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Paraoxonase 1 in Women with Polycystic Ovary Syndrome

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Abstract

Background: Oxidative stress (OS) plays a crucial role in the development of insulin resistance in women with Polycystic Ovary Syndrome (PCOS). Serum paraoxonase 1 (PON1) level is an antioxidant associated with HDL complex. Diminished activity of PON1 enzyme and increased levels of Malondialdehyde (MDA) has been reported in variety of diseases involving oxidative stress.

Objective: To compare the serum levels of PON1, MDA and lipid parameters in obese and normal women with PCOS with their respective controls.

Materials and Methods: Case-control study was carried out at the Biochemistry Department, Koppal Institute of Medical Sciences, Koppal, India from July 2015 to March 2018. It included 100 women with PCOS (50-obese and 50 normal) and 100 control subjects (50-obese and 50 normal), aged 18 to 40 years. Fasting lipid profile, Malonaldehyde (MDA) and PON 1 were estimated.

Results: There was no dyslipidemia in women with PCOS. Serum levels of PON1 were significantly decreased and MDA levels were increased in women with PCOS irrespective of BMI compared to their respective controls with a p value of <0.001, suggesting a significant inverse correlation between PON1 activity and MDA concentrations in women with PCOS irrespective of BMI.

Conclusion: Women with PCOS have increased oxidative stress irrespective of BMI indicated by decreased serum PON1 and increased levels of serum MDA. However, there was no dyslipidemia.

Keywords: ***Polycystic ovarian syndrome; Insulin resistance; MDA and PON 1***

Introduction

Polycystic Ovary Syndrome (PCOS) is a common heterogeneous endocrine metabolic disorder. It is characterized by clinical hyperandrogenism, oligo or anovulation, biochemical changes and Polycystic Ovaries (PCO). It affects 5%-10% of women in reproductive age [1]. Patients with PCOS are at increased risk for the development of diabetes mellitus, hypertension, and atherosclerotic heart disease. Oxidative stress (OS) which plays a key role in the pathogenesis of Cardiovascular Disease (CVD) is also seen in women with PCOS. Serum oxidative stress marker-Malonaldehyde (MDA) produced during the decomposition of polyunsaturated fatty acids, is one of the stable end products of lipid peroxidation that can serve as a good biomarker in women with PCOS [2,3].

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Paraoxonases Enzyme (PON 1) is synthesized by the liver and secreted into serum, where it is associated with High-Density Lipoproteins (HDL). It plays an important role in protection against oxidative damage and lipid peroxidation. It also contributes to innate immunity, detoxification of reactive molecules, bioactivation of drugs, modulation of endoplasmic reticulum, stress and regulation of cell proliferation and apoptosis [4]. Since it has the ability to destroy modified phospholipids and to prevent the accumulation of oxidized lipids in lipoproteins, [1] it possesses anti-atherogenic and anti-inflammatory properties. Lower levels of serum PON1 activity is observed in patients with cardiovascular diseases [5].

The aim of the present study was to compare serum levels of PON1 activity, MDA and lipid parameters in obese and normal women with PCOS with their respective controls.

Materials and Methods

A case-control study was conducted on 100 diagnosed PCOS patients (50-obese (BMI >30) and 50 normal (BMI <25) and 100 controls (50-obese and 50 normal) in the age group of 18-40 years. Fasting blood sample of 5.0 ml was obtained from each participant. Fasting lipid profile (total cholesterol, High-Density Lipoprotein Cholesterol (HDL) and total triglycerides) were estimated using enzymatic kits with biochemistry autoanalyzer (ERBA XL640). Low-density Lipoprotein Cholesterol (LDL) was estimated using the Friedwald formula. Serum Malonaldehyde (MDA) was determined by Thiobarbituric Acid Reactive Substances (TBARS). Ethical approval was obtained from the Ethics Committee of the college. Informed consent was obtained from the participants. A physical examination of each subject was carried out. The height and weight of all individuals were measured. Body Mass Index (BMI) was calculated as kg/m^2. The diagnosis of PCOS was done according to the Rotterdam ESHRE revised consensus 2003. Women suffering from any known diseases like diabetes mellitus, thyroid disease, malignancy, hypertension, cardiovascular diseases, renal failure, Cushing's Syndrome, prolactinoma and history of taking any other medication such as lipid-lowering drug, oral contraceptives pills, ovulation induction, antiobesity drugs antidiabetic and antihypertensive drugs within 6 months were excluded. The control group was composed of female volunteers without the concomitant disease, aged 18-40 years, BMI < 25 and BMI >30, who had regular menstrual cycles and no signs of clinical and biochemical hyperandrogenism.

PON 1 was determined by using p-Nitrophenol Acetate as a substrate and the increase in absorbance at 412 nm due to the formation of p-nitrophenol was read. PON1 activity was assessed by the rate of enzymatic hydrolysis of 1.0 mmol/l paraoxon (O, O-diethyl-O-p-nitrophenyl phosphate; Sigma Chemical Co.) to *p*-nitrophenol in 1 mmol/l $CaCl_2$ and 2 mmol/l NaCl The amount of *p*-nitrophenol generated was monitored with a continuously recording spectrophotometer (UV-1601 Shimadzu) by the increase in absorbance at 412 nm and 25°C. The amount of *p*-nitrophenol generated was calculated from the molar absorbtivity at pH 8.0, which was 17 000/mol/cm. One unit of PON1 activity is defined as 1 nmol of 4-nitrophenol formed per minute, under the above assay condition in 0.1 mol/l Tris-HCl (pH 8.0) at a final concentration of 1.2 mmol/l.

Statistical analysis

The mean ± SE were then compared by one way ANOVA with Student Newman Keul's multiple comparison test. The p-value of <0.05 is considered statistically significant and <0.001 as highly significant.

Results

The levels of TGL in control subjects (Con-Normal, Con-Obese) and PCOS (PCOS-Normal, PCOS-Obese) and were 92.7 ± 36.7, 130.7 ± 36.7, 92.7 ± 36.7 and 167.1 ± 33.1 respectively **(TABLE 1)**. There was no difference in the TGL

levels of control-Normal V/S PCOS- normal, while a significant difference in control-obese v/s PCOS-Normal, control-obese V/S PCOS-Obese, control-obese v/s control-normal, PCOS-obese v/s PCOS-normal and Control-normal v/s PCOS-obese were observed **(FIG. 1 and 2)**.

TABLE 1. **Characteristics in obese and normal women with PCOS and controls.**

	Comparison of serum triglycerides (TG) and total cholesterol (TC), Low-density lipoproteins (LDL), High-Density Lipoproteins (HDL) and Very low-density lipoproteins (VLDL) in control (con) and PCOS women			
S. No.	**Parameter**	**Groups**	**Mean ± SE**	**Statistical analysis**
1	TGL	Con-Normal	92.74 ± 5.19	**FIG. 1**
		Con-Obese	130.70 ± 5.19	
		PCOS-Normal	92.72 ± 5.19	
		PCOS-Obese	167.12 ± 4.68	
2	TC	Con-Normal	113.46 ± 3.57	**FIG. 1**
		Con-Obese	176.96 ± 3.36	
		PCOS-Normal	182.48 ± 5.03	
		PCOS-Obese	177.46 ± 3.46	
3	LDL	Con-Normal	127.97 ± 5.40	**FIG. 2**
		Con-Obese	141.95 ± 4.6	
		PCOS Normal	57.37 ± 3.53	
		PCOS Obese	119.46 ± 3.79	
4	HDL	Con-Normal	28.88 ± 1.19	**FIG. 2**
		Con-Obese	20.84 ± 0.76	
		PCOS Normal	37.02 ± 1.06	
		PCOS Obese	31.92 ± 1.06	
5	VLDL	Con-Normal	26.23 ± 0.85	**FIG. 2**
		Con-Obese	33.26 ± 0.93	
		PCOS Normal	18.56 ± 1.04	
		PCOS Obese	26.05 ± 7.27	
Values are expressed as Mean ± SE, (n=50)				

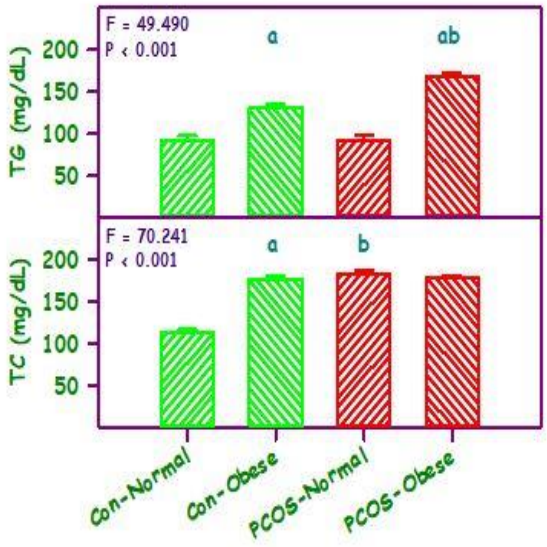

FIG. 1. **Comparison of Serum Triglycerides (TG) and Total Cholesterol (TC) in control (con) and PCOS women. Nonobese without PCOS (Con-Normal), Obese without PCOS (Con-Obese), Nonobese with PCOS (PCOS-normal) and Obese with PCOS (PCOS-Obese). The values are mean+SE (n=50 each) The 'F' and 'P' values are by one way ANOVA with Student Newman Keul's multiple comparison test. (a.) Significantly different from the respective normal and obese groups. (b.) Significantly different from the respective control and PCOS groups.**

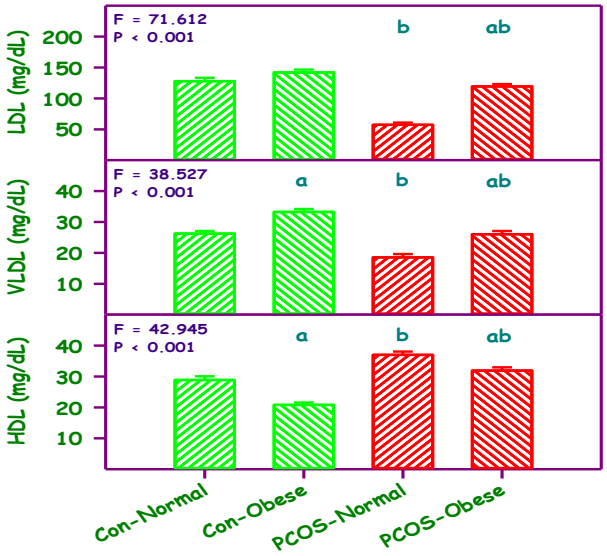

FIG. 2. **Comparison of Low-Density Lipoproteins (LDL), High-Density Lipoproteins (HDL) and Very Low-Density Lipoproteins (VLDL) in control (con) and PCOS women. Nonobese without PCOS (Con-Normal), Obese without PCOS (Con-Obese), Nonobese with PCOS (PCOS-Normal) and Obese with PCOS (PCOS-Obese). The values are mean+SE (n=50 each). The 'F' and 'P' values are by one way ANOVA with Student Newman Keul's multiple comparison test. (a.) Significantly different from the respective normal and obese groups. (b.) Significantly different from the respective control and PCOS groups.**

The levels of TC in control subjects (Con-Normal, Con-Obese) and PCOS (PCOS-Normal, PCOS-Obese) and were 113.4 ± 25.3, 176.9 ± 23.7, 182.4 ± 35.6 and 177.4 ± 24.4 respectively. There was no difference in the TC levels of PCOS-normal v/s control-obese, PCOS-normal v/s PCOS-obese, PCOS-obese V/S control-obese, while, a significant difference in PCOS-normal v/s control-normal, PCOS-Obese v/s control-normal, control-obese v/s control normal.

The levels of LDL in control subjects (Con-Normal, Con-Obese) and PCOS (PCOS-Normal, PCOS-Obese) and were 127.9 ± 38.2, 141.9 ± 33.1, 57.3 ± 24.9 and 119.4 ± 26.8 respectively. There was no difference in the LDL levels of Control-normal v/s PCOS-obese, while a significant difference in control-obese v/s PCOS-Normal, control- obese V/S PCOS-obese, control-obese v/s control-normal, control-normal V/S PCOS- normal, PCOS-obese v/s PCOS-normal were observed.

The levels of VLDL in control subjects (Con-Normal, Con-Obese) and PCOS (PCOS-Normal, PCOS-Obese) and were 26.2 ± 6.0, 33.2 ± 6.5, 18.5 ± 7.3 and 26.0 ± 7.2 respectively. There was no difference in the VLDL levels of Control-normal v/s PCOS-obese, while a significant difference in control-obese v/s PCOS-Normal, control-obese V/S PCOS-obese, control-obese v/s control-normal, control-Normal V/S PCOS-normal, PCOS-obese v/s PCOS-normal was observed.

The levels of HDL were in control subjects (Con-Normal, Con-Obese) and PCOS (PCOS-Normal, PCOS-Obese) and were 28.8 ± 8.4, 2.08 ± 5.4, 37.0 ± 7.5 and 31.9 ± 7.5 respectively. All the groups showed a significant difference.

The levels of MDA in control subjects (Con-Normal, Con-Obese) and PCOS (PCOS-Normal, PCOS-Obese) and were 1.9 ± 0.4, 3.9 ± 0.4, 5.5 ± 0.3 and 7.1 ± 0.5 respectively **(TABLE 2)**. All the groups showed a significant difference. The levels of Paraoxonase in control subjects (Con-Normal, Con-Obese) and PCOS (PCOS-Normal, PCOS-Obese) and were 201.4 ± 0.9, 189.1 ± 10.5, 172.0 ± 6.4 and 156.5 ± 9.6 respectively. All the groups showed a significant difference **(FIG. 3)**.

TABLE 2. **Comparison of Paraoxonase (PON 1) and MDA in control (con) and PCOS women.**

S. No.	Parameter	Groups	Mean ± SE	Statistical analysis
1	PON 1	Con-Normal	201.45 ± 0.13	**FIG. 3**
		Con-Obese	189.19 ± 1.49	
		PCOS-Normal	172.02 ± 0.90	
		PCOS-Obese	156.54 ± 1.35	
2	MDA	Con-Normal	1.92 ± 0.05	**FIG. 3**
		Con-Obese	3.96 ± 0.06	
		PCOS-Normal	5.56 ± 0.04	
		PCOS-Obese	7.14 ± 0.07	
Values are expressed as Mean ± SE, (n=50)				

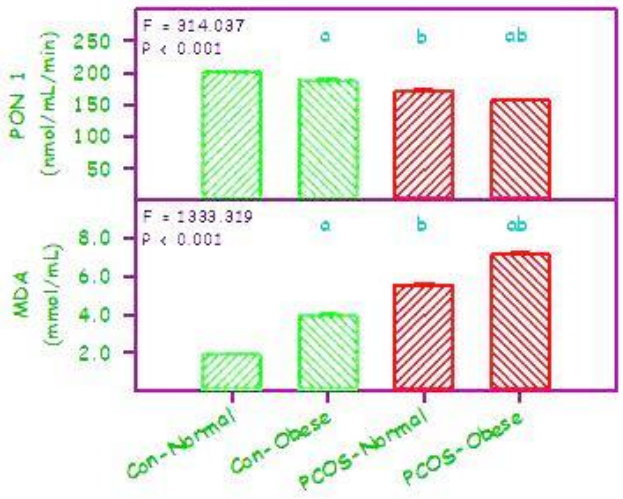

FIG. 3. **Comparison of MDA and Paraoxinase (PON 1) in Nonobese without PCOS (Con-Normal), Obese without PCOS (Con-Obese), Nonobese with PCOS (PCOS-Normal) and Obese with PCOS (PCOS-Obese). The values are mean+SE (n=50 each). The 'F' and 'P' values are by one way ANOVA with Student Newman Keul's multiple comparison test. (a.) Significantly different from the respective normal and obese groups. (b.) Significantly different from the respective control and PCOS groups.**

Discussion

Generally, dyslipidemia in women with PCOS is characterized by increased triglycerides and decreased HDL. In the present study for total cholesterol, there was no statistical significance except between PCOS normal and PCOS obese and control obese and PCOS obese. Total cholesterol was mainly increased in obese women irrespective of PCOS indicating obesity may be an important determinant of hypercholesterolemia rather than PCOS. In the present study LDL, VLDL and HDL showed statistical significance between all the groups. On the contrary, LDL and VLDL were reduced compared to controls and HDL was increased. Similar observations were made by Swetha et al. [6], who revealed that hypertriglyceridemia is relatively uncommon, and decreased LDL is not seen in all women with PCOS. However, contrasting observations were made by Desai et al., Zuo et al., Karabulut et al., Macut et al., Valkenburg et al., Kalra et al., and Unni et al. [7-13].

PCOS patients have demonstrated oxidative stress due to hyperglycemia, IR and low-grade chronic inflammation. Hyperglycemia produces Tumour Necrosis Factor-alpha (TNF α) from Multinuclear Cells (MNC). Women in reproductive age having hyperglycemia. Increased androgen generates ROS from leukocytes, p47phox gene expression, and formation of MDA. The presence of OS in the absence of obesity could be due to diet-induced OS and hyperandrogenism. OS increases low-grade chronic inflammation and vice versa [14].

Serum levels of MDA were significantly elevated in both obese and non-obese PCOS (P=<0.005). Similar observations were made by various other studies like Karabulut et al. [9], Mandal et al. [15], Zhang et al. [16], Palacio et al. [17], and Fenkci et al. [18].

MDA an oxidative marker correlates with the extent of lipid peroxidation. Free radicals in the body before achieving stability, collide with another molecule to either receive or donate an electron, in the process, they generate another free radical (ROS). These ROS target proteins, carbohydrates, nucleic acids and Polyunsaturated Fatty Acids (PUFA) present in the cell membrane known as lipid peroxidation forming various end products. Normally this process is opposed by antioxidant enzymes thus redox balance of the cell. In women with PCOS, there is an imbalance between oxidants and antioxidants leading to oxidative stress [19].

Serum levels of PON 1 an anti-inflammatory marker was significantly decreased in both obese and non-obese PCOS (P=<0.005). Similar observations were made by various other studies. Karabulut AB et al. [9], Mandal B et al. [15], Zhang D et al. [16], Palacio JR et al. [17], and Fenkci V et al. [18].

Oxidative stress and reduced serum paraoxonase lead to insulin resistance and type 2 diabetes mellitus [19]. LDL particles can be protected from free radical-induced oxidation by an HDL linked enzyme, paraoxonase 1 (PON1) [20]. PON1 can be used as a biomarker of diseases involving oxidative stress; inflammation and liver diseases. PON1 inhibits LDL oxidation and stimulates cholesterol efflux from macrophages and plays an important role in preventing oxidative stress and controlling inflammation. Hence the absolute or relative lack of PON1 activity contributes to PCOS. PON1 is inversely associated with atherosclerotic processes, in which ox-LDL plays a significant role [20].

Conclusion

In the present study, we found increased OS indicated by elevated levels of MDA and decreased antioxidant indicated by decreased levels of PON1 in women with polycystic ovary syndrome irrespective of BMI. Hence PCOS women should be evaluated for oxidant and antioxidant markers and supplemented accordingly thus reducing the overall morbidity and enhance the prognosis of PCOS.

Conflict of Interest

Nil

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