

## Article

### Article information:

Received on June 2019  
Accepted on September 2019  
DOI: <https://doi.org/10.3329/jbsp.v14i2.44784>

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### Cite this article:

Relationship between parasympathetic reactivity and oxidative stress in Polycystic ovary syndrome  
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J Bangladesh Soc Physiol 2019;14(2): 48-55

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## Relationship between parasympathetic reactivity and oxidative stress in Polycystic ovary syndrome

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### Abstract

**Background:** Polycystic ovary syndrome (PCOS) is a very common reproductive hormone disorder. Altered cardiovagal autonomic modulation and oxidative stress may predispose PCOS patients to increased cardiovascular morbidity. **Objective:** To assess the relationship between parasympathetic reactivity and oxidative stress in patients with PCOS. **Methods:** This cross-sectional study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbagh, Dhaka from September 2018 to August 2019 on 35 newly diagnosed obese (BMI  $\geq 25\text{kg/m}^2$ ) PCOS patients aged 20-35 years. Age and BMI matched 35 apparently healthy women were also enrolled as control. Three noninvasive conventional autonomic function tests, such as heart rate response to deep breathing, standing and the Valsalva maneuver, were used for evaluation of parasympathetic reactivity. For assessment of oxidative stress, plasma malondialdehyde level (oxidant) and plasma catalase level (antioxidant) were measured in all subjects by spectrophotometry. Data were expressed as mean  $\pm$  SD. For statistical analysis Independent sample “t” test, Pearson’s correlation test and multiple regression analysis were done as applicable. **Results:** In this study PCOS patients had significantly higher ( $p < 0.01$ ) resting heart rate, systolic and diastolic blood pressure than that of healthy control. But Expiration: Inspiration ratio, Expiration: Inspiration difference and 30:15 ratio during standing were significantly lower ( $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$  respectively) in PCOS compared to control. In addition, plasma catalase level was significantly lower ( $p < 0.01$ ) and plasma

malondialdehyde level significantly higher ( $p < 0.001$ ) in PCOS in comparison to healthy control. Multiple regression analysis showed plasma catalase as a significant positive predictor ( $p < 0.05$ ) of the Valsalva ratio in PCOS. Also, Valsalva ratio showed significant negative association ( $p < 0.05$ ) with plasma malondialdehyde ( $p < 0.01$ ) in PCOS. **Conclusion:** Based on these results it is concluded that impaired parasympathetic reactivity showed inverse relationship with oxidative stress in PCOS.

**Keywords:** Autonomic reactivity, polycystic ovary syndrome, oxidative stress

### Introduction

**P**olycystic ovary syndrome (PCOS) is a very common reproductive hormone disorder associated with anovulatory infertility affecting 8-13% of women worldwide<sup>1</sup>. Research evidence suggested that associated metabolic co-morbidities such as obesity, insulin resistance, dyslipidaemia, hypertension confer significant risk for poor cardiovascular health in these women in the long term<sup>2-4</sup>.

Previous studies observed impaired balance between the sympathetic and parasympathetic activity in PCOS which predisposes them to various cardiovascular disorders<sup>5-6</sup>. Deranged resting autonomic activity evaluated by heart rate variability (HRV) and autonomic reactivity assessed by conventional autonomic function tests was reported in PCOS<sup>7-13</sup>. Autonomic reactivity refers to the response of the ANS to various physiological provocations<sup>6</sup>. Almost all of these studies reported autonomic dysfunction characterized by parasympathetic insufficiency and sympathetic hyperactivity in PCOS compared to normal women. Very few authors found no alteration in cardiac autonomic tone analyzed by HRV in PCOS compared to healthy control<sup>14-15</sup>.

Conventional autonomic function tests (CAFT) were introduced by Ewing in 1982, for bedside evaluation of the integrity of parasympathetic and sympathetic reflex response to physiological provocations. The heart rate response to deep

breathing, heart rate response to active standing and the Valsalva maneuver are very sensitive tools for detection of poor cardiovagal autonomic modulation<sup>16</sup>. The Expiration: Inspiration ratio (E:I ratio) and Valsalva ratio were found decreased in a group of PCOS patients when compared to age and BMI matched healthy control<sup>12-13</sup>.

Recent studies reported increased oxidative stress in PCOS patients which could be an additional risk factor for cardiovascular morbidity<sup>17-20</sup>. Oxidative stress implies an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system of the body leading to damage of important biomolecules like DNA, lipids and proteins and ultimately cellular dysfunction and apoptosis. Peroxidation of polyunsaturated fatty acids by ROS yields a very stable and toxic compound known as malondialdehyde (MDA). It is frequently measured as a biomarker of increased pro-oxidant levels in blood<sup>21</sup>. High serum MDA level was observed in PCOS compared to healthy control<sup>19-20, 22-23</sup>. Enzymatic antioxidants including superoxide dismutase, catalase and glutathione peroxidase are the first line of defense against oxidative stress in the body. Catalase is present in peroxisomes of all cells. It efficiently reduces  $H_2O_2$  to water and  $O_2$ , thus protecting the body from its harmful effects of  $H_2O_2$ <sup>21</sup>. Serum catalase level was found lower in PCOS compared to normal women in several studies<sup>24-26</sup>.

Though there is ample evidence that compromised cardio-vagal modulation and oxidative stress may contribute to cardiovascular morbidity in PCOS patients, the relationship between them is yet to be explored. Therefore this study aimed to investigate the possible relationship between oxidative stress and parasympathetic autonomic reactivity in the pathogenesis of PCOS.

### Methods

This cross-sectional study was carried out from September 2018 to August 2019 at the Department of Physiology, BSMMU, Shahbag, Dhaka to assess the relationship between cardiovascular autonomic reactivity and oxidative stress in 35 newly diagnosed PCOS patients, aged 20-35 years with BMI  $\geq 25\text{kg/m}^2$ . For comparison 35 age and BMI matched apparently healthy women with regular menstrual cycles were taken as control.

The patients were enrolled from the outpatient department of the Department of Endocrinology, BSMMU by purposive sampling and the control subjects were recruited by personal contact. The protocol of this study was approved by the Institutional Review Board of BSMMU. After briefing about the study, informed written consent was taken from each subject. Detailed personal and medical history was taken. All the subjects were free from any systemic disease. Women who were pregnant, lactating or on any hormonal therapy were not included in the study. After enrollment, venous blood sample from antecubital vein was collected from all the subjects with all aseptic measures, for estimation of plasma catalase and malondialdehyde (MDA) and fasting blood sugar, serum creatinine, serum TSH and serum ALT. Plasma catalase and plasma MDA were measured by spectrophotometry in the Department of Physiology with the preserved plasma of the subject within 28 days of collection. Subjects who fulfilled the inclusion and exclusion criteria were advised to follow some instructions on the previous night of conventional autonomic

function tests. They were advised to finish their meal by 9:00 pm the night before and to avoid any type of stress or any sedative or hypnotic medication. They were also requested to take a light breakfast without tea and coffee the next morning and afterwards attend the autonomic nerve function laboratory in the Department of Physiology, BSMMU between 8:00 am to 10:00 am. A thorough physical examination was done and height, weight, hip and waist circumference of all subjects were measured. BMI and waist-hip ratio was calculated. The subject was advised to take rest for 15-20 minutes in a controlled laboratory environment. Then her resting pulse and blood pressure was taken. Four limb leads of the MAC 400 3 channel ECG were attached appropriately and resting ECG was done for 30 seconds. For the heart rate response to deep breathing test, the subject was asked to breathe deeply and maximally at a rate of six breaths per minute for one minute while continuous ECG monitoring was done. Then after 5 minutes of supine rest, the subject was asked to stand up quickly with all the ECG leads in place for assessment of heart rate response to standing. For the Valsalva maneuver the subject blew into a rubber tube connected to a dial manometer and maintained a pressure of 40 mmHg for 15 minutes. ECG was recorded for 5 seconds before and 40s after the maneuver. After completion of all three tests, RR interval of ECG was analyzed to carefully. To determine the heart rate response to breathing deep breathing, the ratio of longest RR interval during expiration and shortest RR interval during inspiration (E:I ratio), the difference between longest RR interval during expiration and shortest RR interval during inspiration ( $E-I_{\text{diff}}$ ) was calculated. To determine heart rate response to lying to standing and ratio of longest RR interval around 30 beat and shortest RR interval around 15 beats (30:15 ratio) after standing was calculated. To determine heart rate response to Valsalva maneuver, Valsalva ratio was calculated.

Data were expressed as Mean  $\pm$  SD. Statistical analysis was done by SPSS version 22. Independent sample 't' test was done for comparison of data between PCOS and control. Pearson's correlation test and multiple regression analysis were done to assess the relationship of conventional autonomic function variables with plasma catalase and MDA. A p value of  $< 0.05$  was considered as statistically significant.

### Result

In this study all PCOS patients were comparable to healthy control by age, BMI and waist-hip ratio (Table I). Resting heart rate, SBP and DBP were significantly higher ( $p < 0.01$ ) in PCOS compared to control (Table I). In addition, plasma

catalase levels were significantly lower ( $p < 0.01$ ) and plasma MDA levels were significantly higher ( $p < 0.001$ ) in PCOS when compared to healthy control (Table I). PCOS patients had significantly lower E:I ratio, ( $p < 0.001$ )  $E:I_{diff}$  ( $p < 0.01$ ) and 30:15 ratio, ( $p < 0.05$ ) than that of control (Table II).

E:I ratio,  $E:I_{diff}$ , Valsalva ratio and 30:15 ratio were positively correlated with plasma catalase and negatively correlated with plasma MDA in PCOS but all the relationships were statistically non-significant ( $p > 0.05$ ) (Table III). In multiple regression analysis, Valsalva ratio showed significant positive relationship with plasma catalase levels ( $p < 0.05$ ) and significant inverse relationship with plasma MDA ( $p < 0.05$ ) in PCOS patients (Table IV).

**Table I:** Anthropometric, clinical and biochemical data in PCOS and control (N=70)

Variable	Control (n=35)	PCOS (n=35)
Age (years)	27.69 $\pm$ 4.00 (22-33)	26.97 $\pm$ 5.0 (20-35)
BMI (kg/m <sup>2</sup> )	28.63 $\pm$ 2.56 (25.38-34.58)	29.22 $\pm$ 3.42 (25.09-38.28)
Waist-Hip ratio	0.86 $\pm$ 0.05 (0.76-0.96)	0.88 $\pm$ 0.06 (0.75-1.01)
Resting heart rate (beats/min)	79.98 $\pm$ 10.52 (60-96.77)	88.14 $\pm$ 8.88** (75-103.45)
Resting SBP (mmHg)	115.77 $\pm$ 6.96 (100-130)	121.83 $\pm$ 7.51** (110-135)
Resting DBP (mmHg)	75.91 $\pm$ 5.32 (65-88)	79.94 $\pm$ 4.73** (70-88)
Plasma catalase (U/ml)	226.60 $\pm$ 79.68 (101-389)	172.06 $\pm$ 64.88** (59-282)
Plasma MDA (ng/ml)	203.00 $\pm$ 92.54 (82.04-478.80)	541.88 $\pm$ 223.35*** (101.30-952.50)

Data were expressed as Mean  $\pm$  SD. Values in parentheses indicate ranges; Statistical analysis was done by Independent sample t-test; PCOS-polycystic ovary syndrome; BMI-Body Mass Index (kg/m<sup>2</sup>); SBP-systolic blood pressure; DBP-diastolic blood pressure; MDA-Malondialdehyde; N-total number of subjects; n-number of subjects in each group; . \*\*= $p < 0.01$ .; \*\*\*= $p < 0.01$

**Table II:** Measures of parasympathetic reactivity in PCOS and control (N=70)

Variable	Control(n=35)	PCOS(n=35)
E:I ratio	1.31 $\pm$ 0.13 (1.13-1.59)	1.22 $\pm$ 0.08*** (1.1-1.40)
E-I <sub>diff</sub> (beats/min)	20.05 $\pm$ 7.00 (10.25-38.96)	16.21 $\pm$ 5.06* (10.44-29.14)
Valsalva ratio	1.32 $\pm$ 0.17 (1.11-1.82)	1.30 $\pm$ 0.15 (1.07-1.67)
30:15 ratio	1.31 $\pm$ 0.10 (1.103-1.750)	1.24 $\pm$ 0.13* (1.067-1.680)

Data were expressed as Mean  $\pm$  SD. Values in parentheses indicate ranges; Statistical analysis was done by Independent sample t tests; PCOS-polycystic ovary syndrome; E:I ratio-Expiration: Inspiration ratio; E-I<sub>diff</sub>-Expiration: Inspiration difference; 30:15 ratio-ratio of RR interval at 30th beat to 15th beat after standing; N-total number of subjects; n-number of subjects in each group; ns-non-significant ( $p > 0.05$ ); \*= $p < 0.05$ ; \*\*\*= $p < 0.001$ .

**Table III:** Correlation of E:I ratio, E-I<sub>diff</sub>, Valsalva ratio and 30:15 ratio with plasma catalase and MDA levels in PCOS (n=35)

Dependent	Catalase		MDA	
	r value	p value	r value	p value
E:I ratio	0.053	0.762	-0.009	0.958
E-I <sub>diff</sub>	0.025	0.886	-0.13	0.441
Valsalva ratio	0.196	0.258	-0.129	0.460
30:15 ratio	0.002	0.989	-0.049	0.782

PCOS-polycystic ovary syndrome; E:I ratio= Expiration: Inspiration ratio; E-I<sub>diff</sub>-Expiration Inspiration difference; 30:15 ratio-ratio of RR interval at 30th beat to 15th beat after standing n; MDA Malondialdehyde-number of subjects in PCOS group; ns-statistically non-significant (p>0.05).

**Table IV:** Multiple regression analysis of E:I ratio, E-I<sub>diff</sub>, Valsalva Ratio, 30:15 Ratio (dependent variable) with plasma catalase and MDA levels (independent variables) in PCOS (n=35)

E: I Ratio	Coefficients		95% CI		P value
	B	b	Lower limit	Upper limit	
Constant	1.209	-	1.123	1.294	0.000
Catalase (U/ml)	0.000	0.099	-0.000	0.001	0.380
MDA(ng/ml)	-0.00003	-0.272	-0.000	0.000	0.249
E-I <sub>diff</sub>					
Constant	16.774	-	11.492	22.057	0.000
Catalase (U/ml)	0.016	0.207	-0.021	0.053	0.380
MDA (ng/ml)	-0.006	-0.272	-0.017	0.005	0.249
Valsalva Ratio					
Constant	1.266	-	1.122	1.409	0.000
Catalase (U/ml)	0.001	0.508	0.000	0.002	0.026*
MDA(ng/ml)	0.000	-0.468	-0.001	0.000	0.039*
30:15 Ratio					
Constant					
Catalase (U/ml)	0.000	0.066	-0.001	0.001	0.782
MDA (ng/ml)	-0.00005	-0.090	0.000	0.000	0.707

PCOS-polycystic ovary syndrome; MDA-malondialdehyde; E-I<sub>diff</sub>- Expiration-Inspiration difference in heart rate; CI-confidence interval; n-number of subjects; ns-statistically.\*-p<0.05

## Discussion

In this study, significantly higher resting heart rate, SBP and DBP were observed in PCOS patients compared to control. Saranya and colleagues reported a similar observation<sup>12</sup> and Hashim, Hamdan and Al-Salihi observed a higher resting heart rate but similar SBP and DBP in PCOS compared to control<sup>7</sup>. Other investigators found these measures to be comparable in PCOS and healthy control<sup>10,11,19, 22</sup>. It has been suggested that since resting heart rate and blood pressure are principally under vagal modulation and sympathetic control respectively, patients with PCOS might have decreased vagal tone and increased sympathetic tone, thus, an altered sympathovagal balance at rest<sup>12</sup>. In our study, all the autonomic function test variables assessing parasympathetic reactivity with the exception of the Valsalva ratio, were found significantly lower in PCOS women compared to healthy control. Few researchers also observed a significantly low E: I ratio in PCOS<sup>12,13</sup>. Significantly lower Valsalva ratio in obese and non-obese PCOS women compared to BMI matched control was also found in a separate study<sup>13</sup>. These results indicate a clear deficit in parasympathetic or vagal reflex response in women with PCOS when compared to healthy women of same age and BMI. To explore the oxidative stress in the present study, the observed lower plasma catalase levels in PCOS patients agrees with other previous investigators suggesting a lower anti-oxidant status in these series of patients<sup>17,24-26</sup>. In a similar study, no effect of insulin resistance and body weight on catalase level was found in nonobese PCOS and it was concluded that antioxidant levels may not be decreased in non-obese PCOS women without insulin resistance. In contrast, higher plasma catalase in PCOS patients was also reported which was explained by the adequate counterbalance attributed to compensatory antioxidant response in these patients<sup>27-28</sup>. In this study, higher levels of malondialdehyde (MDA) in PCOS patients agrees to many previous

authors<sup>17, 19, 20, 22, 23</sup> but contrast to reports published almost similar MDA level in PCOS and healthy women<sup>29-31</sup>.

In the present study, significant inverse relationship of Valsalva ratio was noted with plasma MDA levels after adjustment with plasma catalase in PCOS patients. Moreover significant positive association of Valsalva ratio was seen with plasma catalase level after adjustment with plasma MDA level in PCOS. It is obvious from this result that increased oxidant level and decreased antioxidant level, consequently, oxidative stress, is associated with a decline in vagal reactivity.

The role of oxidative stress on impaired autonomic reflex response has been explained by altered nitric oxide signaling in the central and peripheral nervous system which might play a key role in cardiovagal autonomic dysfunction due to oxidative stress.<sup>32</sup> Reactive oxygen species, especially superoxide inactivates NO and thereby reduces NO induced facilitation of baroreceptor mediated vagal activation particularly in vasomotor center. In addition, as NO enhances vagal cholinergic discharge in cardiac autonomic ganglia and facilitates bradycardia<sup>32-33</sup> so increased oxidative stress could cause alteration in autonomic response in PCOS patients.

From analysis of the findings of this study it may be summarized that there was alteration in parasympathetic autonomic reactivity leading to the conclusion of deficient parasympathetic response in the present series of PCOS patients when compared to age and BMI matched healthy control. As none of the measures of parasympathetic reactivity were found abnormal in the PCOS patients in this study, there was no evidence of autonomic neuropathy in these PCOS patients. But the measures showed significant difference from healthy control which suggests attenuated parasympathetic reflex response in these patients. In addition, increased oxidative stress, supported by lower plasma catalase and

higher plasma MDA was found associated with PCOS patients in this study. Further the results of regression analysis suggest, parasympathetic autonomic reactivity in the current series of PCOS patients was associated with higher oxidative stress.

### Conclusion

The results of this study concludes that weak parasympathetic reactivity in polycystic ovary syndrome was related to higher oxidative stress.

**Conflict of interest** None

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