinically normal healthy women with BMI<25 as Group III (controls, n=30). Resting Heart rate variability at 5-minute epoch was recorded for the study group. **Results:** Significant decreases in time RMSSD, SDNN, mean RR was observed in women (obese and non-obese) with PCOD. A significant negative correlation was found between RMSSD with other variables in the study. RMSSD/SDNN ratio strongly agrees with LF/HF ratio by agreement analysis, whereas by linear regression it was found to be y (RMSSD/SDNN)=1.533 + (0.234)LF/HF. **Conclusion:** Women with PCOS showed features of autonomic dysfunction with sympathetic predominance and parasympathetic withdrawal. Whether the robustness of SDNN/RMSSD as surrogate for LF/HF needs more confirmation and is suggested for further evaluation, since it had strong correlation in our results with p-value smaller than 0.001.

Keywords: HRV-5-Minute epoch, polycystic ovarian disease, RMSSD/SDNN ratio, surrogate

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Introduction

Metabolic and cardiovascular disorders have been shown to be related with autonomic dysfunction.[1,2] PCOD is a common endocrine disorder associated with long-term health risks, including insulin resistance, diabetes mellitus, dyslipidemia, hypertension, and premature atherosclerosis.[3] It has long been proven that the significant indicator for atrial fibrillation (AF) and epilepsy is RMSSD (the root mean square differences of successive RR intervals).[4,5] Besides, SDNN, pNN50% and TINN are

other essential time domain measures of HRV whereas the frequency domain measures are LF and HF.[6] Few studies suggested that SDNN/RMSSD could be a good surrogate of LF/HF for healthy subjects.[7,8] Whether this statement is affirmative for patients diagnosed of PCOD, would be revised by analysis of cardiac measurements in this study.

The inter beat variations between successive sinus rhythms and the instantaneous HR fluctuations which are technically difficult to detect without intracardiac atrial electrograms, can now be assessed using HRV measurements.[9,10] Heart rate variability (HRV), a conventionally accepted non-invasive cardiovascular assessment tool to describe variations of both instantaneous heart rate (HR) and R-R intervals, are recorded from a digital ambulatory Holter ECG monitoring.[11,12] Premature contractions are mostly the additional confounders while recording HRV. In the time domain analysis, standard deviation of all normal RR intervals (SDNN) and the root mean square successive differences (RMSSD) have been used to index vagal activity where the RMSSD measure has been scientifically proved to be a surrogate marker for the cardiac vagal tone. In calculating SDNN (measured between consecutive sinus beats), any RR interval that begins or ends with a premature contractions is simply deleted from the sequence.[13] HRV is not a stationary recording, i.e. the mean and variance are independent of record length, hence values obtained by 5-minute recording (SDNN=30ms) cannot be compared to values based on 24-hour longer recordings (SDNN=70-100msec) which are accepted normal range.[12,13] The time domain analysis (SDNN, RMSSD) of HRV reports the activity of the cardiac system. The frequency domain analysis (LF, HF) reflects sympathovagal balance of the ANS.[13]

Aim and Objectives

1. To analyse and compare the variations in time domain measures of HRV analysis between women (obese and non-obese) with PCOD and non-obese women without PCOD.
2. To assess the correlation and reliability analysis between SDNN/RMSSD ratio and LF/HF ratio.

Methodology

Study Design and Population

This study is a descriptive cross-sectional study conducted after obtaining Institutional ethics clearance and a written informed consent from the subjects. 90 women of age group 15-39 years were recruited for this study. Of which, based on the BMI, the study group was divided into (i) Obese women who were clinically diagnosed as PCOD with BMI>25 (n=30); (ii) Non-Obese women who were clinically diagnosed as PCOD with BMI<25 (n=30); (iii) Non-Obese, age matched clinically normal healthy women with BMI<25 as control group (n=30).

Inclusion criteria

Selection of cases

With the help of clinical experts from the Department of Endocrinology, a sample size of n=60 women of 15-39 years in age, who were clinically diagnosed of PCOD with duration of illness more than six months were recruited based on Rotterdam criteria. Only those patients who fulfilled 2 out of 3 Rotterdam criteria were included in the study group.

Rotterdam criteria for Diagnosis of PCOD

1. Oligo and/or anovulation
2. Biochemical and/or clinical signs of Hyperandrogenism
   - Clinical: Hirsutism, Acne, Acanthosis nigricans or male pattern alopecia
   - Biochemical: Total Testosterone > 70ng/dl, Androstenedione > 245ng/dl, DHEA-S > 248 ug/dl.
3. Polycystic ovaries on USG:
   ≥12 or more follicles (2-9mm diameter) in each ovary or ovarian volume >10ml).

Selection of Controls

A sample size of n=30 clinically normal and healthy women of 15-39 years with BMI 18.5-25 were recruited from the Department of Master Health Check-up, as control group.

Exclusion Criteria

Those women who were diagnosed of Pregnancy, who were on oral contraceptive pills, ovulation induction drugs, Steroids, anti-diabetics, anti-androgens and other hormonal drugs, and also those who were diagnosed of hypothyroidism, any benign uterine or ovarian conditions, and any chronic

hepatic or renal or cardiac illness were all excluded from the study.

Procedure

The study was started after clear explanation and demonstration of the procedure to all the participants. The participants were asked to fill up a pro-forma consisting of their socio-demographic details and clinical history. A thorough clinical examination was done, following which the BMI (Body mass index) of each participant was calculated with their weight (kgs) and height (m) as measured, using Quetelets index [BMI = weight (Kg) / Height (m^2)]. The waist hip ratio (WHR) was calculated by the ratio between the waist circumference to the hip circumference. [WHR = Waist circumference/Hip circumference]. Their waist circumference was measured in centimeters at the level of umbilicus or midway between lower ribcage and pubic symphysis; and their hip circumference which was measured as widest circumference at the level of greater trochanter. Waist circumference more than or equal to 88cm was considered as obesity whereas WHR more than 0.9 for females was considered as obesity. Vital parameters such as pulse rate, blood pressure, temperature and respiratory rate for every participant was noted.

Measurement of heart rate variability[14,15]

All participants were instructed about the experimental procedure and an adequate time was ensured before and during recording. They were subjected to recording of HRV using Physiopac 8 channel HRV recorder in the morning hours to avoid any circadian influence at room temperature of 24°C-28°C. Participants were instructed to abstain from alcoholic beverages or caffeine/tannin during the 24h prior to the recording and were asked to have a light diet 3h before the recording. After confirming the participant's good sleep and doing a clinical examination, with a 15 min rest in supine position, the blood pressure and heart rate were measured to ensure the basal conditions.

Electronic signals were continuously recorded, transmitted and stored via an electromagnetic field for analysis and interpretation of HRV. Data thus obtained were transferred by means of a signal emission to the computer. HRV was assessed in both time and frequency domains. The region of greatest stability for the gathering of the R-R intervals was used for this measurement, so that at least 256 consecutive beats were presented. The time domain measures were mean RR-the mean of the RR intervals, SDNN-the standard deviation of all RR intervals and RMSSD-the square root of the mean of the sum of the squares of differences between the adjacent normal divided by the number of RR intervals within a given time minus one RR interval (RMSSD). SDNN reflected overall HRV, whereas RMSSD was considered to be an index of parasympathetic modulations of heart rate (HR). For the frequency domain, spectral analysis was performed and the power spectral components obtained were LF: 0.04–0.15 Hz and HF: 0.15–0.4 Hz in absolute units (ms). The LF band was modulated by both the sympathetic and the parasympathetic nervous systems, and the HF band was correlated with vagal cardiac control.

Statistical analysis

Data analyses were performed with the statistical software package SPSS Version 24.0 for windows (USA). The normality of distribution of all variables was checked using the Kolmogorov–Smirnov test. All the data were expressed in mean and standard deviation (SD) for variables with parametric distribution. Since the HRV data were not normally distributed, these results were expressed as median (minimum – maximum). The significance of differences between groups was determined using one-way ANOVA and Post-hoc analysis was done with LSD as appropriate. Correlation analyses between RMSSD and biochemical parameters were tested using Pearson's rank correlation(r). For all analyses, $p<0.05$ was considered statistically significant.

Results

About 30 clinically healthy subjects, 30 obese women with PCOD and 30 non-obese women with PCOD were tested for HRV analysis. Among the 60 women (obese/non-obese) with PCOD, 48 women had diabetes, 38 women had hypertension and 41 had both diabetes and hypertension. The age between the three groups was almost the same (table.1). The BMI and WHR were significantly higher in obese women with PCOD group compared to that of non-obese women with PCOD and the control group as mentioned in Table.1.

A slight increase in the systolic blood pressure (p<0.001), diastolic blood pressure (0.125) and the basal heart rate (<0.001) were observed in obese/non-obese women with PCOD group compared to the control group as shown in Table.I.

Time Domain Measures of HRV analysis among the study group

In a short-term recording (5-minute) of HRV, considering the reliability and accuracy of heart rate variability measurements into account, the total heart rate (THR), mean of RR intervals (MRR), standard deviation of normal to normal RR intervals (SDNN), and root mean square of successive NN interval differences (RMSSD) are the most reliable time domain measurements.[6] They were calculated by the following equations: THR = X.

$$MRR = \bar{I} = \frac{1}{X-1}\sum_{n=2}^{X} I(n)$$

$$SDNN = \sqrt{\frac{1}{X-1}\sum_{n=2}^{X}[I(n) - \bar{I}]^2}$$

$$RMSSD = \sqrt{\frac{1}{X-2}\sum_{n=3}^{X}[I(n) - I(n-1)]^2}$$

Intergroup analysis showed significant differences (p<0.05) between the groups, with lower SDNN and RMSSD in obese & non-obese women with PCOD when compared to that of the non-obese women without PCOD. Though not significant, a consistent increase in TINN (triangular index) was observed in obese & non-obese women with PCOD when compared to that of the non-obese women without PCOD. Also Table.II illustrates the comparison of mean RR interval traces among the three groups, where a decrease in mean RR intervals was clearly seen in the women with PCOD (obese and non-obese) compared to that of the non-obese women without PCOD.

Compared with the non-obese control group, women (obese and non-obese) with PCOD had lower FSH levels (p<0.0001), higher LH levels (p<0.0001) and higher fasting glucose (p<0.004) levels as depicted in Table.II.

Correlation between RMSSD and other variables

Table.III shows the correlation analysis between time domain parameters of HRV and other variables. There was a significant negative correlation between RMSSD with BMI and WHR indicating that vagal modulation decreases as BMI increases. This was more observed in obese women with PCOD than non-obese women with PCOD. A significant negative correlation was observed between RMSSD with SDNN and mean RR. We also observed significant negative correlation between RMSSD with LH and FBS in women (obese/non-obese) with PCOD.

Reliability (agreement) analysis and linear regression model between SDNN/RMSSD ratio and LF/HF ratio

In obese women with PCOD, the agreement analysis and linear regression were done to find out whether SDNN/RMSSD could be an alternate for LF/HF. The intraclass coefficient was 0.412 (p=0.029) and the 95%CI (0.555-0.714) are quite close, smaller intervals tells us that the rater agreement can be extrapolated well in population also. The measure of agreement by kappa was 0.612 (p<0.01), which means that SDNN/RMSSD ratio strongly agrees with LF/HF ratio. By linear regression model, considering the dependent variable SDNN/RMSSD as y, the intercept constant *a*, slope *b* and the independent variable LF/HF as x, the linear regression equation has been calculated as y=1.533 + (0.234)x.

Discussion

HRV Recordings from 30 healthy normal subjects and 60 women diagnosed with PCOD were assessed to find out the significant variations in time domain measures of HRV and also to find out whether SDNN/RMSSD ratio reliably agrees with that of the LF/HF ratio in women with PCOD. The analysis of HRV at rest in supine position purely based on ECG recordings helped us to study the parasympathetic modulation and the sympathetic-vagal balance in young women with PCOD.[15]

Previous studies evaluating HRV in PCOD showed impaired cardiac autonomic modulation at rest condition and during 24 h recording by comparing the (frequency domain measures) LF/HF ratio with controls.[16] However, neither the changes in time domain measures nor its association with other

modifiable/nonmodifiable cardiovascular risk factors were studied in women diagnosed of PCOD.

Epoch/Reliability assessment of HRV

Recent literature on HRV pointed up the relationship between variables and epoch lengths. RMSSD, SDNN and HF were considered more reliable HRV measures than other metrics for various epochs.[17-21] This study mainly focus on the variations in the time domain measures of HRV analyses. The normal sinus rhythm RR interval as recorded was used. The segment durations varied from 10 seconds and 9 metrics were employed, of which rMSSD and mean heart rate(MHR) were more reliable as per the updated analysis of HRV.[18] In our study we used 5-minute epoch that was suggested for the short-time HRV measurements.[21] Reliability is a factor of reproducibility and stability of values in any research.[13,22-24] Of all, the total power, the mean RR interval and RMSSD were proven the highest stability, SDNN with least stability and highest reliability was considered towards LF and HF than LF/HF ratio.[23-24]

In our study, the RMSSD measure showed consistent stability in various epoch lengths and hence its correlation with other parameters were tested, whereas SDNN was consistently unreliable. For the epoch effect, we were more interested in analysing the impact of PCOD on time domain of HRV and deriving SDNN/RMSSD calculation. Hence the correlation coefficients and reliability analysis between SDNN/ RMSSD ratio and LF/HF ratio were calculated in our work. However, the selection of various epoch lengths at varied time periods could still bring changes in the result.

In a study conducted in patients with PCOD had an unfavourable cardiac autonomic innervation profile with increased sympathetic activity and authors suggested that this result was caused by differences in the hormonal profile.[25] In this study, we observed that young women with PCOD showed a decrease in HRV indexes in the time (SDNN and RMSSD) and frequency (LF, HF) domains compared to ovulatory women in the same age range.

Time domain measure of HRV in PCOD

Our study clearly states that women with PCOD have a higher level of sympathetic nerve activity (as detected through lower SDNN, RMSSD and TINN measurements) than the women without PCOD. This finding harmonizes with other studies in patients with PCOD stating the presence of diminished vagal and an increased sympathetic modulation of sinus node.[26-29] Supporting this, a study experimented exercise stress test in PCOD patients, and the authors observed a decrease in SDNN in PCOD patients, suggesting the role of compromised cardiovascular modulation.[30] Apart from SDNN, reports of other time domain measures were not detailed in that study. Though it was reported that HRV analyses was not altered in non-obese patients with PCOD, our study showed a decreased time domain measures in the same population.[29] The presence of concomitant cardiovascular risk factors like diabetes and hypertension in our study patients might partly explain the observed findings.

Variations of mean RR and mean HR in PCOD

Of note, all the time domain HRV indices strictly are measures of variability in RR interval and have units of time(ms) except pNN50, where heart rate(HR) has units of beats per minute(bpm) and RR has units of ms. To be exact (HR=60,000/RR), where fluctuations in RR interval and HR are closely related, but not linear. Thus, a doubling in RR interval variability does not mean HRV would necessarily double if measured from the sequence of corresponding instantaneous HR values. Even though instantaneous HR may correspond closely to autonomic tone and have greater physiologic significance than RR interval, time domain measurements are traditionally calculated from the RR (or NN) interval sequence.

The basal HR which is mainly under vagal modulation is reported to be high in women with PCOD as a result of decreased vagal activity.[33] Moreover, researchers observed raised systolic and diastolic blood pressure (BP) in those women and attribute this increment to increased sympathetic drive as regulation of BP is mainly under sympathetic control.[34] This increment in HR could be due to cardiac sympathetic stimulation that mainly reflects augmented compensatory activation in response to excessively decreased venous return to the heart.[35,36] Our study also showed an increased mean HR and mean RR interval in obese women with PCOD whereas in non-obese women with PCOD only a slight variation has been observed compared with the controls. Also the systolic and diastolic blood pressure in women with PCOD were within

physiological limits, even though a slight increase was seen compared with controls.

Weight gain and time domain of HRV in PCOD

It has been known, obesity causes autonomic dysfunction by enhancing adrenergic discharge and modulating vagal tone, hence it is considered as a major determinant of sympathetic discharge in women.[37] An increased prevalence of metabolic syndrome was reported in women with PCOD.[38] It is important to point out that a significant increase in BMI and WHR with co-existing negative correlation between the RMSSD with BMI and WHR was found in women (obese & non-obese) with PCOD. Though this finding of increased weight gain could be substantiated due to age, hypoestrogenism, it becomes even more important as the negative correlation is also seen among non-obese women with PCOD, where we enrolled only women(PCOD) of 20–39years old.[39-41] some studies have reported a significant negative correlation between BMI and SDNN in obese male and female subjects and quoted a probable reason to be of decreased parasympathetic activity and autonomic modulation of HR with increase in weight.[42,43] However, the insulin resistance, glucose intolerance, levels of total cholesterol, triglycerides and CRP were not assessed in this study.

Some research investigated the relationship between RMSSD and other important variables.[44-47] No significant correlation between RMSSD with LH and FSH has been observed in our study except the fact that LH and FSH were significantly increased in obese and non-obese women with PCOD. PCOD must be suspected in every adolescent girl with menstrual irregularity, hirsutism, obesity, persistent acne vulgaris, scalp hair loss and hyperhidrosis .[48] Moreover, Kim and Rosenfield stated that cutaneous signs of androgen excess were acne, seborrhea, alopecia, or hyperhidrosis.[49] This emphasize that sympathetic autonomic cholinergic over activity could be involved in the pathogenesis of patients with PCOD.

SDNN/RMSSD and LF/HF in PCOD

The main point of this paper is to study the relationship between SDNN/rMSSD ratio and LF/HF ratio. It was stated in a work that RMSSD cannot model HF power, the mapping of vagal control onto heart rate.[47] Although this was disagreed by some, SDNN/RMSSD and LF/HF seemed to exhibit balance between long-term(24-hour recording) and short-term(5 minute recording) variability.[7] Furthermore, Two scientific work has been done on this topic, where SDNN/RMSSD ratio were reported to have a strong correlation coefficient with LF/HF in normal healthy subjects (r = 0.90) tested in supine state and after 70◦tilt test (HUT) and cortical injury patients(r=0.94) experimented in sitting, standing and with mental tasks.[7,8] By reliability analysis we could only say that SDNN/RMSSD ratio moderately agrees with LF/HF and can be extrapolated to the population, but to prove it as a surrogate marker for LF/HF needs more in depth evaluation with more sample size study in patients with PCOD.

Conclusion

In our work, there were 60 women with PCOD, all the subjects were tested with HRV. The results were observed by 5-minute epoch with normal distribution. With reference to others (4,5) whether the robustness of SDNN/RMSSD as surrogate for LF/HF needs more confirmation and is suggested for further evaluation, since it had strong correlations with p-value smaller than 0.001.[7, 8] Although the time domain variables of HRV are related to the cardio vagal modulation of the heart in obese and non-obese PCOD patients, this study has its limitations. Despite our results, we insist the need for more studies with larger samples and detailed assessment of variables in HRV to corroborate and validate the results presented here. In this sense, it is relevant to emphasise that HRV technique so far can be viewed as a gold standard in evaluating adrenergic effect on cardiovascular system.

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Conflict of interest: Nil

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Table 1. Comparison of age, anthropometric characteristics and cardiovascular factors between groups.

Parameter	Obese PCOD (n=30)	Non-obese PCOD (n=30)	Control group (n=30)	*p
Age (years)	25.3±5.01	24.53±4.72	23.9±4.19	0.539
Weight (kg)	78.8±5.82	50.9±2.43	51.63±2.61	<0.001
Height (m)	1.56±0.08	1.55±0.06	1.58±0.06	0.168
BMI (kg/m2)	31.82±1.93	21.19±1.15	20.61±1.62	<0.001
WHR	0.98± 0.04	0.79±0.03	0.81±0.04	0.01
SBP (mm Hg)	118.33±7.59	110.53±7.39	112.2±9.03	<0.001
DBP (mm Hg)	76.27±7.61	72.07±8.56	73.8±7.55	0.125

Data presented as mean ± standard deviation. BMI is body mass index; WHR is Waist Hip Ratio; SBP and DBP were Systolic and Diastolic Blood Pressure respectively. *p<0.05: one-way ANOVA.

Table.II: Comparison of time domain variables of HRV analysis between groups.

Parameter	Obese PCOD (n=30)	Non-obese PCOD (n=30)	Control group (n=30)	*p
Time domain-HRV:				
SDNN	52.7± 6.42	65.03± 18.15	75.23± 14.55	<0.001
RMSSD	33.76± 4.59	34.29± 5.73	35.17± 6.86	0.003
TINN	114.56± 4.79	85.32 ± 6.04	60.83± 5.72	0.256
mean RR (ms)	869.03±112.6	807.17±124.49	799.87±130.47	0.041
Mean HR (beats per minute)	80.43±4.45	74.4±2.67	75.3±2.83	<0.001
Biochemical:				
FBS (mg/dl)	130.8±13.59	108.83±8.78	109.43±9.38	<0.004
LH(mIu/ml)	10.01 ±1.39	8.82±1.34	6.66±1.05	<0.0001
FSH (mIu/ml)	5.01±0.89	6.13±0.89	7.41±0.84	<0.0001

Data presented as mean ± standard deviation. *p<0.05: one-way ANOVA. SDNN: standard deviation of all NN interval; RMSSD: Standard deviation of differences between adjacent; NN: Square root of the mean of the sum of the square of the differences between adjacent NN intervals; mean RR: mean RR intervals; mean HR: mean Heart Rate; TINN: triangular interpolation of NN interval. LH and FSH were Leutinizing hormone and Follicle Stimulating hormone respectively. FBS is fasting blood sugar

Table.III: Pearson's rank correlation between RMSSD versus other variables among the study group.

Parameter RMSSD Vs	Obese PCOD (n=30)	Non-obese PCOD (n=30)	Control group (n=30)
BMI	-0.227*	-0.041*	0.120
WHR	-0.568*	-0.216*	-0.131
SDNN	0.420	-0.191	-0.331
FBS	-0.463	-0.214	-0.376
LH	-0.58*	-0.43	0.31
FSH	0.313	0.325	0.111

Pearson correlation test. *p<0.05: