ORIGINAL ARTICLE

Oxidative stress in polycystic ovary syndrome: a case-control study

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Abstract

Objective: To compare the serum levels of biochemical and oxidative stress markers i.e., malondialdehyde (MDA) and paraoxonase-1(PON1) in polycystic ovary syndrome (PCOS) patients and healthy female individuals of reproductive age group (18-40 years).

Methods: This case-control study was conducted in Dow University of Health Sciences (DUHS), Karachi from June 2019 to October 2020. Seventy Subjects including 35 PCOS patients that have primary subfertility problem (cases) and 35 healthy and fertile females (controls) were recruited. Serum samples were collected for analysis of insulin, sex hormone-binding globulin, testosterone, fasting blood glucose and lipid profile. PON 1 and MDA levels were estimated by ELISA. Comparison between the two groups was done using independent t-test.

Result: The patients had significantly increased mean body mass index (28.5+4.6 kg/m^2 vs 25.7+4.5 kg/m^2, p=0.014), systolic (129.6±13.9 mm of Hg vs 113±7.7 mm of Hg, p<0.001) and diastolic (78.7±8.8 mm of Hg vs 74.6±6.7 mm of Hg, p=0.032) blood pressures compared to controls. The high-density lipoprotein cholesterol levels were significantly lower in PCOS (42.2±8.6mg/dl) than controls (48.8±11.8mg/dl, p=0.009, p=0.009). Serum insulin (14.3±5.8 uIU/mL) vs (10.0±5.2 uIU/mL), p=0.002 and testosterone levels (1.3±0.9 nmol/L) vs (0.82±0.3 nmol/L), p<0.001 were significantly higher whereas sex hormone binding globulin (SHBG) levels (35.2±19.7nmol/L vs 58.8±31.0 nmol/L) were significantly lower in patients than healthy controls (p<0.001). Both oxidative stress markers, paraoxonase 1 (7.7±2.4 vs 6.4±2.6 µg/mL, p=0.04) and malondialdehyde (2.5±1.0 vs 1.9+0.51µg/mL, p=0.034) levels were significantly elevated in PCOS patients than controls. No significant correlation was found between dietary habits and life style between cases and controls.

Conclusion: The study reported significantly elevated levels of oxidative stress markers in PCOS patients.

Keywords: Blood Glucose, Blood Pressure, Testosterone, Oxidative stress, Malondialdehyde, Lipoproteins, Cholesterol, Lipids, Enzyme.

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Introduction

PCOS is complex autosomal dominant, familial disorder affecting women's health worldwide.[1] The clinical features are irregular menstrual cycle, insulin resistance, hyperandrogenism, obesity, hirsutism and subfertility.[2]

It is being reported that 105 million females are diagnosed as PCOS,[3] which is one the major cause of anovulatory subfertility affecting 6-20% women during child bearing age.[3]

Consanguineous marriages, environmental and nutritional factors could be the causes of high prevalence of PCOS.[4]

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Aetiology of PCOs is unknown but environmental and genetic factors do lead to PCOS incidence. According to studies increase level of androgens in PCOS can impair hypothalamus- pituary-ovarian axis.[5,6]

The hormonal cascade with HPO axis is controlled by feedback system, Gonadotropin releasing hormone (GnRH) after being secreted from the hypothalamus, acts on pituitary for FSH and LH secretion which then stimulates estrogen and progesterone from ovaries that further inhibit FSH and LH production.[6,7]

Multiple causative agents such as environmental, genetic or dietary factors might impair the hypothalamus pituitary ovarian (HPO) axis that leads to disproportionate release of LH and FSH causing formation of immature ovarian follicles. Also, the increased levels of androgen and decreased sex hormone binding globulin (SHBG) by liver, contributes to PCOS.[8]

At Rotterdam, European Society for Human Reproduction and Embryology (ESHRE) and The American Society for Reproductive Medicine ASRM in 2013 form PCOS

diagnosing criteria – Rotterdam criteria[9], which is presence of 2 or more of the following 1) Oligo-anovulation 2) hyperandrogenism 3) polycystic ovaries on ultrasound. Classification of PCOS by Rotterdam and Androgen Excess and PCOS Society (AE-PCOS) is based on physical appearance of phenotypes- Frank PCOS, Ovulatory PCOS and Non-PCO PCOS.[9,10]

Frank PCOS is characterized by hyperandrogenism, oligomenorrhea and PCO. Ovulatory PCOS consists of regular menstrual cycle with hyperandrogenism and PCO and Non-PCO present with oligomenorrhoea and hyperandrogenism. In Normoandrogenic class, androgen levels are normal with PCO and oligomenorrhoea, as defined by Rotterdam.[10]

Oxidative stress (OS) refers to imbalance in antioxidant defense system and increase level of free radicals; reactive oxidative species (ROS), reactive nitrogen species (RNS) and peroxides produced by endogenous system. Moreover, certain environmental toxins and dietary factors are also involved in ROS formation.[11] Antioxidant defense system counters free radicals by involving enzymatic and non-enzymatic antioxidants.[12] Mostly Enzymatic antioxidants are metal binding providing non-reactive state and non-enzymatic antioxidants are chain breaking antioxidants of lipid oxidation reaction hence prevent cell damage by free radicals. OS can lead to tissue damage by impairing structures and functions of proteins, lipids and DNA.[13]

A calcium dependent antioxidant enzyme, Paraoxonase-1 (PON1) has 354 amino acids and 43kDa. It is formed in liver and is related with high density lipoprotein (HDL) in blood. It prevents HDL oxidation and inflammation. PON1 includes enzymatic activity of paraoxonase, aryl esterase, lactonase and Hcy-thiolactonase.[14] It hydrolyzes lipid peroxides, organophosphates and aromatic ester thus preventing cell damage. PON1 inhibits LDL oxidation and homocysteinylation of proteins.[15] In PCOS the activity of PON1 is still not completely understood yet, but PON1 activity does decrease in disorders related to oxidative stress and plays an important role in antioxidant defense system, indicating a link between local and systemic oxidative stress in PCOS.[16]

Malondialdehyde (MDA) is a biomarker of oxidative stress with an end product of lipid peroxidation. It is a highly reactive product formed by oxidation of poly-unsaturated fatty acid (PUFA).[17]

The oxidative degradation of lipids forms ROS. These reactive oxygen species causing cellular membrane damage and altering cell signalling pathway. MDA binds to plasma protein, amino acids (lysine and histidine) nucleic acids and lipids covalently and interferes in insulin signalling pathway leading to diabetes mellitus, atherosclerosis, cancer, chronic inflammation, PCOS and subfertility. Compromised state of PON1 and increase in serum MDA levels might contribute in pathogenesis of PCOS but it still needs to be further studied.[18]

Due to limited regional literature available regarding the risk factor causing disease, this study determined the levels of oxidative stress markers (MDA and PON1) in the PCOS to fill the gaps in understanding of the PCOS pathogenesis and help in improving the reproductive life of women. In Pakistan, burden of PCOS related primary subfertility is increasing and becoming a major public health issue. There is a need to fully understand the environmental factors responsible for disease complications. Our study tried to understand different factors that contribute in the oxidative stress in PCOS related primary subfertility.

Materials and Methods

This case control study recruited 70 subjects among which 35 were married PCOS diagnosed cases recruited from Gynaecology OPD of DUHS hospital and 35 non PCOS primary fertile females as controls. Convenient sampling method was used and computed sample size for this case control study was 66 (n=33 in each group). Sample size (n=66) was calculated using open EPI.com with 80% power of the test and 95% confidence interval.[19] This study was carried out after obtaining approval from Institutional Review Board (IRB) of DUHS (IRB-1215/DUHS/APPROVAL/2019). This study was conducted in Dow University Hospital, Dow University of Health Sciences (DUHS), Karachi. Rotterdam criteria and ESHRE PCOS guideline 2018 was used as inclusion criteria for PCOS. The controls were healthy female, multigravida with regular menstrual cycle Exclusion criteria in this study were pregnant, >40 years age women or women with any chronic illness like thyroid dysfunction, diabetes mellitus, chronic liver disease, malignancy, hyperprolactinaemia, Cushing's syndrome, adrenal tumours and patients with subfertility due to tubal factors. Also, patient with secondary subfertility or using any contraceptives or steroid were also excluded. Participants were briefed and consent was taken. Their detailed menstrual and personal history including lifestyle and dietary habits was collected. Anthropometric measurements like height, weight, hip and waist circumference were recorded. BMI was calculated and classified according to South Asian cutoff points into four categories as underweight (<18.5 kg/m^2), normal (18.5-22.9 kg /m^2), overweight (23-24.9 kg /m^2) and obese (≥ 25

kg /m^2).[20] Waist-to-hip ratio (WHR) was determined from data of waist and hip circumference. Cut off value for WHR was 0.89 for men and 0.81 for women.[21] Systolic and diastolic blood pressure was recorded. Blood pressure of study subjects was also recorded. Blood pressure <120 mm of Hg systolic and <80 mm of Hg diastolic was considered as normal.[22] Blood samples were collected and all the biochemical and laboratory investigations were performed for both PCOS patients and healthy controls. Pelvic ultrasound was also done for all study groups.

Blood sample (10mL) was collected from participants after overnight fasting. Venous blood was drawn for clinical testing and biochemical investigation for oxidative stress markers (PON 1 and MDA). Process of centrifugation was held at 2500 rpm. Serum was collected and aliquoted into two separate tubes for assays and stored at –70°C until analysis.

Biochemical parameters like fasting blood glucose, insulin, lipid profile (including serum triglycerides, total cholesterol, HDL-C, LDL-C and VLDL-C), total testosterone and SHGB were also measured.

Serum Malondialdehyde (MDA) levels were estimated by competitive enzyme immunoassay technique. Paraoxonase-1 (PON1) levels in serum samples were quantified by sandwich ELISA. All data were analyzed through Statistical Programme for Social Sciences (SPSS) version 26. Normality was assessed by using Shapiro-wilk test. The mean differences of study variables were assessed by using independent t-test and Mann-Whitney U test based. Independent t-test was for normally distributed variables and Mann-Whitney U test for non-normal distributed variables. Chi- square test was used for categorical data. p-value of less than 0.05 was considered statistically significant.

Results

Mean age of the participants was 28.2±5.0 years. Polycystic ovaries were observed in pelvis ultrasound of cases 30(89.2%) as compared to controls (0.0%, p<0.001). When compared for body mass index (BMI), cases were found significantly more overweight or obese 30 (88.6%) than controls 24 (68.6%), p=0.041. The ethnicity, consanguinity and family history of PCOS was not significant. Comparing dietary habits of cases and controls revealed no significant difference.

The mean Body mass index (BMI) was significantly higher in PCOS (28.5+4.6 kg/m^2) in comparison to healthy controls (25.7+4.5 kg/m^2, p =0.014). The waist-to-hip ratio (WHR) was found similar between the cases (0.89±0.1) and control (0.89±0.1) group (p=0.993). The mean systolic (129.6±13.9mm of Hg) vs (113±7.7 mm of Hg) and diastolic (78.7±8.8 mm of Hg) vs (74.6±6.7 mm of Hg) blood pressure were found significantly higher in cases than healthy controls (p<0.001 and 0.032 respectively) as shown in table 1.

The comparison of metabolic and hormonal profile of PCOS, showed the lipid profile (including triglycerides, cholesterol, and LDL and VDL cholesterol) of patients and healthy controls, was not statistically significant, although the levels were higher in PCOS. HDL-cholesterol was significantly lower in patients (42.2±8.6mg/dl) than controls (48.8±11.8mg/dl, p=0.009). No significant difference was observed in fasting blood glucose between the cases (91.6±9.2mg/dl) and controls (88.1±10.4mg/dl, p=0.212), however serum insulin (14.3±5.8 uIU/mL) was significantly higher in patients as compared to healthy controls (10.0±5.2 uIU/mL), p=0.002. Serum testosterone of patients (1.3±0.9 nmol/L) as compared to healthy controls (0.82±0.3 nmol/L) was significantly higher (p<0.001). HOMA-IR and free androgen index (FAI) were found significantly higher (p<0.001) in patients, whereas sex hormone binding globulin (SHBG) levels (35.2±19.7 nmol/L vs 58.8±31.0 nmol/L) was significantly lower in patients than healthy controls (p<0.001).

The levels of antioxidant paraoxanse-1 (PON1) were found significantly higher in cases (7.7± 2.4 µg/mL) than controls (6.4± 2.6 µg/Ml), p<0.05 respectively as shown in fig 1. Oxidative stress marker malondialdehyde (MDA) was found significantly higher (2.5±1.0 µg/mL vs 1.9±0.51 µg/mL) in cases than controls (p<0.05).

Table-1: Comparison of anthropometric and physiological parameters between cases and controls (n=70).

Variables	Cases n=35		Controls n=35		
	Mean±SD	Range	Mean±SD	Range	p-value*#
Body mass index (BMI) (kg/m^2)	28.5±4.6	19.8-37.7	25.7±4.5	17.3-35.5	0.014
Waist circumference (cm)	91.2±9.6	74-113	88.2±10.1	72.0-122.0	0.214
Hip circumference (cm)	102.2±9.3	88-130	98.6±9.5	71.0-125.0	0.117
Waist-to-Hip ratio (WHR)	0.89±0.1	0.7-1.1	0.89±0.1	0.80-1.2	0.993
Systolic blood pressure (mmHg)	129.6±13.9	110-163	113±7.7	99.0-130.0	<0.001
Diastolic blood pressure (mmHg)	78.7±8.8	62-112	74.6±6.7	60.0-90.0	0.032

*p<0.05 is considered statistically significant
#p-value refers to independent t-test

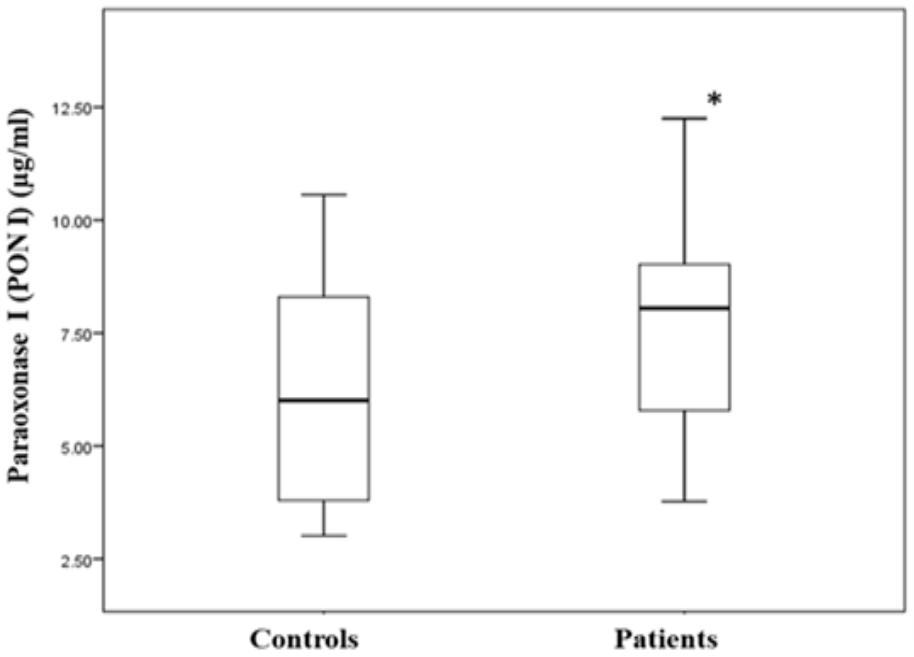

Figure-1: Box plot analysis of Paraoxonase-1 (PON1) levels in PCOS patients and controls.
Box plot analysis of PON1 levels in PCOS patients and healthy controls demonstrated that the PON1 levels were higher in patients than controls. Significant difference was found between the two groups.
*p<0.05, statistically significant difference

Discussion

The present study focussed on the various life style, dietary factors and oxidative stress markers which affect female reproductive health and can contribute in PCOS development.

This study observed that the PCOS was also prevalent in lean or normal weight women (BMI <22.9 kg/m^2), leading to the conclusion that PCOS is not restricted to obese or overweight women. The result was similar to the research by Daghestani et al 2018,[23] which concluded that in lean women the metabolic disorders can lead to PCOS.

This study also highlights the increased blood pressure of PCOS patients than controls (table 1). Hypertension is associated with high sympathetic nerves activity and endothelial dysfunction in PCOS. Various studies have reflected the relation between hyperandrogenaemia and hyperinsulinaemia, obesity, hypertension and endothelial dysfunction in PCOS.[24]

Dyslipidaemia is more evident in PCOS. The results of this study are in agreement with previous studies that dyslipidaemia is common in PCOS and decreased HDL-C levels together with obesity and insulin resistance predispose complications in PCOS.[23]

Paraoxonase-1 (PON1) is an enzyme which has antioxidant, anti-inflammatory and athero-protective properties. Very few studies highlighted the PON1 expression in PCOS patients as opposed to its activity in PCOS patients and related complications. Cetin M et.al, [25] stated that serum paraoxonase-1 level increases in response to the oxidative stress in the early stage of PCOS.[25] Our results are in agreement with these studies that reported higher PON1 levels in PCOS group compared to healthy controls, and the elevated PON1 levels might indicate a compensatory antioxidant mechanism for increased oxidative stress.[26]

Present study reported that the mean MDA levels were significantly higher in PCOS patients than in controls. Moreover, higher MDA levels showed increased oxidative stress in PCOS patients.[27] These findings are in consistence with the worldwide published studies that reported increased OS in PCOS patients.[27, 28]

The study showed the significant role of oxidative stress in PCOS as both the antioxidant (PON1) and oxidant (MDA) levels are increased in PCOS. Hence, investigation of oxidative stress markers in PCOS can lead to early diagnosis and prognosis of disease.

Conclusion

PCOS is the most common hormonal disorder in reproductive women which presents with various clinical features although, its etiology is unknown. It is considered to be a complex multifactorial disorder, which is also influenced by environmental and genetic factors. Various studies highlighted the levels of OS markers found in PCOS patients. This study reported significantly elevated levels of oxidative stress markers MDA and PON1 in PCOS patients.

Moreover, OS has been found to contribute in the risk factors of metabolic syndrome, reproductive failure, CVDs and cancer. However, further studies are required to standardize biomarker's measurements of OS influencing PCOS, as status of oxidants and antioxidants vary between individuals due to diverse dietary and life style habits.

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