Original Article

Analysis of Poincare plot of heart rate variability in the assessment of autonomic dysfunction in patients with polycystic ovary syndrome

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Abstract

Background and Aim: Polycystic ovary syndrome (PCOS) due to its constant association with obesity poses a significant cardiovascular (CV) risk. Heart rate variability (HRV) has been a noninvasive marker of autonomic dysfunction and CV risk. This study was designed to assess the nonlinear dynamics of HRV using Poincare plot in patients with PCOS and elucidate its importance in predicting the CV risk.

Methods: A total of 45 women with newly diagnosed PCOS and 45 controls were recruited for the study. Waist-hip ratio, body mass index (BMI), basal CV parameters such as basal heart rate (BHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and rate pressure product (RPP) were recorded. HRV analysis was done using both linear (time domain and frequency domain) and nonlinear measures (Poincare plot).

Results: The cases had increased basal heart rate, BMI, SBP, DBP, MAP, and RPP. In linear measures of HRV, the total power (TP), which depicts overall HRV was reduced, and the ratio of low-frequency to high-frequency (LF-HF) was significantly increased in cases. In nonlinear measures, the standard descriptors (SD1 and SD2) and area of the ellipse (S) were decreased, which signifies decreased HRV. There was a significant correlation of linear measures (TP, LF-HF ratio) and nonlinear measures (SD1, SD2, S) with elevated RPP. Both linear and nonlinear measures had independent contribution to elevated RPP, observed from regression analysis.

Conclusion: Decreased HRV and autonomic dysfunctions in the form of increased sympathetic drive and decreased vagal activity were observed in PCOS patients that may herald CV risks. Poincare plot analysis can independently quantify the magnitude of autonomic dysfunction in PCOS.

Key words: Autonomic dysfunction, heart rate variability, Poincare plot, polycystic ovary syndrome

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder seen in the reproductive years of women, with a prevalence of around 5–10%.[1] It is characterized by menstrual irregularities, biochemical or clinical hyperandrogenism, and polycystic ovary.[1,2] Obesity has been a very common clinical feature in these women, affecting 50% of them.[1,3] Obesity has been associated with cardiovascular (CV) autonomic dysfunction in the form of increased sympathetic activity.[4] With this potent comorbid factor, PCOS poses a significant CV risk, which needs to be assessed early in these patients.[5] Autonomic dysfunction has been related to adverse CV events.[6]

Heart rate variability (HRV) has been used as a noninvasive marker of cardiac autonomic activity and in CV risk stratification.[7] Conventionally, there are two main approaches to HRV analysis, the time domain analysis using the various statistical measures and the

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frequency domain analysis using the spectral analysis by fast Fourier transform. Both of these measures are linear models of HRV analysis. There are previous studies on the linear measure of HRV, and we have also reported, CV autonomic involvement in the form of decreased HRV, increased sympathetic tone and reactivity in patients with PCOS using the linear methods.[8-10]

Genesis of HRV also involves the nonlinear phenomena, which are determined by complex interactions of hemodynamic, electrophysiological and humoral variables, as well as by autonomic and central nervous regulations.[7] Hence, the analysis of the nonlinear dynamics of HRV would enable a better physiological interpretation of the HRV and for the assessment of the risk of sudden death.[7] The analysis of Poincare plots or sections of RR intervals is an emerging method of nonlinear dynamics applied in HRV analysis.[11] Poincare plot of RR intervals is an useful visual tool, which is capable of summarizing an entire RR time series derived from an electrocardiogram (ECG) in one picture, and a quantitative technique which gives information on the long- and short-term HRV.[12] However, no studies have examined the nonlinear component of HRV in PCOS. Therefore, in this study, an attempt has been made to assess specifically the nonlinear dynamics of HRV using Poincare plot in patients with PCOS and compare it with that of the HRV assessed by linear measures.

MATERIALS AND METHODS

Study design

This was an analytical cross-sectional study, conducted in the autonomic function testing (AFT) laboratory, Department of Physiology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India. The approval of the Institute Research Council and Institute Ethics Committee for human studies was obtained prior to the commencement of the study.

Subjects

A total of 90 participants were included in the study. 45 cases from the outpatient department of Obstetrics and Gynecology of JIPMER, Puducherry, India as per ESHRE/ASRM criteria[1] and 45 controls were recruited for the study. The cases included patients with newly diagnosed PCOS in the age group of 15–35 years. Patients already on treatment for PCOS were excluded from the study. Age-matched healthy regularly menstruating and nulliparous women were included as controls. Women with menstrual irregularities, hypothyroidism, diabetes, and women on any hormonal therapy or drugs were excluded. Written informed consent was obtained from all the subjects prior to the commencement of the study.

Procedure

The study was conducted during the follicular phase of the menstrual cycle in control subjects to avoid the influence of ovarian hormones on autonomic function and HRV.[13] In the study group, the test was conducted during amenorrheic period.[8] The subjects were asked to report to AFT laboratory at 07.00 h after overnight fasting.

Anthropometric measurements and metabolic parameters

Waist circumference was measured as the circumference of the abdomen at its narrowest point between the lower costal (10th rib) border and the top of the iliac crest. Hip circumference was measured at the level of the greatest posterior protuberance of the buttocks. Subject's height was measured to the nearest millimeter by a wall-mounted stadiometer and weight was measured with a spring balance to the nearest half a kilogram avoiding zero and parallax errors. Body mass index (BMI) and waist-hip ratio (WHR) were calculated. BMI was calculated by Quetelet's index. Asian criterion for BMI was followed for classifying the subjects as obese.[14]

Baseline cardiovascular parameters

After 5 min of sitting rest, basal heart rate (BHR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded by oscillometric method using automated blood pressure monitor Omron MX3 (Omron Healthcare Co. Ltd, Kyoto, Japan). Rate-pressure product (RPP), a determinant of myocardial oxygen consumption and workload was calculated using the formula,[15]

$$RPP = (BHR \times SBP) \times 10^{-2}$$

Short-term heart rate variability analysis

The subjects were explained about the tests. The room temperature at 23°C and the humidity between 25% and 35% were maintained.[16]

Data acquisition

Short-term HRV recording was done using lead II ECG, following the standard procedure as per the recommendation of Task Force.[7] The data acquisition were done using 16 bit, 16 channel data acquisition system BIOPAC MP100 (BIOPAC Inc., Goleta, CA, USA) with AcqKnowledge 3.8.2 software (BIOPAC Inc., Goleta, CA, USA). Sampling rate was kept at 500 samples/s/channel. Raw ECG was filtered using band pass filter (2–40 Hz). The RR tachogram was acquired for further analysis of linear and nonlinear dynamics.

Linear dynamics

Frequency domain analysis

Frequency domain analysis of the RR tachogram was done by power spectral analysis using fast fourier

transformation and time domain measures using the software from Biomedical signal analysis group, version 1.1 (Kuopio, Finland). The frequency domain indices included low frequency (LF; 0.04–0.15 Hz), high frequency (HF; 0.15–0.4 Hz), total power (TP), LF in normalized units (LFnu), HF in normalized units (HFnu) and the ratio of LF to HF (LF-HF ratio).

Time domain analysis

The time domain indices computed using statistical methods on RR tachogram, included mean-RR (mean of RR interval), SDNN (standard deviation [SD] of RR interval), RMSSD (the square root of the mean of the sum of the squares of the differences between adjacent NN intervals), NN50 (the number of pairs of adjacent NN intervals differing by >50 ms in the entire recording) and pNN50 (the percentage of NN50 counts, given by NN50 count divided by total number of all NN intervals).

Among these indices from linear dynamics of short-term HRV, the HF, HFnu, TP, SDNN, RMSSD, NN50 and PNN50 of HRV indices represent the cardiac parasympathetic drive (vagal tone). The LF and LFnu represent sympathetic tone. The LF-HF ratio depicts the sympathovagal balance.[7]

Nonlinear dynamics: Using Poincare plot

Using the RR tachogram, the Poincare plot was plotted using the software from Biomedical signal analysis group, version 1.1 (Kuopio, Finland). Poincare plot is a visual presentation of time series signal to recognize the hidden patterns. It is a two-dimensional graphic representation of the correlation between consecutive RR intervals, in which each interval is plotted against the following interval and its analysis can be done in a qualitative way, by assessing the shape formed by its attractor, which shows the degree of complexity of RR intervals, or in a quantitative way. The quantitative analysis is done by fitting an ellipse to the shape formed by the plot and measure the dispersion along the major and minor axis of the ellipse.[17,18]

There are two standard descriptors of Poincare plot namely:

- Standard deviation 1
  - It is the standard deviation (SD) of the instantaneous (short-term) beat-to-beat R-R interval variability (minor axis of the ellipse or SD1).
- Standard deviation 2
  - It is the SD of the long-term R-R interval variability (major axis of the ellipse or SD2).[17,18]

Furthermore, additional parameters were computed which included:

- Area of the ellipse (S)
  - It is given as the amount of area covered by the ellipse
  - It is calculated by doing the product of π, SD1, and SD2.
  - It represents total HRV.[19]

Statistical analysis of data

Sample size was calculated using PS program version 3.0.43. Sample size was estimated for three parameters LFnu, HFnu, and LF-HF ratio. The calculation with LFnu yielded the highest sample size of 30, with an expected mean difference of 13 from the previous study done for a power of 0.8 and type I error of 0.01.[7] Statistical analysis was done using SPSS Statistics software, Version 19 (SPSS Software Inc., Chicago, IL, USA). For data analysis, all values were expressed as mean ± SD. The data were subjected to Kolmogorov–Smirnov normality test. The inter-group differences between the controls and cases were compared using Student's unpaired *t*-test for normally distributed data and Mann–Whitney U-test for nonparametric data. Association of HRV parameters with RPP was assessed by Pearson correlation for parametric data and Spearman's Rank correlation for nonparametric data. Multiple regression analysis was done to assess the contribution of individual factors to RPP. $P < 0.05$ was considered statistically significant.

RESULTS

Both the cases and control subjects belonged to the same mean age group ($P = 0.2008$) [Table 1]. The cases had significantly high ($P < 0.001$) BMI and WHR compared to that of controls. The CV parameters, that is, BHR, SBP, DBP, mean arterial pressure (MAP) and RPP were significantly high ($P < 0.001$) in cases compared to that of controls.

TP and HFnu were significantly reduced ($P < 0.001$); LFnu and LF-HF ratio were significantly increased ($P < 0.001$)

Table 1: Comparison of age, anthropometric and basal cardiovascular parameters between controls and PCOS women (*n*=45)

Parameters	Controls	PCOS women	*P*
Age (years)	26.71±3.19	25.47±5.61	0.2008
BMI (kg/m^2)	22.93±5.27	29.17±7.49	<0.001
WHR	0.782±0.037	0.851±0.073	<0.001
BHR (beats/min)	63.75±7.14	86.4±12.16	<0.001
SBP (mmHg)	110.61±9.49	119.27±11.9	<0.001
DBP (mmHg)	72.8±4.61	86.63±7.29	<0.001
MAP (mmHg)	81.1±4.3	94.9±9.7	<0.001
RPP (mmHg/min)	70.64±11.93	103.27±16.51	<0.001

Values expressed as mean±SD. Statistical analysis was done by Student's unpaired *t*-test. Controls: Women with regular menstrual cycle. BMI: Body mass index, WHR: Waist-hip ratio, BHR: Basal heart rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MAP: Mean arterial pressure, RPP: Rate pressure product, PCOS: Polycystic ovary syndrome, SD: Standard deviation

in PCOS women compared to the controls. All the time-domain indices (mean-RR, SDNN, RMSSD, NN50, and pNN50) were significantly decreased ($P < 0.001$) in PCOS women compared to the controls [Table 2].

SD1, SD2, and S were significantly reduced ($P < 0.001$) in PCOS women compared to controls [Table 3, Figure 1].

There was a significant negative correlation of RPP with BMI ($P = 0.031$), SD1 ($P = 0.019$), SD2 ($P = 0.029$), S ($P = 0.041$), TP ($P = 0.028$) and a significant positive correlation with LF-HF ratio ($P = 0.021$). There was no significant correlation with RMSSD ($P = 0.081$) [Table 4].

SD1 ($P = 0.023$) and SD2 ($P = 0.031$), S ($P = 0.047$), TP ($P = 0.017$) and LF-HF ratio ($P = 0.028$) had independent association with RPP. There was no significant contribution of BMI to RPP [Table 5].

DISCUSSION

In this study, the baseline CV parameters (BHR, SBP, DBP, and MAP) were significantly elevated in women with PCOS compared to the controls ($P < 0.001$). The increase in BHR indicates attenuated vagal tone, as BHR is mainly under vagal modulation.[20] The raised SBP, DBP, and MAP observed in patients with PCOS could be attributed to increased sympathetic activity, as regulation of BP is mainly under sympathetic modulation.[21]

This alteration in autonomic modulation was further substantiated by the findings of linear dynamics of short-term HRV analysis. In frequency domain analysis, TP, which is an index of overall HRV was significantly

Table 2: Comparison of linear measures of HRV indices parameters between controls and PCOS women (n=45)

Parameters	Controls	PCOS women	*P*
Frequency domain indices			
TP (ms^2)	992.73±217.73	341.72±91.6	<0.001
LFnu	31.27±12.85	53.61±17.92	<0.001
HFnu	68.73±12.85	46.39±17.92	<0.001
LF-HF ratio	0.454±0.5	1.156±0.8	0.0011
Time domain indices			
Mean RR (ms)	912.7±116.19	716.2±94.5	<0.001
SDNN (ms)	68.92±29.62	28.74±12.57	<0.001
RMSSD (ms)	81.47±47.92	31.15±16.81	<0.001
NN50	123.18±64.9	48.37±29.95	<0.001
pNN50[‡]	39.06±7.49	15.57±4.07	<0.001

Values expressed as mean±SD. Statistical analysis was done by Student's unpaired *t*-test. Controls: Women with regular menstrual cycle. TP: Total power, LF: Low frequency, HF: High frequency, nu: Normalized units, SDNN: Standard deviation of NN intervals, RMSSD: Square root of the mean squared differences of successive NN intervals, NN50: Number of pairs of adjacent NN intervals differing by more than 50 ms, pNN50: Percentage of NN50, PCOS: Polycystic ovary syndrome, HRV: Heart rate variability, SD: Standard deviation

Table 3: Comparison of nonlinear measures of HRV indices parameters between controls and PCOS women (n=45)

Parameters	Controls	PCOS women	*P*
Poincare plot			
SD1	54.74±11.4	24.8±4.7	<0.001
SD2	83.17±15.7	47.4±10.57	<0.001
S	14581±495.16	3714±281.53	<0.001

Values expressed as mean±SD. Statistical analysis was done by Student's unpaired *t*-test. Controls: Women with regular menstrual cycle. SD_1: Minor axis of the ellipse, SD_2: Major axis of the ellipse, S: Product of π, SD_1 and SD_2, PCOS: Polycystic ovary syndrome, HRV: Heart rate variability, SD: Standard deviation

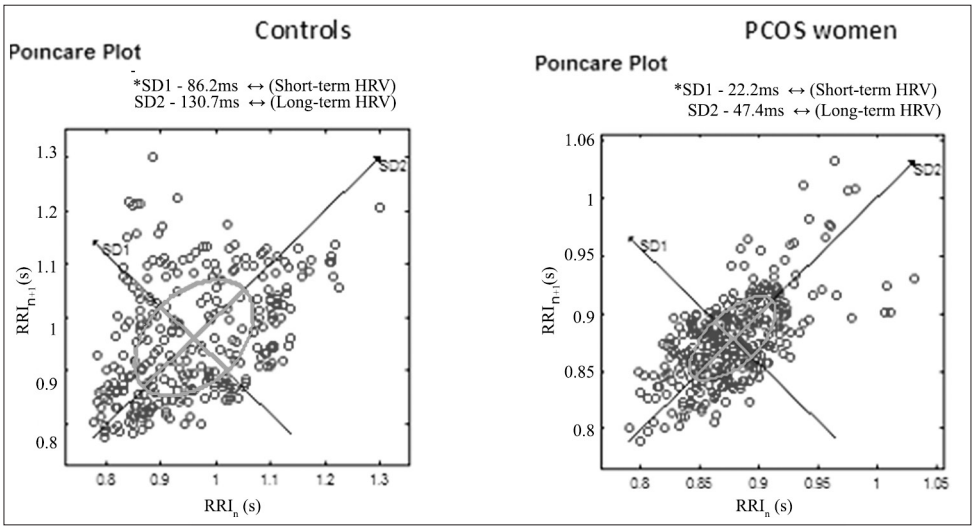

Figure 1: A sample of Poincare plot in controls and polycystic ovary syndrome women, with its numerical descriptors, SD1 and SD2. Controls: Women with regular menstrual cycle, SD1: Minor axis of the ellipse, SD2: Major axis of the ellipse

Table 4: Correlation analysis of RPP with HRV parameters among the controls and PCOS women

Parameters	Controls (*n*=45)		PCOS women (*n*=45)	
	r	*P*	*r*	*P*
BMI	0.094	0.294	0.369	0.031
SD1	-0.195	0.112	−0.437	0.019
SD2	-0.074	0.304	−0.381	0.029
S	-0.183	0.217	−0.294	0.041
TP	-0.156	0.264	−0.389	0.028
RMSSD	-0.219	0.192	−0.197	0.081
LF-HF ratio	0.317	0.097	0.415	0.021

Controls: Women with regular menstrual cycle. BMI: Body mass index, SD_1: Minor axis of the ellipse, SD_2: Major axis of the ellipse, S: Product of π, SD_1 and SD_2, TP: Total power, RMSSD: Square root of the mean squared differences of successive NN intervals, LF: Low frequency, HF: High frequency, PCOS: Polycystic ovary syndrome, RPP: Rate pressure product, HRV: Heart rate variability

Table 5: Multiple regression analysis of RPP with HRV parameters among the PCOS women

Parameters	Standardized beta	*P*
BMI	0.273	0.379
SD1	0.619	0.023
SD2	0.579	0.031
S	0.537	0.047
TP	0.673	0.017
LF-HF ratio	0.583	0.028

RPP: Rate pressure product, BMI: Body mass index, SD_1: Minor axis of the ellipse, SD_2: Major axis of the ellipse, S: Product of π, SD_1 and SD_2, TP: Total power, LF: Low frequency, HF: High frequency, PCOS: Polycystic ovary syndrome, HRV: Heart rate variability

reduced in the cases [Table 2]. Decreased HRV depicts decreased cardiovagal modulation, which is a potential CV risk.[6] Furthermore, significantly increased LFnu and decreased HFnu in PCOS subjects depicted their increased sympathetic drive and attenuated vagal tone.

LF-HF ratio, the marker of sympathovagal imbalance (SVI) was increased in PCOS cases, depicting increased sympathetic activity in these patients as increase in LF-HF ratio represents accentuation of sympathetic activity.[7] As time-domain indices of HRV depict the cardiac vagal drive, considerable reduction in these indices (mean RR, RMSSD, SDNN, NN50, pNN50) in PCOS subjects further establish decreased vagal modulation of cardiac functions in them.[7] Hence, in women with PCOS, there is a decrease in overall HRV, with an increased sympathetic tone and decreased vagal tone. These findings are similar to our earlier report of the nature of SVI in PCOS patients.[9,10]

From the nonlinear dynamics, it could be inferred that there was a decrease in short-term and long-term HRV in patients with PCOS, as observed from a significant decrease in SD1 and SD2, respectively. Furthermore, a significant reduction in S, the area of the ellipse indicates a reduction in total HRV.[19]

The RPP, an indirect measure of a myocardial load and oxygen demand was found to be significantly elevated among the cases indicating that PCOS patients are constantly under the stress of increased myocardial performance.[15] The linear measures of HRV, TP, and LF-HF ratio showed a significant association with RPP. This suggests that altered autonomic modulation can strain the myocardium. Similarly, a negative correlation of RPP with nonlinear measures, that is, SD1, SD2, and S, depicts that attenuated short-term, long-term and total variability could increase the stress on myocardial performance. The significant independent contribution of both linear and nonlinear dynamics to elevated RPP observed in multiple regression analysis further confirms this.

There was a positive correlation of RPP with BMI. In our study, the women with PCOS were obese as assessed by Asian criteria and had increased WHR, which suggests that they had android type of obesity. Obesity has long been known to cause derangement in autonomic functions in the form of increased adrenergic and decreased vagal modulation.[4] However, on multiple regression analysis there was no independent contribution of BMI to RPP. This suggests that, obesity *per se* in PCOS may not contribute to the myocardial stress rather the derangement in autonomic modulation could be a potential risk for elevated RPP.

Hence, we observed that, the findings of the nonlinear dynamics of HRV are corroborative with the observations from linear dynamics. Information about HRV has been commonly obtained using linear methods.[7] However, RR intervals fluctuate in a more complex pattern exhibiting patterns suggestive of nonlinear processes. In recent years, the use of nonlinear dynamic methods has become widely applied to the quantitative analysis in many temporal physiological signals including the R-R interval time series.[22] Visual inspection of the Poincare plot has been largely used in the analysis of HRV.[23] It is capable of summarizing an entire RR time series derived from an ECG in one picture, and a quantitative technique, which gives information on the long- and short-term HRV.[12] The nonlinear analysis offers the advantage of not requiring any preprocessing or stationarity of the data, which is needed in linear analysis.[23] Thus, Poincare plot analysis that depicts decreased cardiovagal modulation can assess the magnitude of susceptibility to CV dysfunctions in PCOS patients.

Limitations of the study

The sample size in each group in the present study was modest. Therefore, we could not perform logistic regression analysis to assess the contribution of Poincare plot indices to autonomic dysfunction and its predictive role in the assessment CV risks. Studies should be conducted in larger sample size to further establish the

predictive and investigative importance of Poincare plot analysis in PCOS.

CONCLUSION

Observations from the present study suggest that nonlinear analysis of HRV can independently quantify the alteration in HRV and can be used for CV risk stratification. When used along with linear measures, it would enable a better interpretation of the various physiological correlates of HRV abnormalities in PCOS patients.

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