Genetic Variations of (SOD2; Rs5746136) and (PON2; Rs705379) are Associated with the Risk of PCOS and Influence Metabolic Profile Among the Iranian Population

Rawaa Allawi Kadhim[1], Gholamreza Dehgan[1],*, Mohammad Khalaj-Kondori[1], Kobra Hamdi[2]

[1]*Department of Biology, Faculty of Natural Sciences, University of Tabriz, 51666−16471, Tabriz, Iran*
[2]*Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

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ABSTRACT

Polycystic ovarian syndrome (PCOS) is one of the most prevalent disorders affecting women's metabolic, psychological, and reproductive features. This study investigated the roles of superoxide dismutase 2 (SOD2; rs5746136) and paraoxonase 2 (PON2; rs705379) gene polymorphisms on the risk of PCOS and oxidative stress and clinical indices in Iranian women. This cross-sectional study included 56 patients (female) with PCOS and 54 healthy women (control group), and single nucleotide polymorphisms (SNPs) of rs5746136 and rs705379 were genotyped. The results showed that there were no detectable variations in frequency between PCOS and control groups in SOD2 genotypes and alleles ($p>0.05$). Compared with controls, the frequency of rs705379-CC genotype was higher in PCOS individuals [adjusted OR (95%CI) = 2.171 (1.098-4.313), P=0.016], which showed a significant association. While the frequency of the TT genotype was higher in the reference group (OR= 0.464 (0.236-0.97), P=0.015). Patients with the A and C alleles exhibited considerably greater serum levels of luteinizing hormone (LH) and LH/FSH (follicle-stimulating hormone) than the control group. Our results revealed that the PON1 gene (C>T) polymorphism is related to the risk of PCOS. However, the SOD2 gene (G>A) polymorphism in our study population did not show any relation to PCOS. Importantly, oxidative stress parameters showed a significant relationship with PON1 rs705379-genotype.

1. Introduction

Polycystic ovary syndrome (PCOS) is the leading cause of ovarian infertility among women of reproductive age. It is one of the most prevalent multifactorial reproductive endocrine metabolic illnesses. The rate of this syndrome is estimated to be 5-15% of reproductive-aged women around the world [1, 2]. PCOS development is influenced by a variety of factors, including genetic, epigenetic, environmental, follicular, and lifestyle factors. However, the exact cause of PCOS is unknown. The clinical consequences of PCOS include anovulation, obesity, hyperandrogenism (hirsutism, acne), ovulatory dysfunction (oligomenorrhea, amenorrhea), polycystic ovarian morphology (PCOM), insulin resistance, the risks of type 2 diabetes (T2DM), chronic inflammation, dyslipidemia, cardiovascular disease, oxidative stress (OS), and most importantly, endometrial cancer [3-5]. Some studies have also shown that hormonal imbalances of LH and FSH can be important in the etiology of PCOS. In normal women, the FSH hormone can induce the growth of ovarian follicles. It stimulates the development and maturation of follicles in the ovaries. FSH helps the follicles grow and produce estrogen. LH works in synergy with FSH to stimulate follicular growth and ovulation. LH stimulates the production of estradiol, a form of estrogen, by the growing follicles [6, 7]. When FSH levels are lower due to an elevated LH/FSH ratio, it can impair follicle maturation. This can lead to the development of immature follicles that are unable to release a mature egg during ovulation [8].

Oxidative stress (OS) is the overproduction of reactive oxygen species (ROS) and an imbalance between oxidative markers and the antioxidant defense system. ROS include superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2) [9]. OS is associated with diabetes mellitus, aging, various cancers, neurodegenerative diseases, e.g. Parkinson's and Alzheimer's, cardiovascular diseases, and chronic kidney disease. It has been demonstrated that OS plays a main role in the pathogenesis of PCOS [10, 11]. Some studies have revealed that ROS production and OS in PCOS patients is most dominant compared to healthy women. OS can lead to cell damage and changes in steroidogenesis in the ovaries, which subsequently increase the level of androgens to help with follicular growth disorders and infertility [12, 13].

Paraoxonase 1 (PON1) and superoxide dismutase 2 (SOD2) in humans are the two most significant intracellular antioxidant defense systems. SOD2 with a tetrameric structure is present in the mitochondrial matrix, as the primary site of ROS generation. SOD2, also called manganese SOD (MnSOD), is an isozyme of SOD located in mitochondria and catalyzes the transformation of $O_2^{\bullet-}$ to O_2 and H_2O_2. Catalase and glutathione peroxidase then destroy H_2O_2 [14-16]. Several genetic variants have been identified in the SOD2 gene. One of the common polymorphisms of the SOD2 gene is rs5746136 (A/G substitution) which is located <1 kb upstream from SP1 and NF-κB transcription element sequences (located in the 5th intron near 3′-UTR at 65 base pairs downstream of the poly-A site, and <1 kb upstream from SP1 and the NF-kB transcription element sequences) [16]. The polymorphism in this region may change the expression level of SOD or may cause the loss or reduction in the function of the expressed enzyme. Furthermore, a study showed that antioxidant supplementation has a good and significant impact on pregnancy rates in PCOS individuals. Therefore, it can be considered that ROS is a potential inducer of PCOS pathogenesis [17].

The PON1 gene, located in the chromosomal region 7q213, has 9 exons and comprises 26 kb. Polymorphism in promoter region PON1-108 (c.–108C>T; C: cytosine, T: thymine) has a significant impact on PON1 expression and activity [18]. Human PON1 is synthesized mainly in the liver [18], and its activity has been reported to be lower in diseases such as T2DM that are linked to insulin resistance [19], coronary atherosclerotic disease [20], and PCOS [21-23]. In this case-control study, we studied the frequencies and the association of polymorphisms of SOD2 (rs5746136 G>A) and PON1 (rs705379 C>T) genes with risk of PCOS and investigated the impacts of the genotypes on hormonal, clinical, and OS indexes in Iranian women.

2. Materials and methods

2.1. Subjects

In this case-control study, 56 women with PCOS with an average age of 28.48±5.84 years and 54 healthy volunteer women without PCOS (with normal menstrual cycles and normal circulating androgen levels) with an average age of 30.46±6.73 years participated. Patients with PCOS were recruited from the Al-Zahraa Hospital in Tabriz, Iran. The Rotterdam criterion was used to diagnose PCOS. Hyperandrogenism, oligomenorrhea, amenorrhea, hirsutism, and acne are some of these requirements. The serum samples were taken from healthy and PCOS patients for our research purpose, and the ethical code issued by the ethics committee of Tabriz University (IR.TABRIZU.REC.1403.017) was used for samples in the present study. Acute or chronic conditions such as autoimmune/inflammatory diseases, cardiovascular disease, infections, tumors, thyroid dysfunction, hyperprolactinemia, endometriosis, hypogonadotropic, hypogonadism, premature ovarian insufficiency luteal phase, and smoking were not present in any of the participants. The degree of hirsutism (Ferriman-Gallwey score) was between 8 and 15 and in some people it was higher. All participants were from North Western Iran. PCOs were diagnosed and confirmed by the existence of 12 or more follicles with 2–9 mm in diameter and/or increased ovarian volume (> 10 mL). All individuals had clinical characteristics measured, such as body mass index (BMI, kg/m^2), height (cm), age (years), and the severity of acne and hirsutism. After an overnight fast, blood samples were obtained in the morning. Plasma or serum and blood cells were prepared by centrifugating at 1500 g for 15 min at 4 °C. The aliquots of the prepared samples were stored at −80 °C for further investigations and DNA extraction.

2.3. Genomic DNA extraction and genotype analyses

Using the Pure Gene purification kit (Qiagen, Hilden, Germany), DNA was isolated from peripheral blood leukocytes, by the manufacturer's protocol. The purity and quantity of the extracted DNA were evaluated using NanoDrop ND-2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA) by measuring the ratio of absorbance at 260 nm and 280 nm (260/280) and then, stored at −20 °C until analysis.

Genotype analyses of the subjects for the polymorphisms of *SOD2* (rs5746136 G>A) and *PON1* (rs705379 C>T) were determined by using PCR-RFLP. The following primers were used to amplify a 513-bp fragment for the SOD2 genotype [24]: forward, 5′-GCATTTCTTGATGTTGCTTAGTCAC-3′ and reverse 5′-GCTGTTGAAGTTTGCCTTTACTGTG-3′. On the other hand, for the rs705379 C>T genotype, a 240-bp fragment was amplified with the following primers: forward, 5′-AGCTAGCTGCCGACCCGGCGGGGAGGAG-3′ and reverse 5′-GGCTGCAGCCCTCACCACAACCC-3′. The digestion of rs5746136 G>A and rs705379 C>T products was performed using Taq I (Thermo Fisher Scientific, Inc.) or Bsrb I (New England BioLabs, Inc.), respectively. The *G* allele's 346-bp and 167-bp fragments and the *A* allele's non-digested 513-bp fragment were produced by the digestion of SOD2 gene products. Digestion of *PON1* gene products resulted in 212-bp and 28-bp fragments for the *C* allele and a non-digested 240-bp fragment for the T allele. A gel document was used to visualize the products after they underwent electrophoresis analysis on a 2-4% agarose gel.

2.4. Analysis of hormonal, metabolic, and oxidative stress markers

Plasma glucose concentration and lipid profile (including cholesterol (Chol), triglyceride, HDL, and LDL) were estimated using enzymatic colorimetric methods (Bionik kit, Iran) with a biochemistry autoanalyzer. Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH) (DiaZist kit, Iran), testosterone and free testosterone (Monobind, Inc. kit, USA), and $DHEA_{SO4}$ (Calbiotech. Inc. kit, USA) were measured using the ELISA technique. Total antioxidant capacity (TAC), malondialdehyde (MDA) levels, PON1, and SOD2 enzyme activity were measured using a colorimetric method (Randox Kit, United Kingdom).

2.5. Statistical analysis

The statistical analysis was performed using GraphPad Prism version 9.2 (GraphPad Software Inc., LaJolla, CA). The odds ratio (OR) and 95% confidence interval (CI) were estimated using logistic regression analysis. In order to test deviations from the Hardy–Weinberg equilibrium in the genotype distribution and to evaluate the frequencies of SOD2 and PON1 gene polymorphisms between patients and controls, chi-square (χ^2) analysis was utilized. Hardy-Weinberg equilibrium analysis of alleles was carried out using the Fisher test. Student's t-test were used to determine whether group variance was significant or not. The Pearson coefficient r value was employed to assess correlation. Quantitative parametric data were subjected to the Shapiro-Wilk test to confirm the normal distribution and were expressed as mean ± standard deviation (SD) and statistical differences were defined as * $p < 0.05$ and ** $p < 0.01$.

3. Results

3.1 Demographic characteristics of patients and controls

Data were obtained from 56 and 54 women with and without PCOS. Table 1 shows the demographic characteristics of the PCOS patients and control subjects. As depicted in this table, the age, height, weight, and BMI of the PCOS and healthy groups did not differ significantly.

Table 1: Clinical and hormonal parameters in PCOS patients and control women.

Parameter	Mean Value ± SD		Sig.	*P* value
	Control	Patients		
Age (years)	30.35±6.7	28.14±5.8	NS	0.0701
BMI (kg/m^2)	25.24±4.71	26.47±4.1	NS	0.1535

BS (mg/dl)	89.87±10.38	91.04±14.11	NS	0.6253
Cholesterol (mg/dl)	85.47±9.316	91.39±13.73	*	0.0101
TG (mg/dl)	121.9±57.27	195.3±154.4	**	0.0016
HDL (mg/dl)	41.47±5.3	37.63±8.5	**	0.0059
LDL (mg/dl)	90.70±24.42	108.4±31.1	**	0.0015
LH (IU/mL)	5.998±4.5	8.319±6.4	*	0.0321
FSH (IU/mL)	5.0±2.436	6.482±4.289	*	0.0314
LH/FSH	1.234± 0.9261	2.358± 1.294	**	<0.0001
TEST (ng/dL)	0.6±0.5	0.82±0.56	*	0.0334
Free TEST (ng/dL)	1.504 ±0.9	1.945 ±1.2	*	0.0371
$DHEA_{SO4}$ (µmol/L)	2.075 ± 0.8394	2.582 ± 1.547	*	0.0399
SOD2 (U/L)	77.94 ± 15.24	71.28 ± 12.54	*	0.0137
PON1 (U/L)	153.2± 65.1	127.1± 59.33	*	0.0321
TAC (mmol/L)	1.164± 0.3	1.151± 0.3240	NS	0.8228
MDA (µmol/mL)	1.866± 0.7	2.420± 1.1	**	0.0025

Values are presented as mean±SD

NS; Non-significant, ** (P <0.01), * (P <0.05)

3.2 Investigating the relationship between *SOD2* and *PON1* polymorphisms with PCOS

In the present work, PCR-restriction fragment length polymorphism (PCR-RFLP) was utilized to evaluate the SOD2 and PON1 polymorphisms in PCOS. Digestion of the PCR products was performed with the use of TaqI and BspPI restriction enzymes for SOD2 and PON1, respectively. The PCR-RFLP products were separated by 2% agarose gel electrophoresis and visualized under a UV illuminator following ethidium staining and the results confirmed that the enzyme TaqI cleaves the sequence of 5′TCG3′ and gave two fragments of 346bp and 240 bp length (Fig. 1). According to the presence or absence of polymorphism, the genotypes were: AA homozygote with one band of 513 bp, GA heterozygote with three bands of 513, 346, and 167 bp and GG homozygote with two bands of 346 and 167 bp (Fig. 1). Furthermore, the enzyme BsrbI was applied to cleave the gene *PON1*, when allele C is present in this position. The result revealed two fragments with lengths of 212 and 28 bp. Therefore, genotypes TT (240 bp), CT (240, 212, and 28 bp), and CC (212, and 28 bp) were determined (Fig. 1B).

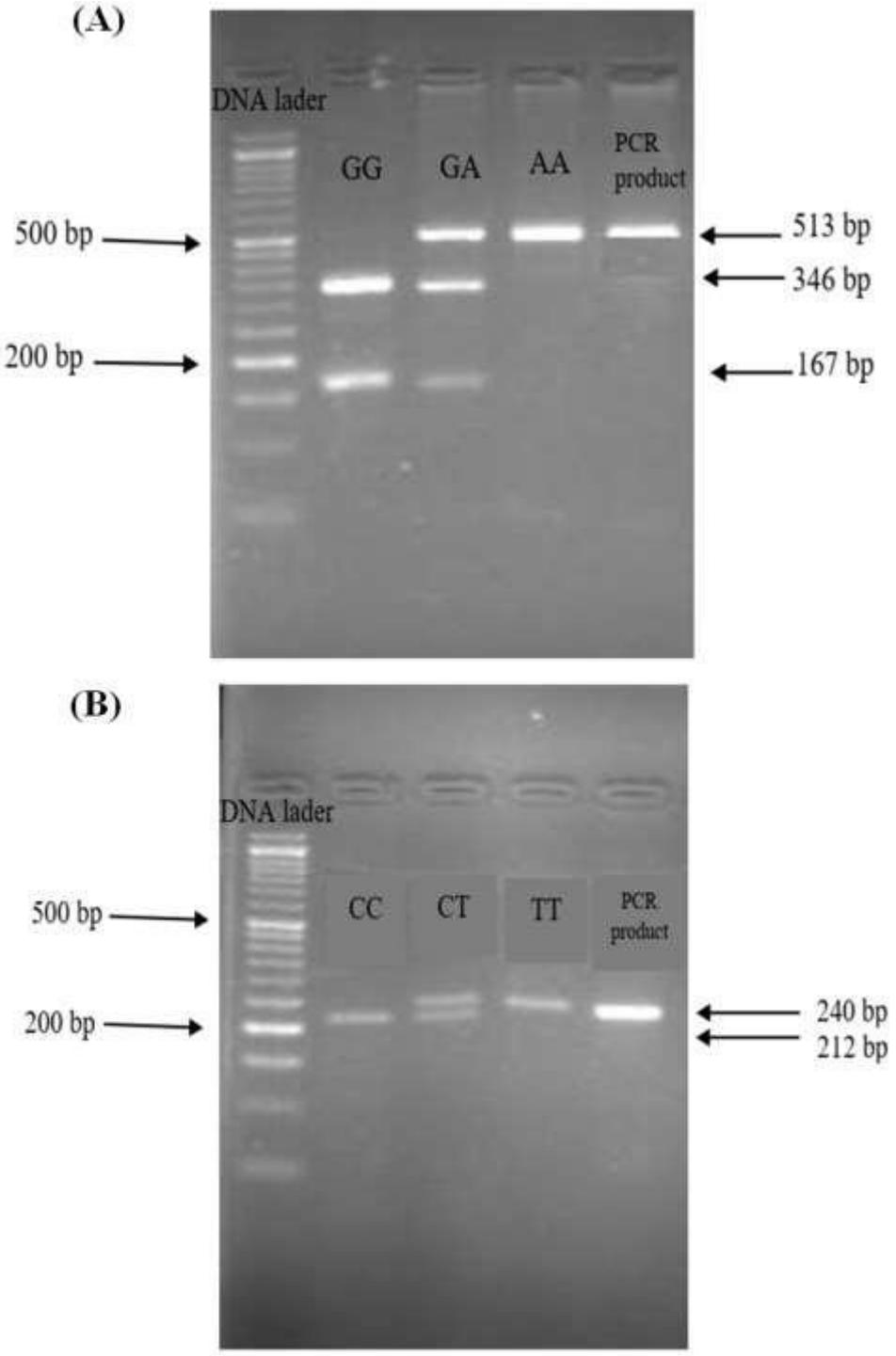

Fig. 1. PCR-RFLP products of (A) SOD2 (rs5746136 G>A) and (B) PON1 gene (rs705379 C>T) polymorphisms.

3.3 Clinical and biochemical characteristics, and oxidative stress markers

Table 2 shows the clinical and biochemical features of the POCS patients and controls. According to this table, no significant differences were observed between the patients and the reference group in age, blood sugar, or BMI. However, compared to the healthy subjects, the POCS patients had significantly higher levels of cholesterol, TG, and LDL (P <0.05 for cholesterol and P <0.01 for TG and LDL), while HDL was significantly higher in the control group (P<0.01). In addition, the PCOS group showed considerably greater levels of LH, FSH, testosterone (TEST), free testosterone (Free TEST), and dehydroepiandrosterone sulfate ($DHEA_{SO4}$) compared to the control group (P <0.05).

The TAC level was slightly lower in the PCOS group than the control group with no significant difference. While the PCOS group depicted significantly higher levels of MDA than the control group (p <0.01). PON1 and SOD2 enzyme activities were significantly lower in the PCOS group (*P*<0.05),

3.4 Genotype and allele frequencies distribution of *SOD2* and *PON1* genes

To investigate the association of the SOD2 G>A (rs5746136) and PON1 C>T (rs 705379) gene polymorphisms with PCOS risk among Iranian women, we have analyzed the genotype and allele frequency distribution of those variants between each group (Tables 2 and 3). Regarding the PON1 gene, PCOS patients exhibited a greater frequency of the CC genotype compared to the control group. Notably, there was a notable rise in CC genotype in the PCOS group (35.71% vs. 20.37%; P=0.016). While the frequency of the TT genotype was higher in the reference group (37.037% *vs*. 21.428%; P=0.015). On the other hand, compared with the control subjects, the C allele was more common among PCOS patients (P=0.029). Further analysis showed that the CC is recessive genotype though the differences was not statistically significant (78.572% *vs.* 62.963%, P=0.055) (Table 2). For SOD2 genotypes and alleles, there were no discernible variations in frequency between PCOS and control groups (p>0.05) (Table 3).

Table 2. Frequencies of PON1 genotype and allele in PCOS subjects and healthy group.

PON1 genotype	PCOS (n=56)	Control (n=54)	OR (95% CI)	*P* value
CC	20 (35.71%)	11 (20.37%)	2.171 (1.098-4.313)	0.016*
CT	24 (42.857%)	23 (42.592%)	1.011 (0.555-1.841)	1
TT	12 (21.428%)	20 (37.037%)	0.464 (0.236-0.97)	0.015*
Allele				
C	64 (57.142%)	45 (41.666%)	1.867 (1.025-3.406)	0.029
T	48 (42.857%)	63 (58.333%)	0.536 (0.294-0.976)	0.029
Recessive				
CC	20 (35.71%)	11 (20.37%)	2.17 (0.92-5.12)	0.057
CT+TT	36 (64.29%)	43 (79.63)	1	-
Dominant				
CT+CC	44 (78.572%)	34 (62.963)	2.15 (0.93-5.02)	0.055
TT	12 (21.428%)	20 (37.037%)	1	-
Over-dominant				
CT	24 (42.857%)	23 (42.592%)	1.076 (0.51-2.28)	0.50
CC+TT	32 (57.13)	33 (57.41)	1	-

Table 3. Frequencies of SOD_2 genotype and allele in PCOS subjects and healthy group.

SOD2 genotype	PCOS (n=56)	Control (n=54)	OR (95% CI)	*P* value
GG	20 (35.714%)	16 (29.629%)	1.319 (0.699-2.493)	0.359
GA	29 (51.785%)	27 (50%)	1.074 (0.594-1.943)	0.801
AA	7 (12.5%)	11 (20.37%)	0.558 (0.242-1.278)	0.123
Allele				
G	69 (61.67%)	59 (54.629%)	1.333 (0.730-2.436)	0.317
A	43 (38.392%)	49 (45.37%)	0.750 (0.410-1.370)	0.317
Recessive				
GG	20 (35.714%)	16 (29.629%)	1.32 (0.59-2.94)	0.317
GA+AA	36 (64.29%)	38 (60.37%)	1	-
Dominant				
GA+GG	49 (87.5%)	43 (79.63%)	1.79 (0.64-5.03)	0.196
AA	7 (12.5%)	11 (20.37%)	1	-
Over-dominant				
GA	29 (51.785%)	27 (50%)	1.074 (0.51-2.27)	0.501
GG+AA	27 (48.21%)	27 (50%)	1	-

3.5. Effects of SOD2 and PON1 genetic variants on clinical, hormonal, metabolic, and OS parameters

The possible impacts of SOD2 and PON1 genetic polymorphisms on the serum levels of clinical, hormonal, metabolic, and OS factors were studied, and the obtained results are shown in Tables 5 and 6. The SOD2 AA genotype resulted in a statistically significant rise in the Chol and $DHEA_{SO4}$ levels (*p*-value <0.01), and LH, FSH, TEST, and free TEST (P <0.05). In addition, in individuals with the SOD2 AG genotype, the serum levels of LDL and MDA significantly increased, however, a decrease was observed in HDL concentration (P <0.01). Furthermore, these subjects showed an increase in TG and LH concentrations and a reduction in PON1 and SOD2 (P <0.05) compared to control groups. On the other hand, the results indicated that the level of PON1 significantly decreased (P <0.01) in individuals with the GG SOD2 genotype. However, the serum levels of TEST and free TEST were significantly increased in the GG subgroup (P <0.05).

The results presented in Table 6 indicated that the concentration of Chol and PON1 was increased and decreased (P <0.05), respectively, in subjects with the PON1 TT genotype when compared to the reference group. In subjects carrying the PON1 TC genotype, the serum levels of TG (P <0.01) and free TEST increased (P <0.05), while the concentration of PON1 decreased (P <0.05). Also, the obtained data revealed that the concentrations of LH, FSH, TEST, and MDA with (P <0.05) and $DHEA_{SO4}$ with *P* value <0.01 significantly increased in subjects with the PON1 CC genotype.

However, these individuals showed a significant decrease in PON1 content with *P* value <0.05.

Table 4. Clinical characteristics and OS parameters of the SOD2 genotypes in PCOS patients and controls.

	AA (Mean±SD)			**GA (Mean±SD)**			**GG (Mean±SD)**		
	Control (n=54)	**PCOS (n=56)**	***p-value***	**Control (n=54)**	**PCOS (n=56)**	***p-value***	**Control (n=54)**	**PCOS (n=56)**	***p-value***
BS (mg/dl)	88.00+10.25	89.70+17.03	0.985 NS	90.83+10.07	90.88+14.30	0.999 NS	91.94+9.68	92.90+12.45	0.993 NS
Chol (mg/dl)	82.73+9.53	99.70+19.52	0.006**	87.17+8.78	90.88+14.30	0.645 NS	85.25+9.69	93.40+11.75	0.143 NS
TG (mg/dl)	110.27+48.30	166.38+83.38	0.407 NS	108.13+44.45	170.52+77.10	0.035*	151.75+73.60	204.95+142.08	0.192 NS
HDL (mg/dl)	40.09+5.82	35.80+6.12	0.299 NS	42.33+5.33	36.72+6.39	0.005**	41.06+5.17	36.45+7.40	0.078 NS
LDL (mg/dl)	84.55+11.11	103.40+34.55	0.313 NS	96.08+27.34	121.21+28.56	0.005**	86.63+27.30	95.74+28.04	0.697 NS
LH (IU/mL)	4.73+3.07	11.21+5.25	0.034*	6.77+5.83	11.22+8.11	0.021*	5.76+2.87	6.46+4.29	0.976 NS
FSH (IU/mL)	5.43+2.43	10.13+4.89	0.014*	5.10+2.45	6.67+4.68	0.329 NS	4.54+2.17	5.99+3.68	0.542 NS
LH/FSH	1.04+0.63	2.17+0.72	0.025*	1.32+0.77	2.46+0.95	0.016*	1.09+0.47	2.62+1.75	0.0003**
TEST (ng/dL)	0.65+0.42	1.36+0.77	0.023*	0.61+0.55	0.90+0.49	0.206 NS	0.55+0.54	1.09+0.69	0.026*
Free TEST(ng/dL)	1.70+0.82	2.90+0.67	0.028*	1.28+0.71	1.62+1.02	0.548 NS	1.76+1.24	2.63+1.15	0.039*
$DHEA_{SO4}$ (µmol/L)	2.35+0.76	4.14+1.20	0.004**	1.88+0.92	2.38+1.32	0.354 NS	2.21+0.78	2.63+1.73	0.674 NS
PON1 (U/L)	90.09+49.05	143.33+66.10	0.125 NS	150.24+61.69	108.25+62.20	0.038*	197.19+44.64	116.89+57.35	0.0003**
SOD2 (U/L)	81.54+7.62	78.83+7.69	0.959 NS	76.91+16.05	67.43+13.26	0.042*	79.08+17.79	70.77+9.87	0.190 NS
TAC (µmol/mL)	1.04+0.20	1.08+0.38	0.986 NS	1.19+0.28	1.20+0.29	0.999 NS	1.21+0.33	1.11+0.34	0.729 NS
MDA (mmol.L)	1.89+0.64	2.71+1.18	0.117 NS	1.99+0.81	2.80+1.10	0.005**	1.72+0.56	1.81+0.80	0.989 NS

Values are presented as mean±SD

NS; Non-significant, ** (P <0.01), * (P <0.05)

Table 5. Clinical characteristics and OS parameters of the PON1 genotypes in PCOS patients and controls.

	TT (Mean±SD)			CT (Mean±SD)			CC (Mean±SD)		
	Control (n=54)	**PCOS (n=56)**	***p-value***	**Control (n=54)**	**PCOS (n=56)**	***p-value***	**Control (n=54)**	**PCOS (n=56)**	***p-value***
BS (mg/dl)	87.11+ 13.14	95.25+16.78	0.222 NS	91.55+6.14	90.29+12.52	0.981 NS	91.60+11.67	89.40+14.47	0.957 NS
Chol (mg/dl)	82.58+10.30	95.25+16.78	0.012*	89.73+6.33	90.71+12.00	0.988 NS	83.90+9.90	89.90+14.00	0.463 NS
TG (mg/dl)	120.21+50.25	157.36+100.70	0.775 NS	112.55+41.28	245.23+213.43	0.0006**	130.20+76.01	161.15+67.67	0.864 NS
HDL (mg/dl)	40.95+5.41	39.08+4.50	0.866 NS	41.73+5.33	37.83+10.98	0.203 NS	41.40+5.56	36.50+7.10	0.234 NS
LDL (mg/dl)	89.21+22.63	99.83+26.27	0.663 NS	90.64+22.06	106.77+31.61	0.163 NS	94.30+30.30	115.25+33.07	0.157 NS
LH (IU/mL)	6.14+3.44	7.68+6.23	0.806 NS	5.96+5.67	9.05+5.58	0.137 NS	4.83+3.00	10.15+5.63	0.031*
FSH (IU/mL)	5.02+2.68	6.13+3.33	0.803 NS	5.06+2.45	6.52+4.02	0.456 NS	4.81+2.39	8.76+5.55	0.021*
LH/FSH	1.10+0.48	2.86+1.82	<0.0001	0.94+0.56	2.05+0.99	0.012	1.56+1.26	2.19+0.81	0.222 NS
TEST (ng/dL)	0.68+ 0.52	0.77+0.48	0.965 NS	0.71+0.55	0.95+0.64	0.353 NS	0.25+0.14	0.89+0.52	0.0123*
Free TEST(ng/dL)	1.36+0.64	1.78+0.89	0.603 NS	1.64+1.01	2.53+1.23	0.014*	1.40+1.28	1.55+1.00	0.976 NS
$DHEA_{SO4}$ (µmol/L)	2.16 + 0.74	2.68+0.93	0.530 NS	2.33+0.79	2.63+1.49	0.779 NS	1.28+0.78	2.88+1.66	0.002**
PON1 (U/L)	142.90+63.11	77.78+38.79	0.013*	181.09+52.76	135.67+61.03	0.020*	173.90+41.15	118.95+56.78	0.037*
SOD2 (U/L)	78.09+14.38	72.78+11.57	0.667 NS	79.65+16.82	70.52+13.12	0.090 NS	73.81+14.82	71.29+12.94	0.956 NS
TAC (µmol/mL)	1.10+0.23	1.06+0.36	0.970 NS	1.21+0.34	1.23+0.35	0.993 NS	1.16+0.25	1.12+0.26	0.976 NS
MDA (mmol.L)	1.94+0.85	2.53+1.21	0.229 NS	1.84+0.58	2.10+1.05	0.731 NS	1.77+0.67	2.74+1.06	0.030*

Values are presented as mean±SD

NS; non-significant, ** (P <0.01), * (P <0.05)

The most prevalent hormonal condition affecting women is PCOS. As a syndrome, the pathophysiology consists of several intricate molecular pathways that affect hormone, inflammatory, metabolic, and hemostatic functions, mainly defined by insulin resistance (IR) and hyperandrogenism [25]. Studies have recently turned to investigating the genetic aspects that influence gene expression and the role of gene polymorphism in the emergence and development of a disease.

The key factor influencing OS sensitivity is genetic background. Numerous researches have studied the association between the risk of diseases and gene polymorphisms in the important redox regulation enzymes [14, 26-28]. The frequency and correlation between the polymorphisms in the SOD2 and PON1 genes and the risk of PCOS were examined for the first time in this study because of their central roles in the antioxidant defense system. Also, the impacts of the studied genotypes on hormonal, clinical, and OS indexes in Iranian women were evaluated. Here, we discovered that the blood sugar level and BMI values did not significantly differ between the PCOS and control groups. The levels of LH and FSH were found to be significantly higher (X% and Y%, respectively) in women with PCOS than in the healthy group. Despite this, the level of LH was found to be higher than the FSH in the same PCOS group. In line with the results of previous studies, deficient follicular development is a result of low FSH to high LH levels [29].

Different studies have reported that the concentrations of Chol, TG, and LDL showed a significant increase and HDL decreased in women suffering from PCOS. In terms of prevalence, dyslipidemia has been reported in up to 70% of women with PCOS. Our findings were in good agreement with these reports (Table 1), and the findings indicated that, compared with the healthy group, the serum concentrations of Chol, TG, and LDL were increased in PCOS. However, the content of HDL decreased, with a significant difference from the control group [30, 31].

The serum levels of TEST, free TEST, and DHEAs increased in women with PCOS. Some evidence supports the role of androgens in the etiology of PCOS. Hyperandrogenism is characterized by elevated levels of androgens [32, 33].

We discovered that there was no discernible difference between the two groups under investigation and that PCOS patients had slightly lower levels of TAC than the control group. It has been reported that TAC can be lower in patients with PCOS compared with the control group [34].

There was a significant variation in the MDA level between PCOS and the reference group. In the context of PCOS, the majority of studies have shown that the level of MDA, a marker of OS, is higher in PCOS patients compared to healthy controls. Also, they refer to a correlation between lipid peroxidation and MDA in patients with PCOs. PCOs are associated with OS, which can lead to increased lipid peroxidation and MDA production [35, 36].

SOD2 and PON1 enzymes were found to be less active in the PCOS group than in the control group, with significant differences [37-39]. This decrease in PON1 activity may contribute to dyslipidemia in PCOS as we found in our results. Some factors may cause a decrease in PON1 enzyme activity, such as genetic factors; the polymorphism in the gene structure of the PON1 gene could affect this enzyme activity; and increased oxidative stress may leads to a decrease in PON1 enzyme activity [40]. Also, the reason for the decrease in SOD2 enzyme activity may be related to errors during the SOD2 gene expression [41], or it may be due to dysfunction in the mitochondria because of oxidative stress (Table 1) [42].

Genetic elements and markers of oxidative stress and biochemical and clinical parameters were investigated in detail in PCOS pathogenesis. Previous studies have proven that the balance between hormones, biochemical parameters, and oxidative stress may be seriously disrupted. Importantly, both genetic factors and markers of oxidative stress, as well as biochemical and clinical factors, may play a role in PCOS risk. Also, positions SOD2 (rs5746136 G>A) and PON1 (rs705379 C>T) show that these genetic variants probably play a role in regulating the expression of these women. Therefore, we hypothesized that these genetic variants may be associated with PCOS risk. Data showed that the polymorphism in PON1 (rs705379 C>T), CC, CT, and TT genotypes were present at rates of 35.71, 42.857, and 21.428% in PCOS patients, and 20.37, 42.592, and 37.037% in controls, respectively (Table 2). The frequency of the CC wild homozygous genotype in PCOS patients was significantly higher than in controls (OR=2.171, CI=1.098-4.313; p = 0.016). Although PCOS patients had a higher incidence of the CT heterozygous genotype than controls, no discernible connection was found (OR=1.011, CI=0.555-1.841; p=1). Our findings were in good agreement with

the previously reported results [16]. Also, the frequency of the TT genotype in controls was significantly higher than in PCOS patients (OR=0.464, CI=0.236-0.9.7; p=0.015).

The PON1 C allele was common among PCOS subjects, and the T allele was common among control subjects (Table 2), which is in accordance with the previous studies [16, 24]. Knebel et al. [43] investigated the relationship between the polymorphism of the PON1 (rs705379 C>T) gene and the risk of PCOS, and they discovered that the distribution of the CC genotype and C allele among women with PCOS were higher than those of control subjects.

According to the polymorphism in SOD2 (rs5746136, G>A), the genotypes of GG, GA, and AA were present at rates of 35.714, 51.785, and 12.5% in PCOS patients, and 29.629, 50, and 20.37% in controls, respectively. The frequencies of the GG wild homozygous genotype and the GA genotype in PCOS patients were higher than in controls (OR=1.319, CI=0.699-2.493, P=0.359, and OR=1.074, CI=0.594-1.943, P=0.801, respectively), while the frequency of the AA genotype in controls was higher than in PCOS patients (OR=0.558, CI=0.242-1.278, P=0.123), but no significant relationship was observed. The G allele was common among PCOS subjects and the A allele was common among control subjects (without a significant *p*-value) (Table 3). Polat et al. [44] investigated the relationship between polymorphism SOD2 (rs5746136 G>A) and the risk of PCOS among Turkish women and they observed that the frequency of the GG genotype among women with PCOS was higher than that of control subjects, and the frequency of the G allele was higher among patients.

As shown in Tables 5 and 6, in PCOS patients carrying the PON1 CC and SOD2 AA genotypes, a significant increase was observed in serum concentrations of LH, FSH, TEST, and MDA. In addition, the content of free TEST and the LH/FSH ratio were significantly increased in subjects with the SOD2 AA genotype. However, no significant changes were detected in the LH/FSH ratio in the PCOS group carrying the PON1 CC genotype. Furthermore, subjects carrying the SOD2 AG and GG genotypes showed a significant increase in the contents of LH, TEST, free TEST, and LH/FSH ratio. Also, $DHEA_{SO4}$ showed an increase in PCOS women with PON1 CC and SOD2 AA genotypes (P <0.01). The PON1 enzyme activity was significantly decreased in patients carrying the PON1 CC, TT, and TC genotypes without any alterations in the SOD1 enzyme. However, a statistically significant decrease was observed in the catalytic activity of these enzymes (SDO2 and PON1) in subjects who carry SOD1 AG and GG genotypes.

In addition, no significant changes in the serum concentrations of Chol, TG, HDL, and LDL were observed in PON1 CC carriers, but patients with PON1 TT and TC genotypes represented significant increases in Chol and TG concentrations, respectively. The SOD1 AA and AG genotypes caused statistically significant changes in the serum levels of Chol, TG, HDL, and LDL compared to the control group. According to these findings, we can suggest that the CC genotype of PON1 and the AA genotype of SOD2 correlate with the increase of LH, FSH, TEST, and $DHEA_{so4}$ levels in PCOS. Thus, we can deduce that women are more susceptible to PCOS if they have the C allele of PON1 and the A allele of SOD2.

5. Conclusion

In conclusion, our study provides evidence of a correlation between PCOS in women and the increased CC genotype of the PON1 gene (rs705379 C>T). This specific genotype was found to be associated with various affected parameters in PCOS, including elevated levels of LH, FSH, $DHEA_{SO4}$, TEST, and MDA, as well as decreased PON1 enzyme activity. These findings suggests that the CC genotype of the PON1 gene may contribute to the pathogenesis of PCOS by influencing hormonal dysregulation and OS. The elevated LH and FSH levels, along with increased TEST and $DHEA_{SO4}$ indicate disruptions in the normal hormonal balance associated with PCOS. The decreased activity of the PON1 enzyme, which is involved in antioxidant defense mechanisms, further supports the presence of OS in PCOS patients. Overall, our study highlights the importance of genetic factors,

specifically the CC genotype of the PON1 gene, in the development and manifestation of PCOS. Further research is needed to elucidate the underlying mechanisms by which this genotype influences PCOS and explore potential therapeutic targets for managing the condition.

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