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ABCA1 gene polymorphism and oxidative stress in polycystic ovary syndrome: Role of antioxidant therapy for prevention and control

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Abstract

Polycystic ovary syndrome (PCOS) is a common health problem that affects women in childbearing age. Recent studies discussed the relation of oxidative stress with PCOS and suggested that the antioxidants may improve the prognosis of PCOS. Changes happened in ATP binding cassette transporter 1 (*ABCA1*) gene encoding a protein regulating entry and exit from cell membrane may contribute to dyslipidemia in patients with PCOS. The role of oxidative stress and *ABCA1* gene polymorphism in the pathogenesis of PCOS was evaluated along with the effect of antioxidants in the prevention and treatment of this disease. The present study included 90 female patients with PCOS and 90 healthy women represented the control group. Patients with PCOS were further divided into two subgroups. The patients included in subgroup 1a were treated with antioxidant and hormonal therapy while patients included in subgroup 1b were treated with hormonal therapy only. *ABCA1*gene polymorphism and oxidative stress parameters were investigated in all groups. The level of the oxidant marker; MDA was higher in PCOS patients when compared to controls. In addition, the levels of GSH, vitamin E, ascorbic acid, and selenium were significantly decreased, while SOD and GPx activities were increased in PCOS patients when compared to controls. The response to treatment among PCOS patients was better in group 1a than group 1b. The frequency of *ABCA1* AA genotype was higher in PCOS patients than the control group (32.2% and 11.1% respectively). It was concluded that oxidative stress may lead to PCOS which raises the possibility of using antioxidants for the prevention and control of PCOS. In addition, the polymorphism in *ABCA*1 gene may be associated with genetic susceptibility for PCOS.

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Introduction

Oxidative stress is the disparity between the amount of oxidants and antioxidants with preference of oxidants that leads to cell damage. Reactive Oxygen Species (ROS) seem to have a significant role in many different processes in female reproductive system such as: oocytes development, fertilization, corpus luteum re-progression, and shedding of uterine endometrium [1]. Functionally active antioxidants may be either enzymatic such as glutathione peroxidase, catalase, and superoxide dismutase; or non-enzymatic such as glutathione, selenium; or vitamin-based such as vitamin A, C and E; or phytochemical such as flavonoids and polyphenols [2]. Lee et al. [3] reported that PCOS is related to oxidative stress and the use of antioxidants may improve the condition .

The human *ABCA1* gene is located on the region q31 of chromosome 9 and is mainly expressed in the liver, lungs, adrenal glands, placenta and fetal tissues. It encodes for the ABCA1 protein [4] which has been shown to mediate the cellular secretion of the antioxidant, alpha-tocopherols (active form of vitamin E) [5]. The aim of the current study was to examine the role of oxidative stress and *ABCA1* gene polymorphism in the development of PCOS and to investigate the possibility of using antioxidant therapy for prevention and treatment of PCOS.

MATERIAL and METHODS

Patients

The current study included 180 females with age ranged from 20 to 40 years, 90 females were diagnosed to have PCOS (group 1) and 90 healthy females represented the control group (group 2). Patients with PCOS were attending the Outpatient Clinic of Gynecology department, Mansoura University Hospital. Diagnosis of PCOS can be done by the presence of two out of the following three criteria: oligo- or anovulation, signs of increased blood androgen levels, and ovaries are polycystic, which approved by the conference (Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group [6]. Patients with any systemic disease including diabetes mellitus, hypertension, or any other disease were excluded from the study.

The current study is a double-blind, placebo-controlled study. The PCOS patients were randomly divided into two subgroups: Subgroup 1a, (n =45 patients) treated with antioxidant therapy plus the hormonal therapy for 3 months. The antioxidant therapy was in the form of vitamin E tablets 400mg once daily, vitamin C effervescence 1gm twice daily and selenium ACE tablets once daily. The hormonal therapy was in the form of (35 µg ethinyl estradiol plus 2 mg cyproterone acetate) with the trade name of Diane-35 was given as a daily tablet starting from day 3 of the menstrual cycle for 21 days every month. Subgroup 1b, (n=45 patients) treated with the same hormonal therapy plus placebo capsules instead of antioxidant therapy. Patients were classified according to response to treatment into good and bad responders. Good response was represented by improvement of hirsutism and restoration of the regularity of menstrual cycles. The control group included 90 healthy women with normal menstrual cycles, an absence of manifestations of hyperandrogenism, not suffering

from any problems during previous pregnancy and labor which resulted in healthy infant. All subjects were informed about the aim of this research and informed consents were obtained. The study was done with the agreement of Faculty of Medicine, Mansoura University local ethics committee [Code (R/16..07.63)] and in accordance with the General Assembly of the World Medical Association Declaration of Helsinki [7].

Biochemical investigations

After 12 hours fasting, one ml blood samples were withdrawn and stored as ethylenediamine tetraacetic acid (EDTA) anti-coagulated blood at −30°C until DNA extraction and *ABCA1* gene polymorphism were carried out. Additional 5 ml blood samples were collected from controls and PCOS patients (before and after treatment) in heparin-treated tubes. The plasma was separated, and stored into aliquots at −30°C until the time of assay of vitamin E [8], selenium [9] and hormonal levels (FSH, LH and testosterone). The buffy coat layer of the centrifuged blood was discarded, and the packed erythrocytes were washed using a physiological saline; washing process was repeated for three times. Preparation of the erythrocytes suspension was carried out according to Quist [10]. Erythrocytes were used for estimation of malondialdehyde (MDA) [11], glutathione (GSH) [12] and ascorbic acid levels [13], and the activities of Glutathione peroxidase enzyme (GPx) [14], superoxide dismutase (SOD) [15], and catalase [16].

Estimation of erythrocyte malondialdehyde (MDA) level

MDA was measured using the thiobarbituric acid (TBA). The reaction between MDA and TBA gives a red compound, which has an absorbance at 535 nm [11]

Estimation of erythrocyte glutathione (GSH) level

Estimation of erythrocyte glutathione level was assayed according to Virgil and George [12]. The principle based on the reduction of a disulfide chromogen, 5, 5-Dithio bis (2-nitrobenzzoic acid) DTNB to a yellow product using glutathione. The absorbance of produced yellow product is detected at 412nm and is positively related to the erythrocyte glutathione level

Estimation of erythrocyte ascorbic acid level

The ascorbic acid level was measured by the method of Tietz [13].

Estimation of erythrocyte glutathione peroxidase (GPx) activity

The activity of Glutathione Peroxidase (GPx) in erythrocytes was assayed by the method Pleban et al. [14], The oxidation of GSH to GSSG by the enzyme in the presence of NADPH and exogenous glutathione reductase regenerates GSH for GSSG. The rate of enzyme activity was detected by the decrease in the NADPH absorbance using a wave length of 340nm.

Estimation of erythrocyte superoxide dismutase (SOD) activity

The assay of SOD activity was done according to Misra and Fridovich [15], which is based on the principle that SOD decreases the rate of

epinephrine auto-oxidation at pH 10.2 to form adenochrome.

Estimation of erythrocyte catalase activity

The activity of catalase in erythrocyte lysates was based on measuring the absorbance of NADH produced from the reaction between ethanol, hydrogen peroxide, and catalase enzyme at 340 nm [16].

Estimation of plasma hormones levels

The levels of testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) in the plasma of all participants were determined by enzyme linked immuno-sorbent assay (ELISA) using kits supplied by Diagnostic Systems Laboratories, Inc., Webster, Texas, USA. (Tecan-sunrise Absorbance Reader, serial number 03930600856 Tecan, Austaria, GmbH- Magellan software).

DNA extraction and investigation of the 2706G /A polymorphism in the ABCA1 gene

Extraction of DNA from peripheral blood was done using Genomic DNA Purification Kit (Qiagen; Hilden, Germany). Two primers were designed for amplification of a 350 base pair (bp) fragment included in the 2706 G /A of the *ABCA*1gene polymorphism [17]. The forward primer sequence was 5`CAA GTG AGT GCT TGG GAT TG-3` and the reverse was 5`TGC TTT TAT TCA GGG ACT CCA-3`. DNA was first de-natured for 5 min at 94°C, followed by 40 cycles of denaturation at 94°C for 45 s, annealing at 52°C for 45 s, and extension at 72°C for 60 s. Final extension was done at 72°C for 7 min to complete DNA amplification. The amplification product was 350-bp in size. The restriction endonuclease used for cleavage was *BsaA I* (Cat. no.R0531S; Life Technologies, Invitrogen; Carlsbad, CA, USA). The PCR product was incubated with 1 U *BsaA I* for 3 h at 30°C. Allele G produced 2 bands of 252 bps and 98 bps, while the A allele remained intact (figure 1).

Statistical Analysis

Excel program and SPSS version 22 was used to analyze the resulting data. Statistical significance was detected between different studied groups. For analysis of quantitative data, Mann-Whitney test was used to compare two groups, the Wilcoxon test was used to compare the two paired groups and Kruskal Wallis test was used to compare more than two groups. To compare qualitative data, χ^2 test was used. $P< 0.05$ was considered statistically significant at 95% confidence interval. The frequencies of genotypes and alleles in both groups were tested for Hardy–Weinberg Equilibrium.

RESULTS

There was a significant increase in the oxidant marker; MDA in PCOS patients compared to the control group. The activities of erythrocyte antioxidant enzymes; SOD and GPx were increased while the erythrocyte GSH and ascorbic acid levels and plasma levels of vitamin E and selenium were decreased in patients with PCOS compared to the control group (Table 1). After treatment, there was significant improvement in the oxidative stress parameters which represented by a decrease in the level of the oxidant marker; MDA and the activities of enzymatic antioxidant (SOD and GPx), while, there was an increase

Table 1. Descriptive and biochemical data in the control group and PCOS patients

Parameters	Control Group N=90 Median (Interquartile Range)	PCO patients N=90 Median (Interquartile Range)	P-value
Age (years)	33 (28-37)	31 (27-37)	**0.384**
Body Mass Index (BMI K/m2)	23.8 (21.4-25.7)	25.8 (24.8-26.9)	**<0.0001***
MDA (nmoles/gm of Hb)	15.7 (13.8-17.3)	38.9 (36.7-41.8)	**<0.0001***
SOD (U/gm of Hb)	726.8 (696.2-748.4)	865.05 (823.5-887.4)	**<0.0001***
Catalase (U/gm of Hb)	9.9 (9.3-10.8)	9.4 (8.7-10.3)	**<0.0001***
GPx (U/gm of Hb)	56.6 (55.1-57.4)	61.2 (59.7-63.8)	**<0.0001***
GSH (mg/gm of Hb)	26.4 (25.6-27.4)	11 (10.3-11.8)	**<0.0001***
Ascorbic acid (mg/dl)	5.6 (4.9-6.3)	5.2 (4.3-5.9)	**0.012***
vitamin E (µ moles/L)	10.7 (9.7-11.5)	3.9 (3.1-4.7)	**<0.0001***
Selenium (ng/ml)	103.5 (100.5-105.4)	71.5 (68.4-75.6)	**<0.0001***
LH (mIU/ml)	15.4 (7.9-20.8)	23 (19.5-28.6)	**<0.0001***
FSH (mIU/ml)	9.9 (8.7-11.9)	12.8 (10.2-20.1)	**<0.0001***
Testosterone (ng/ml)	0.71 (0.54-0.84)	1.48 (0.83-1.84)	**<0.0001***

*= Statistically significant (Mann-Whitney test)

Table II. Biochemical data in PCOS patients before and after treatment

Parameters	PCO patients treated with hormones and antioxidants (subgroup1a) N=45 Median (Interquartile Range)			PCOS patients treated with hormones only (subgroup 1b) N=45 Median (Interquartile Range)		
	Before treatment	After treatment	P-value	Before treatment	After treatment	**P-value**
MDA (nmoles/gm of Hb)	38.9 (36.7-41.9)	22.8 (19.6-27.6)	<0.0001*	38.9 (36.7-41.8)	36.5 (33.5-38.7)	**<0.0001***
SOD (U/gm of Hb)	864.8 (823.5-887.2)	793.8 (743.1-812.8)	<0.0001*	865.2 (828.5-889.2)	857.6 (817.3-894.1)	**0.953**
Catalase (U/gm of Hb)	9.6 (8.4-10.4)	9.8 (9.6-10.8)	0.084	9.3 (8.9-10.2)	9.4 (9.2-10.1)	**0.076**
GPx (U/gm of Hb)	62.4 (60.2-64.1)	58.9 (57.8-59.4)	<0.0001*	60.4 (59.3-63.2)	60.2 (59.5-62.6)	**0.412**
GSH (mg/gm of Hb)	11.1 (10.4-11.8)	21.8 (20.4-23.3)	<0.0001*	10.8 (10.1-11.7)	12.6 (11.1-13.5)	**<0.0001***
Ascorbic acid (mg/dl)	5.2 (4.4-5.9)	6.5 (5.8-6.8)	<0.0001*	5.2 (4.2-6.1)	6.4 (5.8-6.8)	**<0.0001***
vitamin E (µ moles/L)	3.8 (3.1-4.6)	8.9 (8.2-9.4)	<0.0001*	4.1 (3.1-4.8)	4.6 (3.7-4.9)	**<0.0001***
Selenium (ng/ml)	71.8 (68.4-75.2)	96.2 (90.5-99.5)	<0.0001*	71.4 (68.4-75.8)	72.9 (69.3-76.8)	**<0.0001***
LH (mIU/ml)	21.8 (18.8-26.8)	10.1 (7.3-20.8)	<0.0001*	24.3 (19.9-28.9)	13.6 (8.4-26.9)	**<0.0001***
FSH (mIU/ml)	12.7 (9.6-19.3)	11.6 (8.2-17.8)	<0.0001*	13.4 (10.4-20.1)	11.6 (7.8-18.7)	**<0.0001***
Testosterone (ng/ml)	1.46 (0.84-1.82)	0.84 (0.64-0.97)	<0.0001*	1.49 (0.83-1.85)	0.82 (0.54-1.43)	**<0.0001***

*= Statistically significant: Wilcoxon test (compare 2 paired groups)

Table 3. Genotype distribution and allele frequency of the ABCA1 G2706A polymorphism in the control group and PCOS patients

	Control group N=90	PCO cases N=90	Odd Ratio	Relative risk	95% CI	P	
GG(Wild type)	44 (48.9%)	23 (25.6%)	R				**<0.0001***
GA (Heterozygote)	36 (40%)	38 (42.2%)	2.019	1.496	1.023-3.985	0.042*#	
AA(Mutant)	10 (11.1%)	29 (32.2%)	5.548	2.166	2.603-13.349	<0.0001*#	
G allele	124 (68.9%)	84 (46.7%)	R				
A allele	56 (31.1%)	96 (53.3%)	2.531	1.564	1.645-3.892	**<0.0001***	

*= Statistically significant (Pearson Chi-Square and contingency coefficient test)
P value# expresses statistically significant difference between each of genotypes and GG (Wild type) and between A allele and G allele as regard Control group and PCO group.

Table 4. Genotype distribution and allele frequencies of the ABCA1 G2706A polymorphism in PCOS patients according to the response to treatment

Gene Type	Respond to treatment N=52	Not respond to treatment N=38	Odd Ratio	Relative risk	95% Confidence Interval	P-value	
GG (Wild type)	16 30.8%	7 18.4%	R				**0.181**
GA (Heterozygote)	23 44.2%	15 39.5%	1.491	1.297	0.496-4.482	0.476#	
AA (Mutant)	13 25%	16 42.1%	2.813	1.813	0.890-8.892	0.074#	
G allele	55 52.9%	29 38.2%	R				**0.05***
A allele	49 47.1%	47 61.8%	1.819	1.418	0.9996-3.321		

*= Statistically significant (Pearson Chi-Square and contingency coefficient test)
P value# expresses statistically significant difference between each of genotypes and GG (Wild type) and between A allele and G allele as regard bad responsive and good responsive patients.

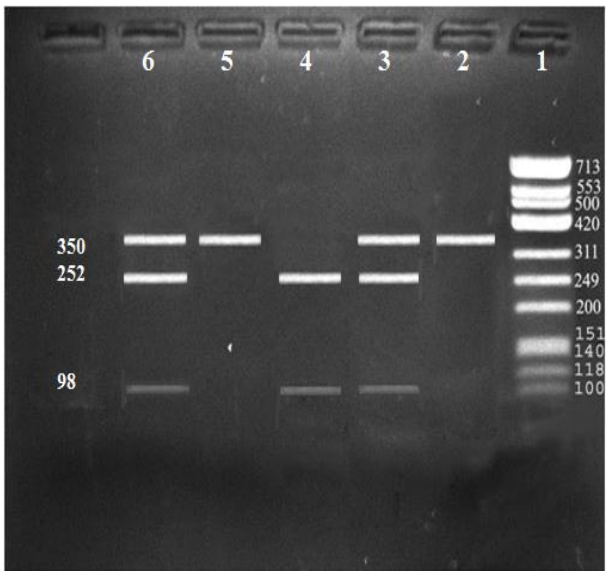

Figure (1): Ethidium bromide stained 2% agarose gel showing enzymatic digestion of G2706A polymorphism of the ABCA1 gene for different groups studied; Lane 1: ΦX174 DNA/Hinf I marker;, lane 2 and 5 (AA genotype);, lane 3 and 6 (AG genotype);, and lane 4 (GG genotype).

Table 5. Biochemical data in different genotypes and alleles of G2706A polymorphism for all subjects

Parameters	G2706A genotype	Median (Interquartile Range)	P-value			G2706A Allele	Median (Interquartile Range)	#
			P1	P2	P3			
MDA (nmoles/gm of Hb)	GG	17.2 (13.8-36.9)	0.069	<0.0001*	0.004*	G	17.6 (14.6-37.6)	**<0.0001***
	GA	34.35 (15.2-39.1)				A	36.2 (17.8-40.4)	
	AA	37.7 (19.2-41.8)						
SOD (U/gm of Hb)	GG	754.4 (715.6-828.5)	0.136	<0.0001*	0.007*	G	756.8 (718.7-833.6)	**<0.0001***
	GA	787.5 (728.9-845.7)				A	819.6 (737.7-884.5)	
	AA	865.2 (743.8-895.3)						
Catalase (U/gm of Hb)	GG	10.4 (9.3-10.8)	<0.0001*	0.007*	0.659	G	9.9 (9.2-10.7)	**0.001***
	GA	9.6 (8.9-10.3)				A	9.6 (9.0-10.4)	
	AA	9.6 (9.2-10.5)						
GPx (U/gm of Hb)	GG	57.3 (55.3-59.8)	0.018*	<0.0001*	0.015*	G	57.6 (55.7-60.3)	**<0.0001***
	GA	58.5 (56.8-60.9)				A	59.5 (57.3-62.8)	
	AA	60.3 (58.2-63.4)						
GSH (mg/gm of Hb)	GG	25.6 (11.9-26.7)	0.158	<0.0001*	0.065	G	25.2 (11.4-26.7)	**<0.0001***
	GA	12.3 (10.6-26.6)				A	11.8 (10.7-25.8)	
	AA	11.5 (10.7-24.8)						
Ascorbic acid (mg/dl)	GG	5.6 (4.8-6.2)	0.507	0.200	0.450	G	5.5 (4.8-6.2)	**0.165**
	GA	5.4 (4.5-6.2)				A	5.4 (4.5-6.1)	
	AA	5.2 (4.4-6.1)						
vitamin E (µ moles/L)	GG	9.7 (4.5-10.6)	0.730	<0.0001*	0.014*	G	9.2 (4.2-10.9)	**<0.0001***
	GA	5.2 (3.9-11.3)				A	4.8 (3.7-9.6)	
	AA	4.8 (3.5-8.7)						
Selenium (ng/ml)	GG	101.7 (77.1-105.1)	0.031*	<0.0001*	0.008*	G	99.8 (74.3-104.4)	**<0.0001***
	GA	78.8 (72.3-103.3)				A	75.6 (70.2-100.2)	
	AA	72.4 (69.3-97.6)						
LH (mIU/ml)	GG	19.5 (14.0-24.8)	0.549	0.467	0.722	G	19.8 (14.4-24.6)	**0.382**
	GA	20.2 (14.7-24.3)				A	20.3 (14.6-24.9)	
	AA	20.8 (12.3-25.8)						
FSH (mIU/ml)	GG	11.3 (9.4-12.8)	0.749	0.083	0.134	G	11.1 (9.2-13.2)	**0.151**
	GA	10.5 (8.7-14.8)				A	11.6 (9.1-17.9)	
	AA	12.4 (9.7-19.2)						
Testosterone (ng/ml)	GG	0.77 (0.64-0.95)	0.293	0.033*	0.181	G	0.79 (0.64-1.21)	**0.021***
	GA	0.86 (0.64-1.49)				A	0.92 (0.67-1.76)	
	AA	0.97 (0.68-1.84)						

P1= between GG and GA genotypes P2= between GG and AA genotypes
P3= between GA and AA genotypes P#= between G and A alleles
*= Statistically significant (P by Kruskal Wallis test & P1, P2, P3 and P# by Mann-Whitney test)

in the levels of non-enzymatic antioxidant markers (vitamin E, ascorbic acid, selenium and GSH). The improvement was significantly better in patients who were treated with antioxidants plus hormonal therapy than those treated by hormonal therapy alone (Table 2).

PCR determination of G2706A polymorphism of the ABCA1 gene revealed that the frequency of AA genotype and A allele was higher in PCOS patients compared to the controls (Table 3). Hardy-Weinberg equilibrium was done to show the expected frequency of different genotypes in both groups, there was no significant difference between

observed and expected genotypes frequencies in the control group (p=0.52) and PCOS group (p=0.14) (data not shown in table).

Patients with PCOS who carried the G allele were good responders to treatment when compared to patients who carried the A allele (Table 4).The level of the oxidant marker; MDA and activities of enzymatic antioxidants (SOD and GPx) were significantly elevated, while the levels of non-enzymatic antioxidants (vitamin E, ascorbic acid, selenium and GSH) were significantly decreased in individuals with A allele compared to those of G allele (Table 5).

Discussion

The higher level of MDA might be due to increased production of ROS as a result of increased oxidative stress in PCOS patients [18]. The increase in the activity of the antioxidant enzymes (GPx and SOD) might be a compensation mechanism to cope with the increased oxidative stress [19]. The decrease in the levels of non-enzymatic antioxidants (GSH, vitamin E, ascorbic acid and selenium) could be due to the increased turnover as a defense against oxidant status in those patients [18]. These results were in agreement with the study done by Sugino et al. [1] who found that the concentration of MDA was significantly increased in patients with PCOS compared with control women. Also, Dinger et al. [20] found that the levels of glutathione and vitamins C and E were lowered in PCOS patients. Murri et al. [21] found that serum levels of non-enzymatic antioxidant markers, such as glutathione, were decreased in patients with PCOS compared to control group. Glutathione is considered as a cofactor of many antioxidant enzymes as glutathione peroxidase and reductase and also repair the antioxidant vitamins, ascorbic acid and tocopherols to their functional forms [21].

Regarding therapy of PCOS, Wright and Sutherland [22] suggested that correction for oxidative stress through improving antioxidant defense might have beneficial effects in the treatment of PCOS. The present work reveals a significant improvement both in the oxidative stress parameters and clinical response in PCOS patients and this improvement was significantly better in the subgroup of PCOS patients that were treated by antioxidants plus hormonal therapy more than the subgroup that was treated by hormonal therapy only, which suggests that antioxidant therapy might be used to improve PCOS if given as adjuvant therapy in cases suffering from this condition. Hosseinzadeh et al. [23] reported that the insulin resistance; the main feature in PCOS patients, may occur as a result of increased oxidative stress. Also, they found that selenium intake that reduce insulin resistance and therefore play an important role in preventing and treatment of PCOS.

Polymorphisms in *ABCA1* gene may participate in the development of abnormal lipid changes in patients with PCOS [17]. In the current study, the percentages of the A allele and AA genotype of *ABCA1* G2706A gene were higher in PCOS patients than the control group and this result was in agreement with the results of a study conducted by Karadeniz et al. [17] who reported that non-obese females suffering from PCOS have decreased levels of high density lipoproteins in comparison to controls, whereas obese women with

PCOS had increased triglyceride levels. The polymorphism in *ABCA1* gene can result in hyperlipidemia and Tangier disease [24].

In the present work, there was a statistically significant increase in the oxidative stress in individuals with A allele compared to those with G allele. Also patients with PCOS that carried the G allele were good responders to treatment when compared to patients that carried the A allele. This result was in contrast with the findings of a previous study done by Karadeniz et al. [17] who reported that *ABCA1* gene G2706A polymorphism was not related to oxidative stress in PCOS patients.

Conclusion

In conclusion, the ABCA12706 AA genotype and A allele may be associated with genetic susceptibility for PCOS. Oxidative stress might play a role in pathogenesis of the PCOS and hence the usefulness of using antioxidant therapy plus hormonal therapy in treatment of PCOS. Further large scale studies about other genes involved in the development of abnormal lipid profile in PCOS should be evaluated.

Declaration of interest

All authors declare they have no conflicts of interest.

References

[1] **Sugino N, Karube-Harada A, Taketani T, Sakata A, Nakamura Y.** Withdrawal of Ovarian Steroids Stimulates Prostaglandin F2. ALPHA. Production Through Nuclear Factor-KAPPA. B Activation via Oxygen Radicals in Human Endometrial Stromal Cells: Potential Relevance to Menstruation. Journal of Reproduction and Development.; 50(2):215-25. 2004

[2**] Sekhon LH, Gupta S, Kim Y, Agarwal A.** Female infertility and antioxidants. Current Women's Health Reviews.; 6(2):84-95. 2010

[3] **Lee YJ, Baw CK, Gupta S, Aziz N, Agarwal A.** Role of oxidative stress in polycystic ovary syndrome. Current Women's Health Reviews.; 6(2):96-107. 2010

[4] **Langmann T, Klucken J, Reil M, Liebisch G, Luciani MF, Chimini G, Kaminski WE, Schmitz G.** Molecular cloning of the human ATP-binding cassette transporter 1 (hABC1): evidence for sterol-dependent regulation in macrophages. Biochemical and biophysical research communications.; 257(1):29-33. 1999

[5] **Andrikovics H, Pongrácz E, Kalina Đ, Szilvási A, Aslanidis C, Schmitz G, Tordai A.** Decreased frequencies of ABCA1 polymorphisms R219K and V771M in Hungarian patients with cerebrovascular and cardiovascular diseases. Cerebrovascular Diseases.; 21(4):254-9. 2006

[6] **ESHRE TR, Group AS. Revised 2003** consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertility and sterility.;81(1):19-25. 2004

[7] **General Assembly of the World Medical Association.** World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. The Journal of the American College of Dentists.; 81(3):14. 2014

[8] **Baker H, Frank D and Winley NC** .*Clinical Vitaminology*.; Page 772. 1968

[9] **A.O.A.C.** Official Method of Analysis, 15th ed. Method 968.08 and 968.09.*Association Official Analytical Chemists*, Inc., Arlington.VA. 1990

[10] **Quist EE.** Regulation of erythrocyte membrane shape by Ca^{2+}. Biochemical and biophysical research communications.; 92(2):631-7. 1980

[11] **Jain SK, McVie R, Duett J, Herbst JJ.** Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes.; 38(12):1539-43. 1989

[12] **Virgil F and George G** .Biochemical aspects of hematology. Tietz textbook of clinical chemistry by corl A and Edward R, 2nd ed. WB Saunders Company. USA ch. 37;; pp 1982-1994. 2000

[13] **Tietz NW.** Methods of determination of ascorbic acid. Text book of clinical biochemistry.:960-2. 1986

[14] **Pleban PA, Munyani A, Beachum J.** Determination of selenium concentration and glutathione peroxidase activity in plasma and erythrocytes. Clinical Chemistry.; 28(2):311-6. 1982

[15] **Misra HP, Fridovich I.** The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry.; 247(10):3170-5. 1972

[16] **Beers RF, Sizer IW.** A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol chem.; 195(1):133-40. 1952

[17] **Karadeniz M, Erdoğan M, Ayhan Z, Yalcın M, Olukman M, Cetinkalp S, Alper GE, Eroglu Z, Tetik A, Cetintas V, Ozgen AG.** Effect Of G2706A and G1051A polymorphisms of the ABCA1 gene on the lipid, oxidative stress and homocysteine levels in Turkish patients with polycystıc ovary syndrome. Lipids in health and disease.;10(1):1. 2011

[18] **Mohan SK, Priya VV.** Lipid peroxidation, glutathione, ascorbic acid, vitamin E, antioxidant enzyme and serum homocysteine status in patients with polycystic ovary syndrome. Biology and Medicine.; 1(3):44-9. 2009

[19] **Chandra R, Aneja R, Rewal C, Konduri R, Dass SK, Agarwal S.** An opium alkaloid-Papaverine ameliorates ethanol-induced hepatotoxicity: diminution of oxidative stress. Indian Journal of Clinical Biochemistry.; 15(2):155-60. 2000

[20] **Dinger Y, Akcay T, Erdem T, Ilker Saygi˙ li˙ E, Gundogdu S.** DNA damage, DNA susceptibility to oxidation and glutathione level in women with polycystic ovary syndrome. Scandinavian journal of clinical and laboratory investigation.; 65(8):721-8. 2005

[21] **Murri M, Luque-Ramírez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF.** Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. Human reproduction update.:dms059. 2013

[22] **Wright D, Sutherland L.** Antioxidant supplementation in the treatment of skeletal muscle insulin resistance: potential mechanisms and clinical relevance. Applied Physiology, Nutrition, and Metabolism.; 33(1):21-31. 2008

[23] **Hosseinzadeh FM, Hosseinzadeh-Attar MJ, Yekaninejad MS, Rashidi B.** Effects of selenium supplementation on glucose homeostasis and free androgen index in women with polycystic ovary syndrome: A randomized, double blinded, placebo controlled clinical trial. Journal of Trace Elements in Medicine and Biology.; 34:56-61. 2016

[24] **Oram JF.** Tangier disease and ABCA1. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids.; 1529(1):321-30. 2000