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ORIGINAL ARTICLE

Clinical Presentations of PCOS in Association with Insulin Resistance and Serum Esterase Enzymes: A Case-control Study

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ABSTRAK

Sindrom ovari polisistik (PCOS) ialah penyakit endokrin-metabolik yang mempengaruhi wanita dalam usia subur mereka. Walaupun prevalensinya tinggi (3-10%), etiologi yang tepat masih kurang difahami. Ketahanan insulin (IR) terlibat dalam patofisiologi PCOS, dan beberapa enzim ester seperti butirilkolinesterase (BuChE) dan paraoksonase 1 (PON1) telah ditemui terjejas dalam IR. Tujuan kajian ini adalah untuk mengkaji enzim ester serum dan hubungannya dengan IR dan manifestasi klinikal PCOS. Dalam kajian kes-kawalan ini, 56 wanita dengan PCOS dan 62 wanita tanpa PCOS telah direkrut. Parameter demografi dan klinikal termasuk status bekerja, perkahwinan, jumlah anak dan status haid, indeks jisim badan (BMI), IR, aktiviti BuChE dan PON1 telah diukur dan dibandingkan di antara dua kumpulan. Status perkahwinan dan jumlah anak bagi wanita PCOS adalah lebih rendah berbanding kumpulan kawalan. Berbanding dengan kawalan, wanita PCOS mempunyai ketidakteraturan haid, IR, dan aktiviti BuChE yang signifikan lebih tinggi serta aktiviti PON1 yang lebih rendah. Terdapat korelasi yang signifikan antara aktiviti BuChE dan BMI dalam wanita PCOS. Apabila dibandingkan dengan IR, aktiviti BuChE masih lebih tinggi dalam wanita PCOS berbanding dengan kawalan. Hasil ini menunjukkan bahawa enzim ester serum boleh mengalami perubahan pada PCOS dan IR mungkin terlibat dalam perubahan ini.

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Kata kunci: *Ketidakteraturan menstruasi; kolinesterase; paraoxonase 1; resistensi insulin; sindrom ovarium policistik*

ABSTRACT

Polycystic ovarian syndrome (PCOS) is an endocrine-metabolic disease affecting women in their childbearing age. Although, it has a high prevalence (3-10%), the exact etiology is poorly understood. Insulin resistance (IR) is involved in pathophysiology of PCOS, and some esterase enzymes such as butyrylcholinesterase (BuChE) and paraoxonase 1 (PON1) have been found to be affected in IR disorders. The aim of this study was to investigate serum esterase enzymes and their relationship with IR and clinical presentations PCOS. In this case-control study, 56 PCOS and 62 non-PCOS women were enrolled. Demographic and clinical parameters including working, marital, parity and menstrual status, body mass index (BMI), IR, BuChE and PON1 activity were measured and compared between the groups. The marital and parity status of PCOS women were lower than control group. In comparison with control, PCOS women had significantly higher menstrual irregularity, IR and BuChE activity as well as lower PON1 activity. There was a significant correlation between BuChE activity and BMI in PCOS women. When justified by IR, BuChE activity was still higher in PCOS women than control. The results indicate that serum esterase enzyme can be changed in PCOS and IR is most probably involved in these alterations.

Keywords: Cholinesterase, insulin resistance; menstrual irregularity; paraoxonase 1; polycystic ovarian syndrome

INTRODUCTION

In the recent years, the growing trend of metabolic and chronic disorders has become one of the most important global issues in healthcare systems. Chronic metabolic disorders include diabetes, metabolic syndrome, polycystic ovary syndrome (PCOS) and more. PCOS is a common endocrine-metabolic disorder affecting women in their reproductive age and its prevalence is estimated to be 3-10% worldwide. The pathophysiology of PCOS is not fully understood, but insulin resistance, which is commonly associated with type 2 diabetes and metabolic syndrome, is involved in PCOS. Epidemiological studies have reported that PCOS not only affects endocrine and reproductive functions in women, but also is associated with higher risk of metabolic disorders such as hypertension, cardiovascular complications, insulin resistance, diabetes and lipid profile disturbance. That's how PCOS can result in different clinical outcomes including reproductive, metabolic, and psychological problems as well

we several cancers. The signs of PCOS such as hirsutism, skin problems, obesity and ultimately infertility, have many negative effects on quality of life in women with this syndrome (Nisa et al. 2024).

The paraoxonase (PON) family of enzymes consists of three proteins, PON1, PON2 and PON3, each of which offers different functions such as protection against oxidative stress and purification of active molecules. PON1, which has been studied more, is synthesised in the liver and enters the circulation where it binds to HDL cholesterol. Serum PON1 prevents the oxidative changes of LDL cholesterol and is believed to be responsible for the antioxidative role of HDL cholesterol. PON1 is also involved in the clearance of foreign toxins such as organophosphorus compounds. Studies have shown that lower enzymatic activity of PON1 is associated with higher risk of insulin resistance and atherosclerotic heart disorders in women with PCOS. It should be mentioned that reduced PON1 activity has been shown to increase the risk of diseases associated with oxidative stress (Meneses et al. 2019; Mostafalou & Abdollahi 2023).

Cholinesterase is an esterase enzyme that hydrolyses choline esters particularly acetylcholine, a well-known neurotransmitter in the nervous system. Inhibition of cholinesterase enzymes is the main pharmacologic mechanism of medicines used in treatment of Alzheimer's disease and myasthenia gravis. Organophosphorus compounds, which are a common class of insecticides in use, act by inhibiting this enzyme. There are two types of cholinesterase enzyme, including acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). BuChE, or pseudocholinesterase, is mainly synthesised in the liver and released into the blood. Although the physiological role of BuChE is not yet fully understood, it is associated with lipid metabolism, serum lipid profile regulation, obesity, and other metabolic disorders (Mostafalou & Abdollahi 2018; Xing et al. 2021).

The aim of this study was to evaluate and compare demographic characteristic (age, weight, height, BMI, working, marital, and parity status), clinical presentations (menstrual regularity, fasting blood glucose, fasting blood insulin and insulin resistance), and enzymatic activity of BuChE and PON1 in PCOS and non-PCOS women.

MATERIALS AND METHODS

Study Design

This case-control study was conducted in Ardabil University of Medical Sciences, Ardabil, Iran. Voluntary participants were registered to participate in the study in two gynecological clinics and one skin and hair clinic from 2019 to 2020. Participants were divided into case group (PCOS patients) and control group (women without PCOS). In this study, the Rotterdam 2003 criteria was used to diagnose PCOS in women (Rotterdam criteria 2004). PCOS was confirmed or rejected through clinical examinations and diagnostic

ultrasound. After biochemical experiments and enzymatic assessments, data were extracted and the variables were compared between the case and control groups.

Sample Size

Based on the statistical formula of sample size calculation at the 95% confidence level with a test error of 0.065% for differences between PCOS prevalence rates reported in various studies ranging from 3% to 10%, the sample size for this study was established as 118 which finally 56 and 62 were determined as the number of samples for cases and controls, respectively.

Study Population

Women in our population ranged in age from 12 to 45 years. Considering the interference of pathophysiological conditions with biochemical and enzymatic parameters objected in this study, it was determined that the participants were free of underlying diseases such as cancer, functional cognitive disorders such as Alzheimer's disease and Parkinson's disease, and not to be pregnant. Participants were checked to ensure they were not taking cholinesterase inhibitors and the medicines that affect PCOS, such as oral contraceptives. Participants were also screened to ensure they had not been exposed to any kind of pesticides in their environment and their spouse's job were confirmed to be not involvd to exposure to pesticides.

Sample Collection

After performing clinical and ultrasound examinations, blood sample (up to 10 cc) was taken from all participants while fasted. The serum was separated from the samples and was used to assess the concentration of glucose and insulin. The remaining sera were stored at -70°C in order to assess enzymatic activity of PON1 and BuChE after collecting all the samples.

Measuring blood glucose concentration

Glucose concentration was measured according to the enzymatic method of glucose oxidase. In this method, glucose is oxidised to gluconic acid under the effect of the enzyme glucose oxidase. As a by-product, hydrogen peroxide is produced during this reaction and can be used to estimate the amount of glucose. Under the effect of peroxidase and in the presence of phenol and 4-aminoantipyrine, hydrogen peroxide produces quinoneimine which can be detected in 505 nm (McMillin 1990). The colorimetric enzymatic assay kit was purchased from the DELTA DARMAN PART CO. (P1019) for single point photometric determination of glucose in the serum samples.

Measuring Blood Insulin Concentration

Insulin concentration was measured based on the method of electrochemical luminescence (ECL) which is very sensitive and accurate. The reaction is initiated by an electric current that

eventually leads to production of light from the ruthenium tris complex. At this stage, electrical voltage is applied to the ruthenium tris immunological complex, which is attached to the streptavidin coated on the surface of the microparticles. The basis of ECL is measurable in the form of light (Bard et al. 2001). The ARCHITECT insulin assay kit was prepared from the Abbott Laboratories (8K41-27) for chemiluminescent determination of insulin in the serum samples.

Measuring Arylesterase Activity of PON1

Arylesterase activity of PON1 was measured according to the Beltowski method based on the initial rate of hydrolysis of phenyl acetate as the substrate in the reaction mixture. The assay mixture composed of phenyl acetate (2 mM), CaCl2 (2 mM) and 10 µl of serum in Tris/HCl (100 mM, pH 8.0). Blank was also prepared using the same ingredients except serum. The absorbance was monitored for 3 minutes at 270 nm. The activity of PON1 was calculated using the below mentioned formula where = 1310 M^{-1} cm^{-1} is the molar absorption coefficient. The results were expressed in U/ml when 1 U of arylesterase hydrolyses 1 mol of phenyl acetate per minute (Mollazadeh et al. 2017).

Measuring BuChE Activity

BuChE activity was measured according to the method developed by Ellman et al. in 1961. Acetylthiocholine, was used as the substrate and the rate of appearance of the enzyme product (thiocholine) was measured by Ellman's reagent or 5,5'-dithiobis-2-nitrobenzoate (DTNB). Liberated thiocholine reduces DTNB into a yellow anion named as 5-thionitrobenzoic acid which can be detected at 412 nm. Briefly, an incubation solution containing DTNB (0.25 mmol/L) in phosphate buffer (75 mmol/L, pH 7.9) was first prepared. 3 ml of incubation solution was mixed with 10 µl of previously melted serum and the tubes were placed at 37°C to establish a temperature equilibrium. Then 10 µl acetyl thiocoline iodide (3 mmol/L) was added to the test tubes while the blank tube received 10 µl of distilled water instead. Finally, the absorbance was read by spectrophotometer at 412 nm (Mostafalou et al. 2012).

Statistical Analysis

SPSS 23.0 was used for statistical analysis. The p-value < 0.05 was considered statistically significant. Data were analysed using statistical methods including chi-square, independent t-test and Pearson correlation in the form of tables.

Ethical Considerations

The study was conducted after approval by the ethics committee and receiving the ethics approval codes. Written consent was signed by all the participants prior to enrolling them in the study and identity of patients was not mentioned anywhere. In this study, no cost was imposed on the patient and all costs were borne by the

counsel.

RESULTS

Demographic characteristics of the study population had been brought in the Table 1. As shown, there was no statistically significant difference in working status between PCOS patients and controls. Among POS patients, 38 (67.9%) were single, whereas among controls 49 (79%) were married so that there was a statistically significant difference in marital status between PCOS patients and controls (p=0.001). 42(75%) of PCOS and 21 (33.9%) of control groups had no parity and a statistically significant difference was found in parity status between PCOS and control groups (p=0.001). In case of BMI, there was no statistically significant difference between PCOS and controls. Instead, PCOS patients had significantly higher menstrual irregularity (p=0.001) and higher insulin resistance index (HOMA-IR) (0.042) compared to the control group (Table 1).

As shown in the Table 2, the age of women in this study ranged from 12 to 45 years. The mean±SD of age for PCOS patients was 22.57±6.77 years,

Table 1: Demographic and clinical characteristics of PCOS women vs. controls

	PCOS ≠ N (%)	Control ≠ N (%)	x^2	P-value
Working status				
Housewife	40(71.4)	35(56.5)	3.65	0.561
Working	6(10.7)	15(24.2)		
Without job	10(17.9)	12(19.3)		
Marital status				
Single	38(67.9)	13(21.0)	26.4	0.001*
Married	18(32.1)	49(79.0)		
Parity status				
0	42(75.0)	21(33.9)		
1	6(10.7)	6(9.7)	25.3	0.001*
2	7(12.5)	20(32.3)		
>3	1(1.8)	15(24.2)		
BMI status				
Underweight	0(0)	5(8.1)		
Normal	31(55.4)	24(38.7)	6.7	0.342
Overweight	16(28.6)	21(33.9)		
Obese	9(16.1)	12(19.4)		
Menstruation status				
Regular	16(28.6)	51(82.3)	34.5	0.001*
Irregular	40(71.4)	11(17.7)		
HOMA-IR				
Normal (<19.5)	12(21.4)	24(38.7)	4.2	0.042*
Insulin resistant (>19.5)	44(78.6)	38(61.3)		
Total	56(100)	62(100)		

BMI: Body Mass Index; HOMA-IR: Homeostatic Model Assessment-Insulin Resistance; N: number; (%): percent; PCOS: polycystic ovarian syndrome.
* significantly different at p-value less than 0.05.

Table 2: Anthropometric characteristics, laboratory findings, BuChE and PON1 activity of PCOS women vs. controls

Variables	PCOS	Control	t	P-value
Age of menarche (years)	13.52 ± 1.36	13.27 ± 1.18	1.04	0.289
Age (years)	22.57 ± 6.77	32.3 ± 7.7	7.2	0.001*
Weight (kg)	67 ± 14.5	67.3 ± 16.1	0.1	0.879
Height (cm)	162.5 ± 7	160.8 ± 7.4	1.3	0.189
FBS (mg/dl)	86.8 ± 0.8	86.2 ± 0.9	0.47	0.641
FINS (µU/ml)	13.9 ± 0.8	11.4 ± 0.7	2.39	0.018*
HOMA-IR	3 ± 0.2	2.5 ± 0.2	2.15	0.034*
BuChE (KU/L)	0.8 ± 0.02	0.75 ± 0.01	2.9	0.004*
PON1 (KU/L)	228.2 ± 16	306.7 ± 22.3	2.8	0.006*

Results were presented as mean ± standard deviation of mean (SD) for age of menarche, age, weight and height and mean ± standard error of mean (SE) for FBS, FINS, HOMA-IR, BuChE and PON1. FBS: Fasting Blood Sugar; FINS: Fasting insulin; HOMA-IR: Homeostatic Model Assessment-Insulin Resistance; BuChE: Butyrylcholinesterase; PON1: Paraoxonase 1; PCOS: polycystic ovarian syndrome.
* significantly different at p-value less than 0.05.

compared to 32.3±7.7 years for controls and this difference was statistically significant (p=0.001). PCOS patients had significant higher fasting insulin level (p=0.018), HOMA-IR (0.034), BuChE activity (P=0.004) but lower PON1 activity (P=0.006) compared to controls (Table 2).

Table 3 presented laboratory and enzymatic findings of women with regular menstruation vs. women with irregular menstruation. Among women with regular menstruation, fasting blood insulin (p=0.025) and HOMA-IR (p=0.045) were significantly higher in the PCOS patients. While among women with irregular menstruation, PON1 activity was found to be significantly lower in the PCOS patients (p=0.004) (Table 3).

Table 3 also showed the comparison of the laboratory and enzymatic results between women with and without insulin resistance. Among insulin resistant women, BuChE activity was significantly higher in PCOS patients (p=0.012) (Table 3).

Correlations of all the quantitative variables including age, BMI, menarche age, FBS, insulin, HOMA-IR, BuChE, and PON1 with each other in PCOS women and controls were also evaluated and were shown in the Table 4. As shown, in the PCOS patients, BuChE activity was significantly correlated with BMI. In addition, PON1 activity was significantly correlated with age (Table 4).

DISCUSSION

This case-control study evaluated demographic characteristic, clinical signs and biochemical parameters such as insulin resistance and serum esterase enzymes in patients with PCOS in comparison with controls. In the present study, majority of study population were housewife and there was no statistically significant

Table 3: Laboratory findings, BuChE and PON1 activity of women based on menstrual irregularities and insulin resistance

Menstrual irregularities				
	Women with regular menstruation			
	PCOS (n=16)	**Control (n=51)**	**t**	**P-value**
FBS (mg/dl)	86 ± 1.8	86 ± 0.9	0.001	0.998
FINS (µU/ml)	14.2 ± 1.6	10.8 ± 0.7	2.3	0.025*
HOMA-IR	3.2 ± 0.4	2.3 ± 0.1	2.04	0.045*
BuChE activity (KU/L)	0.8 ± 0.1	0.7 ± 0.1	1.5	0.128
PON1 (KU/L)	296.2 ± 29.8	297.9 ± 23	0.038	0.962
	Women with irregular menstruation			
	PCOS (n=40)	**Control (n=11)**	**t**	**P-value**
FBS (mg/dl)	87.1 ± 0.9	87.1 ± 3.4	0.02	0.976
FINS (µU/ml)	13.8 ± 0.9	14.2 ± 2.1	0.18	0.854
HOMA-IR	3 ± 0.2	3.2 ± 0.6	0.32	0.738
BuChE activity (KU/L)	0.8 ± 0.2	0.76 ± 0.1	1.28	0.198
PON1 (KU/L)	201 ± 17.4	347.4 ± 68.5	3.03	0.004*
Insulin Resistance				
	Women who are insulin resistant			
	PCOS (n=44)	**Control (n=38)**	**t**	**P-value**
FBS (mg/dl)	87.6 ± 0.9	88.5 ± 1.2	0.6	0.489
FINS (µU/ml)	15.8 ± 0.8	14.1 ± 0.8	1.4	0.122
BuChE activity (KU/L)	0.84 ± 0.02	0.75 ± 0.03	2.6	0.012*
PON1 (KU/L)	224.5 ± 16.4	287.5 ± 29.4	1.9	0.056
	Women who are not insulin resistant			
	PCOS (n=12)	**Control (n=24)**	**t**	**P-value**
FBS (mg/dl)	84 ± 4.4	82.5 ± 5.7	0.7	0.336
FINS (µU/ml)	7.2 ± 1.3	7.2 ± 1.3	0.09	0.879
BuChE activity (KU/L)	0.78 ± 0.12	0.75 ± 0.1	0.87	0.379
PON1 (KU/L)	242 ± 45.7	337.08 ± 33.8	1.6	0.097

Results are presented as mean ± standard error of mean (SE). FBS: Fasting Blood Sugar; FINS: Fasting insulin; HOMA-IR: Homeostatic Model Assessment-Insulin Resistance; BuChE: Butyrylcholinesterase; PON1: Paraoxonase 1; PCOS: polycystic ovarian syndrome
* significantly different at p-value less than 0.05.

difference in working status between PCOS patients and control.

In term of marital status, most of the PCOS patients in our study were single while the controls were mostly married and the difference between two groups was statistically significant. As mentioned earlier, dermatological manifestations and mood variations followed by low self-esteem and decreased quality of life may be involved in this observation. But considering the role of age in marital status and a significant difference found between the mean age of two groups in our study, further confirmatory studies are needed to determine the relation between PCOS and marital status. The present results also showed a statistically significant difference in

Table 4: Correlation between quantitative variables in PCOS and control

Variables	Age	BMI	Menarche age	FBS	FINS	HOMA-IR	BuChE	PON1
PCOS								
Age		r=0.23	r=0.17	r=0.052	r=-0.23	r=-0.22	r=-0.2	r=0.33*
BMI	r=0.23		r=0.01	r=0.12	r=0.39*	r=0.37*	r=-0.3*	r=0.17
Menarche age	r=0.17	r=0.9		r=-0.007	r=0.1	r=0.09	r=0.02	r=0.09
FBS	r=0.05	r=0.12	r=0.007		r=0.39*	r=0.46*	r=0.23	r=0.09
FINS	r=0.23	r=0.39*	r=0.1	r=0.39		r=0.96*	r=0.06	r=0.06
HOMA-IR	r=0.22	r=0.37*	r=0.09	r=0.46*	r=0.96*		r=0.08	r=0.08
BuChE	r=0.19	r=-0.3*	r=0.02	r=0.23	r=0.06	r=0.09		r=0.07
PON1	r=0.33*	r=0.17	r=0.08	r=0.09	r=0.06	r=0.07	r=0.07	
Controls								
Age		r=0.53*	r=0.15	r=0.23	r=-0.21	r=-0.24	r=-0.05	r=0.14
BMI	r=0.53*		r=0.06	r=0.16	r=0.42*	r=0.41*	r=-0.05	r=0.12
Menarche age	r=0.15	r=0.06		r=0.12	r=0.03	r=0.05	r=0.15	r=0.08
FBS	r=0.23	r=0.17	r=0.12		r=0.43*	r=0.56*	r=0.12	r=0.16
FINS	r=0.22	r=0.42*	r=0.3	r=0.43*		r=0.98*	r=0.15	r=0.09
HOMA-IR	r=0.24	r=0.41*	r=0.05	r=0.57*	r=0.98*		r=0.17	r=0.1
BuChE	r=0.05	r=0.05	r=0.15	r=0.13	r=0.15	r=0.17		r=0.06
PON1	r=0.14	r=0.12	r=0.08	r=0.16	r=0.09	r=0.1	r=0.06	

BMI: body mass index. FBS: Fasting Blood Sugar; FINS: Fasting insulin; HOMA-IR: Homeostatic Model Assessment-Insulin Resistance; BuChE: Butyrylcholinesterase; PON1: Paraoxonase 1, PCOS: polycystic ovarian syndrome.
*Correlation is significant between two variables

parity status of PCOS patients with that of controls. Anovulatory, infertility and pregnancy complications are known as the prevalent consequences of PCOS. However, considering unmatched marital status of our groups and principal influence of marital status on parity, this result of our study needed more evidences for confirmation.

Another finding of our study was the significant difference in menstrual regularity between two groups. Menstrual irregularity is one of the main symptoms of PCOS, and our results indicated that PCOS patients had significantly higher menstrual irregularity compared to controls. In term of BMI, our results indicated that the percent of overweight and obese people in the PCOS group was more than control group, but this difference was not statistically significant. As reported by previous studies, PCOS was common among overweight and obese women, but there may be some PCOS patients with normal BMI (Barber et al. 2019). Another explanation for this observation can be the high prevalence of obesity in communities and as shown in our results, 45% of the people in the control group were overweight or obese.

As expected, fasting blood insulin level was significantly higher in PCOS patients than controls in our study. Hyperinsulinemia is a common feature of insulin resistance and studies have shown that women with PCOS mostly have insulin resistance, so that insulin resistance and consequent hyperinsulinemia have been suggested as pathogenic mechanisms of PCOS (Zhao et al. 2023).

The present results about blood esterase enzymes indicated that BuChE activity was significantly higher in PCOS patients than controls. This result confirmed the hypothesis about the role of BuChE activity in the pathogenesis of PCOS.

We also compared these parameters in subgroups of patients based on menstrual regularity and insulin resistance. We compared fasting insulin level, insulin resistance index and BuChE activity between PCOS and control in the women with regular menstruation, and found that fasting insulin level and insulin resistance were higher in PCOS patients while the BuChE activity had no difference in these two subgroups. Similarly, BuChE activity had no difference between PCOS and control among women with irregular menstruation. So, this result indicated that menstrual status had no principal role in the association of BuChE activity with PCOS. On the other hand, among insulin resistant women, BuChE activity was significantly higher in PCOS patients than control. However, in women without insulin resistance, BuChE activity was similar between PCOS patients and controls. Such findings confirmed the effect of insulin resistance on the link between higher BuChE activity and PCOS pathogenesis. Data published by Iwasaki and colleagues (2007) have also confirmed the correlation between insulin resistance and BuChE activity in the Japanese patients with type 2 diabetes and non-diabetic subjects.

Another important finding of this study was the significant difference of PON1 activity between the PCOS and control groups. Serum PON1 activity was significantly lower in the PCOS group than control. In a prospective case-control study conducted on 30 women with PCOS and 20 controls, oxidative stress markers and some antioxidants such as PON1 activity were measured and the results showed increased oxidative stress markers and decreased PON1 activity in PCOS patients. These evidences confirm that patients with PCOS are exposed to oxidative stress which may be involved in development of atherosclerosis in these patients (Perovic Blagojevic et al. 2022).

In the present study, the activity of PON1 in the patients with irregular menstruation was much lower than that in the patients with regular menstruation. Moreover, in the group with irregular menstruation there was a significant difference between the activity of PON1 among PCOS and control groups. These observations indicated that PON1 activity may be affected by irregular menstruation due to ovarian dysfunction in PCOS patients. The results of another case-control study conducted on women in Oman showed that biomarkers of oxidative stress were associated with

PCOS (Sulaiman et al. 2018). Our results also confirmed this association depending on irregular menstruation. This means that irregular menstruation in PCOS may be related to low level of PON1 activity and increased oxidative stress. The results of another case-control study indicated that in women with PCOS and free of metabolic syndrome and insulin resistance, the activity of PON1 was not changed while the other parameters of oxidative stress were increased (Torun et al. 2011). However, this evidence is in parallel with our findings.

Evaluating the link of PON1 and BuChE was another objective of this study. In Alzheimer disease, reduced activity of PON1 has been evidenced and its most common polymorphism at position 192 Q/R has also been shown to affect the therapeutic response to cholinesterase inhibitors. PON1 has been shown to be a potent inhibitor of endogenous cholinesterase, as well (Pola et al. 2005; Zuin et al. 2023). Based on these evidences, the association of PON1 with BuChE was expected but correlational assessment between quantitative variables in our study indicated that there was no significant relationship between the activity of PON1 and BuChE in women with PCOS. In fact, PON1 gene has various polymorphisms which may influence its association with BuChE. It has also been shown that PON1 activity can be modulated under different patterns of nutritional status so that diets having more fruits and vegetables and less fat and sucrose can increase the activity of PON1 (Meneses et al. 2019). Considering the point that our samples were not matched in terms of polymorphisms and nutritional factors, detailed interpretation on this issue cannot be done.

According to these observations, disturbance of homocysteine metabolism can be suggested in PCOS. Homocysteine is known as a risk factor for cardiovascular diseases and previous studies have shown that its serum level is increased in PCOS patient (Saadeh et al. 2018). In addition, the relation of increased serum homocysteine level with hyperinsulinemia and insulin resistance in PCOS patients has already been documented (Diwaker & Kishore 2018). One of the metabolites of homocysteine, homocysteine-thiolactone, is hydrolysed by the enzyme PON1. In fact, thiolactonase activity of PON1 can prevent N-homocysteinylation and subsequent disruptive effects in the structure and function of cellular proteins (Jakubowski 2023). On the other hand, homocysteine has been shown to modulate the activity of AChE and BuChE in different parts of the brain and peripheral tissues. These effects have been attributed to the homocysteine-thiolactone which can further produce in pathologic conditions such as oxidative stress, hyperlipidemia, hyperglycemia and insulin resistance (Scherer et al. 2014).

CONCLUSION

Present data was able to confirm that PCOS is associated with insulin resistance, higher BuChE and lower PON1. Association of PON1 enzyme with insulin resistance independent

of PCOS was also elicited. However, determining the association between BuChE and PON1 enzymes in PCOS requires further studies. However, it can be concluded that insulin resistance most probably is involved in the alterations of PON1 and BuChE in PCOS. In this way, it is recommended to search on new therapeutic strategies for PCOS among factors related to the activity of BuChE, PON1, and oxidative stress. However, small size of the study population and inability to eliminate all the interfering factors involved in the activity of BuChE and PON1 enzymes, were the limitation of our study. It is suggested that in the future studies, larger sample size and evaluating the profiles of lipids and androgens to be considered.

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