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Research Article

Investigation of variants of critically important antioxidant enzyme genes in patients with polycystic ovary syndrome

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ABSTRACT

Aim: To investigate the possible effects of polymorphisms in genes encoding some important antioxidant enzymes such as super oxide dismutase 2 (SOD2), glutathione peroxidase 1 (GPX1), endothelial NOS (eNOS) and catalase (CAT) in patients with polycystic ovary syndrome (PCOS).

Methods: Peripheral blood of 100 patients with PCOS and 100 healthy control group were collected, Polymorphisms in related genes was investigated by using polymerase chain reaction-restriction fragment length polymorphism. In addition, the related biochemical values of the patients were also investigated.

Result: In our study there is no significant results for SOD2 gene but the results obtained between GPX1, eNOS and CAT genes were significant. Fasting blood sugar (FBS), insulin, triglyceride, waist circumference and dehydroepiandrosterone sulphate (DHEAS) were found to be significant with the disease, whereas follicle-stimulating hormone (FSH) was found to be effective in preventing the disease.

Conclusions: These findings suggest that polymorphisms in genes encoding GPX1, eNOS and CAT enzymes may be associated with PCOS. Additionally, it is thought that the genes of FBS, triglyceride, insulin, DHEAS and waist circumference are important in the pathogenesis of the disease in the presence of homozygous mutation.

Keywords: Polycystic ovarian syndrome, antioxidant enzyme genes, polymorphisms.

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Introduction

Polycystic over syndrome (PCOS); is a complex, chronic, metabolic disease characterized by anovulation and hyperandrogenism, affecting approximately 5-10% of women in the reproductive period [1]. Chronic uncommitted estrogen effects in patients with PCOS are features that may increase chronic anovulation, obesity, hyperinsulinemia, endometrial hyperplasia and adenocarcinoma risk. Anovulation causes endometrium to be exposed to mitogenic effects of estrogen, and this effect, which cannot be met by progesterone, is continuous, resulting in atypical or atypical endometrial hyperplasia and increased cancer incidence [2]. Estrogen and its metabolites play a role in tumor development with direct damage to DNA [3]. At the same time estrogen and its metabolites enter the redox cycle and form oxygen radicals, which cause oxidative stress, lipid peroxidation [4] and cause DNA damage. [3-5].

Polymorphisms in genes encoding antioxidant enzymes cause various diseases [6]. Oxidant and antioxidant systems, which are important in the physiological process in organism, have many roles on female reproductivities. Infertility etiopathogenesis plays an important role in oxidative stress in women. Oxidative stress has been shown to play a role in the development of reproductive diseases such as polycystic over syndrome, endometriosis and unexplained infertility [7]. Nitric oxide (NO) is a mediator role in reproductive events; NO is one of the many intraovarian agents involved in the ovary. NO plays a role in the fulfillment of blood-follicular barrier function by gonadotropins. NO as an antioxidant, may play a role in pubertal maturation, ovulation capacity, early embryological development, gestational continuation, and menopause timing, as well as relaxation in vascular smooth muscles, as compared to preliminary studies in humans, and also NO has vasodilation effect [8,9]. Because of all the functions of NO which mentioned above, we think that endothelial NOS (eNOS) polymorphism may be related to PCOS.

Another antioxidant is glutathione peroxidase 1 (GPX1) gene that is expressed in prostate, breast and reproductive tracts cells and protects them against oxidative damage. Low GPX1 activity increases oxidative stress and increases susceptibility to various diseases and cancers [10]. GPX1 is a selenium-dependent cytosolic antioxidant and GPX1gene family has important roles in electron transport and free radical steps [11]. Superoxide dismutase 2 (SOD2) directly converts superoxide radicals to hydrogen peroxide and molecular oxygen. It has been shown that polymorphism-inducing genes encoding SOD enzyme are predisposed to Behçet, diabetes and various types of cancer [12]. Catalase; in the glycoprotein structure, it is a hemoprotein composed of four subunits and mainly found in the cytoplasm and endoplasmic reticulum of the cell. Particularly when the amount of H_2O_2 is excessively increased, it enters the circuit and turns this molecule into water with a great specificity [13,14]. We investigated the effectiveness of the polymorphisms of genes encoding antioxidant enzymes such as SOD2, GPX1, eNOS, and catalase (CAT) in the etiopathogenesis of PCOS.

Methods

The patient group of our study was composed of 100 female patients aged 15-39 years who applied to Bolu Abant Izzet Baysal University Medical Faculty Obstetrics and Gynecology Department and clinically diagnosed as PCOS. Ethics committee approval for the study was

obtained from Istanbul University Clinical Research Ethics Committee (/2015/104). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors. Demographic characteristics of patients are shown in Table 1. Informed consent was obtained from all individual participants included in the study. Informed consent forms were obtained from parents for individuals younger than 16 years of age. When the control group was established, care was taken to ensure that there was no evidence of clinical or biochemical hyperandrogenism with a regular mens cycle, and that they did not have diagnostic criteria for PCOS. Peripheral venous blood of the subjects included in the study were taken with the tubes with ethylenediaminetetraacetic acid (EDTA). Subsequently, genomic DNAs were isolated at molecular genetics laboratory of our department using appropriate isolation kit. Polymerase chain reactions (PCRs) were performed under the appropriate conditions using the isolated DNAs, and primers designed for the gene regions. The PCR, restriction enzyme digestion, and electrophoresis procedures of the study were carried out at Istanbul University, Aziz Sancar Experimental Medical Research Institute, Molecular Medicine Department. In addition, fasting blood sugar (FBS), insulin, thyroid stimulating hormone (TSH) free triiodothyronine (free T3), free thyroxine (free T4), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), very-low-density lipoprotein (VLDL), luteinizing hormone (LH), estradiol 2 (E2), prolactin hormone, progesterone, testosterone, dehydroepiandrosterone sulphate (DHEAS), follicle-stimulating hormone (FSH) and waist circumference were evaluated. Analysis of the biochemical data of the study group was made by Department of Medical Biochemistry, Bolu Abant Izzet Baysal University Medical faculty.

Statistical analysis

Statistical analysis of the data was performed using the SPSS 17.0 package program. Chi-square test was used to compare categorical variables. In comparison of continuous variables between groups, it was determined whether they were parametric or non-parametric by Shapiro Wilk test. Student's t test was then performed for parametric subjects and Mann Whitney U test for non-parametric subjects. The analysis of dependent and independent variables was performed by Binary Logistic Regression analysis. Assessment of risk factors for all genotypes and alleles belonging to the gene was performed using the Kruskall Wallis test, provided that the groups were within the group.

Results

Significant results were found for the weight and waist circumference of patients and control groups ($p < 0{,}001$ and $p < 0{,}001$). There was no significant difference between the groups in terms of age and height ($p = 0{,}085$ and $p = 0{,}243$) (Table 1). In terms of biochemical parameters, TG ($p < 0{,}001$), VLDL ($p < 0{,}001$), free T3 ($p=0{,}027$), FSH ($p<0{,}001$), DHEAS ($p=0{,}006$), testosterone ($p<0{,}001$), fasting blood sugar ($p=0{,}027$), insulin ($p=0{,}001$). Statistically significant difference was observed in terms of values that are given in Table 2. As a result of analysis of the polymorphisms in the genes, polymorphisms in GPX1 ($p = 0{,}002$), eNOS (p

= 0,001), CAT (p = 0,031) genes were found to be significant among the groups. Polymorphism in the SOD2 gene was not found to be statistically significant between the groups (Table 3).

Tablo 1. Evaluation of demographic properties of the groups.

Physical properties	PCOS (n=100)	Control (n=100)	*p*
Size	161,84±5,4	162,84±6,5	0,243
Age	24,14±5,2	25,20±5,3	0,085
Weight	64,95±14,2	57,51±7,4	<0,001
Waist circumference	117±13,5	73,17±8,8	<0,001

Tablo 2. Evaluation of the biochemical parameters of the groups.

Parameters	PCOS (n=100) Ort±SD	Control (n=100) Ort±SD	*p*
Cholesterol	169,93±36,4	163,44±24,37	0,374
Triglyceride	99,16±55,05	71,62±23,58	<0,001
HDL	49,58±11,71	52,48±9,62	0,057
LDL	98,9±28,15	96,57±19,90	0,753
VLDL	19,18±9,20	14,32±4,68	<0,001
Free T3	3,93±0,67	3,73±0,56	0,027
Free T4	1,07±0,12	1,08±0,13	0,652
TSH	1,77±0,87	1,83±1,08	0,644
FSH	5,43±1,94	6,75±2,60	<0,001
LH	6,83±4,40	5,85±3,66	0,128
E2	53,85±35,74	45,77±22,31	0,175
PRL	14,50±7,61	15,63±7,79	0,336
P	0,38±0,37	0,38±0,17	0,137
DHEAS	231,71±96,08	197,17±86,49	0,006
Testosterone	36,59±27,43	27,49±12,39	<0,001
FBS	90,18±10,79	86,91±7,89	0,027
Insulin	7,48±12,09	4,07±4,32	0,001

Table 3. The genotype and allele distributions of genes in the patient and the control group.

	Patient (n)	%	Control (n)	%	*P*
SOD2	CC 19	19.0	17	17.0	
	CT 45	45.0	39	39.0	0.512
	TT 36	36.0	44	44.0	
	C 83	41.5	73	36.5	
	T 117	58.5	127	63.0	0.305
GPX1	CC 32	32.0	34	34.0	
	CT 26	26.0	45	45.0	0.002
	TT 42	42.0	21	21.0	
	C 90	45.0	113	56.5	
	T 110	55.0	87	43.5	0.002
eNOS	GG13	13.0	30	30.0	
	GT 32	32.0	39	39.0	0.001
	TT 55	55.0	31	31.0	
	G 58	29.0	99	49.5	
	T1 142	75.0	101	50.5	0.001
CAT	AA 9	9	21	21.0	
	AT 45	45	46	46.0	0.031
	TT 46	46	33	33.0	
	A 63	31.5	88	44.0	
	T 137	68.5	112	56.0	0.001

When the genotypes and alleles of the genes were evaluated, SOD2 (TT), GPX11 (TT) and eNOS (TT) homozygous mutation genotypes were statistically significant between the groups (p = 0,024 and p = 0003) while CAT (TT) showed no significant difference between the groups for homozygous genotypes (p = 0,262 and p = 0,535). In addition, GPX1 (TT) and eNOS (TT) genotypes alone and in patients and controls were evaluated with other risk factors. Assessment of PCOS risk factors in the presence of the homozygous mutation genotype (TT) of GPX1 gene is given in Table 4. The genotype of homozygous mutation in GPX1 gene was found to be significant among the groups. However, GPX1 homozygote mutation was also found to be significant when compared to PCOS risk factors. In addition, GPX1 homozygote mutation genotype, TG,

FSH and DHEAS were found to be significant in PCOS (p=0,001, p = 0,005 and p = 0,026). Significant results were obtained when the presence of the eNOS homozygous mutation genotype and the risk factors of PCOS were evaluated together. TG, FSH and DHEAS were statistically significant in the presence of the eNOS homozygous mutation genotype (p <0,001, p = 0,014 and p = 0,005).

Table 4: Evaluation of PCOS risk factors in the presence of GPX1 homozygous mutation genotype.

Homozygous mutation model	*P*	OR	%95 CI
GPX1 (TT)	0.011	2,487	1,232-5,019
FBS	0.600	1,012	0,97-1,060
TG	<0,001	1,021	1,010-1,033
FSH	0.005	0,794	0,677-0,933
Waist circumference	0.258	1,016	0,988-1,045
DHEAS	0.026	1,004	1,000-1,008
Insulin	0.235	1,037	0,977-1,100

The evaluation of heterozygous genotypes in the genes by PCOS risk factors was examined by Binary Logistic Regression analysis and given in Table 5. When the risk factors for PCOS were evaluated in the presence of heterozygous genotypes of the genes, it was found that GPX1 CT was significant (p = 0,038), and SOD2 TC, eNOS GT and CAT AT genotypes were not significant. (p = 0,301, p = 0,403 and p = 0,733). TG, FSH and DHEAS are significant risk factors. (p = 0,001, p = 0,007 and p = 0,013). Evaluation of CT genotype of GPX1 by PCOS risk factors is given in Table 6. The GPX1 heterozygous genotype was found to be significant when assessed by PCOS risk factors and heterozygous genotypes of other genes. In addition, when GPX1 CT genotype was analyzed together with PCOS risk factors, TG, FSH and DHEAS were significantly found in the presence of GPX1 heterozygous genotype (p = 0,001, p = 0,005 and p = 0,014).

Table 5. Evaluation of PCOS risk factors in the presence of heterozygous genotype in the genes.

Heterozygous model	*P*	OR	%95 CI
SOD2 (CT)	0,301	1,420	0,730-2,761
GPX1 (CT)	0,038	0,478	0,238-0,961
eNOS (GT)	0,403	0,740	0,365-1,499
CAT (AT)	0,733	1,123	0,576-2,188
FBS	0,684	1,010	0,964-1,058
TG	0,001	1,022	1,009-1,035
FSH	0,007	0,798	0,678-0,940
Insulin	0,332	1,027	0,973-1,084
Waist circumference	0,100	1,031	0,994-1,069
DHEAS	0,013	1,005	1,001-1,009

Assessment of PCOS risk factors with mutant alleles is given in Table 7. The analysis PCOS risk factors, which is significant with the disease-associated mutant alleles, was found to be significant among the mutant allele groups in the eNOS gene (p = 0,007).

Table 6. Significance of PCOS risk factors in the presence of the GPX1 heterozygous genotype.

Heterozygous model	*P*	OR	%95 CI
GPX1	0,034	0,475	0,238-0,947
FBS	0,792	1,006	0,961-1,053
TG	0,001	1,022	1,009-1,035
FSH	0,005	0,795	0,677-0,933
Waist circumference	0,112	1,030	0,933-1,068
DHEAS	0,014	1,005	1,001-1,009
Insulin	0,278	1,032	0,975-1,091

The combined analyzes of the homozygous mutant and heterozygous genotypes of the genes were evaluated by the chi-square test and are given in Table 8. The combination of SOD2 TT homozygous mutation with GPX1 TT, eNOS TT and CAT TT homozygous mutations did not show any significance for PCOS in the patient and control group (p = 0,346, p = 0,577 and p = 1,000). In addition, the association of genotype GPX1 TT homozygous mutation with eNOS TT and CAT TT homozygote mutation genotype was found to be significant for PCOS among the groups (p <0,001, p <0,001). In addition, the combination of the eNOS TT homozygous mutation with the CAT TT genotype was found to be significant (p <0,001). When the combined analysis of SOD2 gene and CAT gene was performed, it was found that there was a significant difference between SOD2 heterozygous genotype carriers and CAT wild type genotype carriers. The proportion of patients with SOD2 heterozygote genotype carriers and CAT wild type genotypes was found to be more significant (p = 0,048). When combined with GPX1 and eNOS genotypes, GPX1 mutant genotype carriers and eNOS mutant genotype carriers showed a significant difference in disease-related groups (p = 0,001). In addition, it can be said that the risk of disease may be low even with the GPX1 heterozygote genotype and eNOS wild type genotype.

Table 7. Evaluation of PCOS risk factors in mutant allele existence.

Mutant Allele Model	*P*	OR	%95 CI
SOD2 (T)	0,377	0,661	0,263-1,659
GPX1 (T)	0,780	1,108	0,539-2,280
eNOS (T)	0,007	3,334	1,381-8,048
CAT (T)	0,074	2,546	0,913-7,098
FBS	0,951	0,999	0,952-1,047
TG	0,001	1,023	1,010-1,036
FSH	0,008	0,797	0,673-0,943
Insulin	0,215	1,044	0,975-1,118
Waist circumference	0,179	1,026	0,988-1,065
DHEAS	0,006	1,005	1,002-1,009

GPX1 heterozygous genotype carriers and CAT wild genotype carriers were found to be significant in terms of protection against disease when combined genetic analysis of GPX1 and CAT gene genotypes were

performed (p = 0,001). GPX1 heterozygote genotype and CAT wild type genotype association; It is found in 10% in control group and 1% in patient group.

Tablo 8. Evaluation of combined analyzes of homozygous mutation genotypes and heterozygous genotypes of genes.

Genotip	PCOS (n)	Control (n)	OR (%95 CI)	*P*
SOD2 CC and GPX1 TT	12	8	0,638 (0,249-1,634)	0,346
SOD2 CC and eNOS TT	19	16	0,812 (0,391-1,688)	0,577
SOD2 CC and CAT 21 TT	16	16	1,000 (0,470-2,130)	1,000
GPX1 TT and eNOS TT	24	5	0,167 (0,061-0,457)	<0,001
GPX1 TT and CAT 21 TT	25	6	0,191 (0,075-0,491)	<0,001
eNOS TT and CAT 21 TT	25	15	0,529 (0,260-1,078)	0,077
SOD2 TC and GPX1 CT	12	17	1,502 (0,677-3,334)	0,315
SOD2 TC and eNOS GT	14	18	1,348 (0,630-2,887)	0,440
SOD2 TC and CAT 21 AT	19	19	1,000 (0,493-2,027)	1,000
GPX1 CT and eNOS GT	9	18	2,220 (0,945-5,214)	0,063
GPX1 CT and CAT 21 AT	16	26	1,845 (0,919-3,703)	0,083
eNOS GT and CAT 21 AT	14	25	2,048 (0,993-4,223)	0,050

The significance of the combinations of heterozygous genotypes in the genes between the groups in terms of PCOS was evaluated by chi-square test. The combination of the SOD2 TT homozygous mutation with the GPX1 TT, eNOS TT and CAT TT homozygous mutations did not appear to be meaningful in terms of PCOS in the patient and control group. There was no statistically significant difference (p = 0,315, p = 0,440, p = 1,000) as a result of the combination of SOD2 heterozygous genotype with GPX1, eNOS and CAT heterozygote genotype. The combination of the GPX1 CT heterozygous genotype with the eNOS GT genotype and the CAT AT genotype did not yield any conclusive results (p=0,063, p= 0,083). In addition, there was no significant result in the co-transformation of eNOS GT heterozygote genotype and CAT AT heterozygote genotype (p = 0,050).

Discussion

It is known that weight-obesity is an important risk factor when assessed in terms of general demographic characteristics of control and patient groups, and that about 50% of cases with PCOS are obese and thus more central and android type [15]. Our study supports this and statistically significant results have been found in terms of obesity and weight in our patient group. It has been reported that several pathological processes such as uric acid, oxidative stress and the formation of oxygen radicals and inflammation are associated with the studies. In a study performed by Havva Keskin et al. [16] a very significant result found between PCOS and uric acid. In our study, we found that the uric acid level was statistically higher in the PCOS group than in the control group. In our study, HDL level in PCOS was found to be lower than control group. TG level was higher in PCOS than

control group and a meaningful result was obtained. Adamska et al. [17] reported that the testosterone concentration in the study with PCOS was higher than the control group. In this study, dehydroepiandrosterone sulphate and testosterone levels were also found to be significant among the groups. In addition, the amount of free testosterone is increasing. Increased free estradiol and free estradiol lead to suppression of FSH levels and increased LH in women with PCOS [18]. In this study, FSH value was found to be lower in patients with PCOS compared to the control group and statistically significant in the positive direction. Based on these data, it can be said that FSH hormone is protective against the disease in PCOS. In our study, there was no significant difference between groups in terms of SOD2 Ala / Val polymorphism. However, the presence of the SOD2 homozygote mutation alone did not pose a risk in PCOS. TG, FSH and DHEAS were found to be significant in the presence of SOD2 homozygous mutation genotype. Evaluation of the combination of the SOD2 TT homozygous mutation with homozygous mutations of GPX1, eNOS and CAT genes did not result in a significant value. When the SOD2 gene is also analyzed with other genes it does not make much sense for PCOS. However, when we evaluated the mutant allele combination of eNOS and T mutant allele in SOD2 gene, significant results were obtained in terms of PCOS. In addition, when combined analysis of SOD2 gene and CAT gene was found, there was a significant difference between groups carrying SOD2 heterozygote genotype and CAT wild type genotype. Glutathione peroxidase, an antioxidant, plays an important role in many cases such as signal transduction, spermiogenesis, regulation of pre-inflammatory cytokine production, and inactivation of inflammatory ROTs [19,20]. GPX1 Pro198Leu polymorphism has been reported to be an important risk factor for cancer formation in studies performed in different countries [21-23]. Regression analysis of the risk factors in PCOS by homozygous mutation of GPX1 and other genes showed that the presence of the GPX1 homozygote mutation genotype (TT) was significant and pose a risk to the disease. In addition, TG, FSH and DHEAS were found to be a risk factor for the concurrent assessment of PCOS risk factors in the presence of GPX homozygous mutation. However, it was found that the measurement of Fasting Blood Sugar, insulin and waist circumference was not statistically significant. In addition, FSH has been shown to be protective against the disease in the presence of GPX1 homozygous mutation. When GPX1 heterozygous genotype and PCOS risk factors were analyzed, TG, FSH and DHEAS were found to be significant in the presence of GPX1 CT genotype, but the measurements of Fasting Blood Sugar, insulin and waist circumference were not significant. In terms of the combination of homozygous mutation genotypes, the association of GPX1 with NOS and CAT was significantly found in PCOS. In addition, the mutant allele combination of the GPX1 and eNOS genes was found to be significant for PCOS. Also GPX1 and eNOS were analyzed in combination, significant differences were found in the groups in terms of disease when GPX1 mutant genotype carriers and eNOS mutant genotype carriers coexisted. It can be said that the risk of disease may be low in GPX heterozygote genotype and NOS wild type genotype bearers. Catalase enzyme plays a crucial role in defense against oxidative stresses that occur in pathological conditions such as Diabetes Mellitus, neurodegenerative diseases, cancer

and nutritional deficiency [24]. Many researchers have investigated catalase polymorphisms and breast cancer, cervical cancer, prostate cancer, pancreatic cancer and colectal cancers and found significant results [25-29]. Significant results were obtained in terms of Catalase-21A / T gene polymorphism between control and patient groups in our study. Together with heterozygous genotype of catalase homozygous mutation genotype and other genes, TG, FSH and DHEAS were found to be statistically significant. Besides, FSH has a protective effect against disease in the presence of CAT homozygous mutation. When the allele (T) associated with the disease in the catalase gene is evaluated together with PCOS risk factors and mutant alleles of other genes, the T allele in Catalase gene is found to be insignificant by itself. Significant results were found in combination with homozygous mutation genotype of catalase gene in combination with GPX1 and eNOS homozygote mutation genotype.

Nitric oxide and endothelium nitric oxide synthase play an important role in endothelial function, regulation: of vascular wall tension and vasculoprotective properties [30-32]. Nitric oxide also has antioxidant properties. Up to now, it has been reported that there are too many reports describing eNOS polymorphisms and possible associations with diseases [33]. Glu298Asp polymorphism is the most frequently studied variant [34]. The Glu 298 Asp polymorphism disrupts the primary structure of the protein and results in functional changes in the enzyme. In our study, there was a statistically significant result in terms of eNOS Glu298Asp polymorphism between patient and control groups. The genotype of the eNOS homozygote mutation was also found to be significant when the eNOS gene was evaluated together with PCOS risk factors and homozygous mutations in other genes. Furthermore, in the presence of the homozygous mutation genotype (TT) of eNOS, together with the risk factors for PCOS, TG, FSH and DHEAS were statistically significant. Statistically significant results were found when different genotypes of eNOS gene and other genotypes of other genes are evaluated together. Besides, significant results were found in cases of NOS3 association with GPX1 and CAT genes homozygous mutation genotypes. Significant results were also found in situation of the combination eNOS, SOD2 and GPX1 mutant alleles.

Conclusion

When the different genotypes in the genes are analyzed in combination, and when the different genotypes come together, the activity in the disease varies greatly. It will be possible to better understand the role of polymorphisms in genes encoding antioxidant enzymes in the etiopathogenesis of PCOS in future studies with a more comprehensive patient and control group.

Ethics Committee Approval: *Ethics committee approval for the study was obtained from Istanbul University Clinical Research Ethics Committee (07.05.2015/104).*

Informed Consent: *Written informed consent was obtained from the patients who participated in this study.*

Conflict of Interest: *No conflict of interest was declared by the authors.*

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***Abbreviations:** SOD2: Superoxide Dismutase 2, GPX1: Glutathione Peroxidase1, eNOS: Endothelial Nitric Oxide Synthase 3, CAT: Catalase, HDL: High Density Lipoprotein, LDL: Low density Lipoprotein, VLDL: Very Low Denstiy lipoprotein, Free T3: Free tiriiyodotironin, Free T4: Free thyroxine, TSH: Thyroid Stimulating Hormone, FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone, E2: Estradiol 2, PRL: Prolactin Hormone, P: Progesterone, DHEAS: Dehidroepiandrosteron.*

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