Review Article

Oxidative Stress and Polycystic Ovary Syndrome: A Brief Review

Masoumeh Mohammadi

Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Polycystic ovary syndrome (PCOS) is one of the most common hormonal disorders, occurring in 5–10% women in reproductive ages. Despite a long history of studies on PCOS, its etiology is still unknown. Oxidative stress is now recognized to play a central role in the pathophysiology of many different disorders, including PCOS. Although intracellular reactive oxygen species (ROS) production and propagation are controlled by highly complex antioxidant enzymatic and non-enzymatic systems, understanding of mechanisms that oxidative stress is important to develop strategies for prevention and therapy of PCOS. This article reviews the literature data related to the mechanisms of oxidative stress in PCOS.

Keywords: *Antioxidant, oxidative marker, oxidative stress, polycystic ovary syndrome, reactive oxygen species*

Introduction

Polycystic ovary syndrome (PCOS) is a public health important disease, affecting at reproductive age and associated with reproductive, metabolic, and psychological dysfunction. PCOS women commonly have features of hyperandrogenism and hirsutism, oligo or amenorrhea, and unovulation. Despite a long history of studies on PCOS, its etiology is still unknown about the pathophysiology of PCOS, have explained the roles of inflammatory state, endothelial injury, oxidative stress, and genetic mechanisms.[1-3]

Oxidative stress is referred as the imbalance between oxidants and antioxidants and the generation of excessive amounts of reactive oxygen species (ROS). A lot of investigations have revealed that oxidative circulating markers are significantly increased in patients with PCOS compared with the normal and are considered as a potential inducement of PCOS pathogenesis.[1]

Oxidative stress is now recognized to play a central role in the pathophysiology of many different disorders, including PCOS. The present review study provides an overview of current knowledge about important oxidative stress roles in the pathogenesis of PCOS.

Methods and Literature Search Strategy

An electronic literature search of databases including PubMed, Medline, Web of sciences, Embase, and Scopus was conducted for publications, in English, up to 2018. The search terms included "Polycystic ovary syndrome," "PCOS," "Reactive oxygen species (ROS)," "Oxidative marker," "Oxidative stress (OS)," "Antioxidant," "Malondialdehyde (MDA)," "Nitric oxide (NO)," "Advanced glycosylated end products (AGEs)," "Xanthine oxidase (XO)," "Superoxide dismutase (SOD)," "Glutathione peroxidase (GPx)," "Total antioxidant capacity (TAC)," "Vitamin E," and "Vitamin C," or their equivalents were used individually or/and in various combinations to retrieve the relevant literatures. In addition, the reference lists of all identified literature were also checked to identify additional relevant articles.

Overview of oxidative stress

Oxidative stress as a general term usually used to describe an imbalance between the production of free radicals and the ability of the body to defense their harmful effects by antioxidants that cause DNA damage and/or cell apoptosis. Disturbances in the normal oxidation reaction of cells and the production of free radicals and peroxides can cause toxic effects that damage the cell.

Address for correspondence:
Dr. Masoumeh Mohammadi, Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
E-mail: m_gorgian@yahoo.com; mgm8362@gmail.com

Access this article online

Website: www.ijpvmjournal.net/www.ijpm.ir

DOI: 10.4103/ijpvm.IJPVM_576_17

Quick Response Code:

How to cite this article: Mohammadi M. Oxidative stress and polycystic ovary syndrome: A brief review. Int J Prev Med 2019;10:86.

The effects of oxidative stress depend on the percentage of these changes from DNA damage and trigger apoptosis and necrosis to cell death.[4]

ROS represent a class of molecules that is derived from the metabolism of oxygen in aerobic organisms. The major ROS with physiological function are superoxide anion ($O2^{-}$), hydroxyl radical (OH), hydrogen peroxide (H_2O_2), organic hydroperoxide (ROOH), alkoxy and peroxy radicals (RO and ROO), hypochlorous acid (HOCl), and peroxynitrite (ONOO-). ROS can act as second messengers in mammalian cells to regulate signal transduction pathways that lead to control gene expression and proteins post-translational changes, involved in cell function, growth, differentiation, and death.[5,6]

There are several sources that generate the ROS. One source of reactive oxygen under normal conditions in humans is the leakage of activated oxygen from mitochondria during oxidative phosphorylation.[7] Other endogenous source of ROS is the leakage of activated oxygen from the detoxification reactions involving the liver cytochrome P-450 enzyme system,[8] peroxisomal oxidases,[9] NAD (P) H oxidases,[10] or XO.[11,12] Exogenous sources include exposure to environmental pollutants; cigarette smoke; consumption of alcohol; exposure to ionizing radiation; and bacterial, fungal, viral infections, etc.[13]

In return, there are molecules in the cells that prevent these reactions by donating an electron to the free radicals without becoming destabilized with the name of antioxidant. In fact, cellular ROS productions are controlled by highly complex antioxidant enzymatic and non-enzymatic systems. The important antioxidant enzymes such as SOD, catalase (CAT), GPx glutathione S-reductase (GSR), Glucose-6-phosphate dehydrogenase (G6PD), and isocitrate dehydrogenase (ICDH) are compartmentalized within the cell by defenses against ROS propagation, oxidative stress, and tissue damage.[14] Non-enzymatic antioxidant systems, system that contains vitamins such as E, C, and A are among the major dietary antioxidants that directly neutralize ROS, provide a major source of protection against the side effects of ROS.[15,16] Trace elements act as cofactors in the regulation of antioxidant enzymes specially Cu, Zn, Mn, and Se are essential constituents of enzymatic antioxidant systems such as Cu-Zn-SOD (SOD1), Mn-SOD (SOD2), and Se-GPx.[14] Polyphenols and ubiquitous, as natural antioxidants, are in diet specially in vegetables and fruits.[17]

Reactive oxygen species diverse actions on cell function

1. Activation of redox-sensitive transcription factors: Redox-sensitive transcription factors such as AP-1, p53, and NF-κB regulate the expression of pro-inflammatory and other cytokines, cell differentiation, and apoptosis. PCOS is associated with low-grade inflammation and raised inflammatory cytokines, which contribute to the pathogenesis of this syndrome. The majority of studies addressing the status of chronic low-grade inflammation in PCOS has focused on the measurement of circulating C-reactive protein (CRP), TNFα, IL-6, and IL-18[18,19]

   Activation of protein kinases: In fact, with activation of protein kinases, cells respond to a variety of extracellular signals and stress that may lead to more extensive cell damage, resulting ultimately in cell death through necrosis or apoptosis.[20] ROS influence the mitogen activated protein kinase signaling pathways that are involved in diverse physiological processes[21] because these pathways are major regulators of gene transcription in response to oxidative stress by promoting the actions of receptor tyrosine kinases, protein tyrosine kinases, receptors of cytokines, and growth factors.[22,23] About pathogenesis of insulin resistance in PCOS patient, several studies indicated that with the increased oxidative stress, various protein kinases are activated to induce serine/threonine phosphorylation of, insulin receptor substrate (IRS), inhibit normal tyrosine phosphorylation of IRS, and finally, induce the degradation of IRS.[24,25] Other pathways that can be activated by ROS include the c-Jun *N*-terminal kinases (JNK) and p38 pathways. JNK is a component of the transcription factor activator protein-1 (AP-1). AP-1 regulates the transcription of numerous genes such as cytokines, growth factors, inflammatory enzymes, matrix metalloproteinase, and immunoglobulins
2. Opening of ion channels: Increase of ROS lead to release of Ca2+ ions from the endoplasmic reticulum and other stores and loss of intracellular Ca2+ homeostasis. Excess the cytosolic Ca2+ ion concentration will adversely affect such as instability in the mitochondrial membrane and also collapse of adenosine triphosphate (ATP) synthesis, finally the cell undergoes primary necrosis.[26,27] In PCOS women, studies showed calcium dysregulation contributes to the development of follicular arrest, resulting in reproductive and menstrual dysfunction[28]
3. Protein oxidation: Amino acids, both in proteins and free, are a target for oxidative damage. Direct oxidation of the side chains leads to the formation of carbonyl products.[29] Carbonyl products lead to a loss of protein function, which is considered an indicator of oxidative damage and disease-derived protein dysfunction.[30] Plasma advanced oxidation protein products (AOPPs) in serum of PCOS patients had significantly higher than control women. AOPPs have been considered as novel markers of oxidant-mediated protein damage and may also act as a novel class of pro-inflammatory mediators[31]
4. Lipid peroxidation: Lipid peroxidation is occurring in the polyunsaturated fatty acid side chains of the plasma membrane or that of any organelle that contains lipid. These fatty acid side chains react with O_2 and create

the peroxyl radical, which can obtain H+ from another fatty acid, creating a continuous reaction.[32] These chain reactions with vitamin E can break and act as an antioxidant because vitamin E has lipid solubility and hydrophobic tail.[27] Levels of markers that could reflect the degrees of lipid peroxidation, such as thiobarbituric acid-reactive substances, oxidized low-density lipoprotein, and malondialdehyde (MDA) increase significantly in the PCOS patients compared with the normal. In addition, serum lipid peroxide concentrations were increased in patients with PCOS[33,34]

5. DNA oxidation: It occurs at guanine residues because of the higher oxidation potential of this base than cytosine, thymine, and adenine. Mitochondrial DNA is particularly vulnerable to ROS attack owing to O^2- generation from the electron transport chain, lack of histone protection, and absence or minimal repair mechanisms that exist.[35] Free radical mediated DNA damage and impaired antioxidant defense have been implicated as contributory factors for the development of cancer. Dinqer *et al*. evaluated H_2O_2-induced DNA damage, a marker of DNA susceptibility to oxidation in PCOS women. They showed a significant increase in DNA strand breakage and H_2O_2-induced DNA damage that may explain the association between PCOS and ovarian cancer.[36]

Oxidative stress biomarkers in PCOS

For investigation of the oxidative stress role in the pathogenesis of diseases, mainly, we have examined oxidative stress biomarkers including MDA and NO also anti-oxidative biomarkers such as TAC, SOD, GPx, and glutathione (GSH). The evaluation of oxidative stress and antioxidant biomarkers have been suggested as useful tools in estimating the risk of oxidative damage and associated diseases[37,38] and help in the prevention and management of oxidative diseases. A number of oxidative stress markers in PCOS are listed in Table 1. These biomarkers in PCOS reported in several studies as follows:

Table 1: Oxidative stress markers in polycystic ovary syndrome (PCOS) patients

Markers reflecting oxidative stress levels	Source	OS levels of PCOS patients compared with the normal
Malondialdehyde (MDA)	Serum; erythrocyte	Higher
Nitric oxide (NO)	Serum	Higher Similar
Advanced glycosylated end products (AGEs)	Serum	Higher
Xanthine oxidase (XO)	Serum	Higher

Table 2: Antioxidative stress markers in polycystic ovary syndrome (PCOS) patients

Markers reflecting antioxidative stress levels	Source	OS levels of PCOS patients compared with the normal
Superoxide dismutase (SOD)	Serum; erythrocyte; and follicular fluid	Higher Lower
Glutathione peroxidase (GPx)	Serum	Lower Similar
Total antioxidant capacity (TAC)	Follicular fluid; serum	Higher Lower Similar
Vitamin E	Serum	Lower
Vitamin C	Serum	Lower

Malondialdehyde

MDA results from lipid peroxidation of polyunsaturated fatty acids[39] is stable and can serve as a good biomarker.[32] MDA level in PCOS reported in several studies. One meta-analysis showed that circulating mean MDA concentrations according to the age and BMI were increased 47% in women with PCOS compared with controls.[1]

Kuscu *et al*. compared blood MDA level in PCOS patients with healthy controls. They showed the MDA level was significantly higher in the PCOS group but was independent of obesity.[40] In another study, Zhang *et al*. demonstrated that serum MDA levels in PCOS patients were significantly higher than the control group, but BMI and age were not recorded.[41] In addition, Dursun *et al*. studied PCOS patients and found serum MDA levels in PCOS patients were similar to those of BMI and smoking status matched controls.[42]

Palacio *et al*. compared PCOS patients with BMI and age matched controls. They demonstrated that higher levels of erythrocyte MDA were seen in PCOS patients compared with controls. These results also were found by Sabuncu *et al*.[43,44]

Nitric oxide

NO is a free radical, and it is an important cellular signaling molecule involved in many physiological and pathological processes, but an excess of NO can be toxic. NO endogenously is biosynthesized by various nitric oxide synthase (NOS) enzymes from L-arginine, oxygen, and nicotinamide adenine dinucleotide phosphate. An alternative pathway, NO synthesis through the sequential reduction of nitrate derived from plant based foods.[45] It is also generated in immune responses through phagocytes by monocytes, macrophages, and neutrophils. NO level in PCOS reported in several studies. Recent meta-analysis study showed that the mean of the NO level had no statistically significant difference in women with PCOS compared with controls.[1]

Hassani *et al*. to evaluate the role of NO in PCOS, Wistar rats treated with L-Arginine, a precursor of NO, and results showed that the ovaries of rats treated with L-arginine had polycystic characteristics in contrast to control and believed that NO may play a major role in the pathophysiology of PCOS.[46]

Karabulut *et al*. investigated relation between PCOS and oxidative stress status by measuring NO in PCOS patients and healthy control. Results showed that statistically higher levels of NO in PCOS patients compared to the control women.[47]

Willis *et al*. compared measures of oxidative stress and NO metabolites in patients with PCOS and age/BMI matched control. Results showed similar nitrite but lower nitrate levels in subjects with PCOS (Nitrite/nitrate concentration is an index of endothelium-derived NO).[48] In addition, Nacul *et al*. reported that NO levels in PCOS patients were similar in age/BMI matched controls. They investigated NO and fibrinogen levels are as two markers of vascular disease that are associated with insulin resistance in PCOS women and showed a significant negative correlation between NO and fasting insulin levels and homeostatic model assessment. They suggested that NO was related to the presence of insulin resistance in PCOS patients.[49]

Advanced glycation end products

AGEs also called "glycotoxins," are the end products of a chemical procedure called the Maillard reaction in which the carbonyl group of carbohydrates reacts non-enzymatically and interacts with lipids or with amino groups of proteins.[50]

ROS catalyze the chemical modification of proteins by Maillard reactions in vivo.[51] AGEs, end product of this reaction, in turn, induce oxidative stress, finally leading to inflammation and the propagation of tissue damage. Thus, the production of AGEs and resultant oxidative stress accelerate Maillard reactions and can initiate an autocatalytic cycle of deleterious reactions in tissues.[52]

AGEs level as oxidative stress marker of PCOS reported in several studies and accumulating evidence has suggested the possibility of AGEs in altering steroid bio-synthesis in polycystic ovaries by affecting enzyme function, induction of inflammatory changes, and insulin resistance.[53] The result of abnormal steroidogenesis in PCOS could lead to elevated androgen synthesis and abnormal folliculogenesis.[54]

Diamanti-Kandarakis *et al*. investigated serum AGEs levels and receptor for AGEs (RAGE) expression in monocytes of PCOS and control group and their correlation with testosterone levels. They demonstrated higher serum AGEs protein levels and RAGE in patients with PCOS compared to control group. In addition, a positive correlation was observed between serum AGEs and testosterone levels, even after controlling for BMI.[55] In another study, the same authors fed Wistar rats diet containing high (H) or low (L) AGEs for 6 months. In H-AGE rats, they found elevated AGE deposition in ovarian theca interna cells, increased RAGE staining in granulosa cells, and higher plasma testosterone compared to L-AGE rats.[56] In addition, Tantalaki *et al*. investigated the role of AGE dietary intake on hormonal status of women with PCOS. They gave women with PCOS isocaloric diet containing high AGE or low AGE for 2 months and found higher serum AGE along with elevated testosterone, free androgen index , and androstendione levels in PCOS patients with the H-AGE diet compared to L-AGE diet.[57] These studies substantiate the association between AGE and hyperandrogenism in PCOS.

Xanthine oxidase

XO is an enzyme that participates in the generation of superoxide anion radicals.

This enzyme catalyzes the oxidation of hypoxanthine to xanthine and oxidation of xanthine to uric acid.[58] Serum XO, which plays an important role in the catabolism of purines in humans and generates ROS, was increased in PCOS in studies.[59] In addition HaticeIsık *et al*. investigated serum XO and SOD activities, hormonal levels, cholesterol levels, fasting plasma glucose (FPG), fasting plasma insulin (FPI), quantitative insulin sensitivity check index (QUICKI), homeostatic model assessment-insulin resistance (HOMA-IR) index, CRP, white blood cell, and neutrophil counts. The basal hormone levels, triglyceride (TG) levels, TG/high density lipoprotein-cholesterol (HDL-C) ratios, FPG, FPI, and HOMA-IR levels were higher in PCOS patients compared to controls. However, CRP, platelet and plateletcrit (PCT) values, and XO activity were significantly increased, and SOD activity was decreased in PCOS patients. The XO activity was positively correlated with CRP, PCT, FPG, FPI, LH/FSH, TG/HDL ratios, and HOMA-IR, and negatively correlated with QUICKI levels. They suggested that the XO is a useful marker to assess oxidative stress in PCOS patients.[60]

Total antioxidant capacity

TAC is the ability of serum to scavenge free radical production. TAC level in PCOS reported in several studies. One meta-analysis showed that there was no significant difference in TAC in women with PCOS compared with controls.[1] Fenkci *et al*. investigated TAC level in PCOS patients compared with the age, BMI, and smoking status matched controls. They demonstrated that the TAC level was significantly lower in PCOS patients.[61]

However, Verit *et al*. reported that TAC levels were significantly higher in PCOS patients compared with age and BMI matched controls. Although the complete mechanism of this elevation is unknown, they proposed that TAC was increased to restitution of oxidative stress elevation.[62]

Results of studies about antioxidant concentration are conflicting; therefore, further studies of oxidative stress in PCOS are needed to clarify the association PCOS with antioxidants. A number of antioxidative stress markers in PCOS are listed in Table 2.

Superoxide dismutase

SOD is an enzyme and an important antioxidant defense that eliminates superoxide anions (O2-), as a major oxygen radical, by catalyzes of them to H_2O_2 and final by GPx converted to water.[6] Several common forms of SOD exist depending on the metal cofactor and the protein fold such as the Cu/Zn type, Fe and Mn types, and the Ni type.[63]

SOD activity in PCOS reported in several studies. Sabuncu *et al*. determined antioxidant status in women with PCOS evaluated blood SOD level in PCOS patients compared with healthy controls. They showed that women with PCOS had higher SOD levels than normal subjects.[44] Moreover, Zhang *et al*. showed that the serum SOD level in PCOS patients was significantly lower than the control group.[41]

Kuscu *et al*. determined the oxidative stress role in endothelial dysfunction in a young, non-obese group of PCOS patients, measured blood SOD level. They demonstrated that SOD levels were significantly higher in a PCOS group than in control group.[40] In 2013, the result of one meta-analysis also showed that the mean SOD activity was 34% higher in PCOS patients than in controls.[1]

In another study, Seleem *et al*. examined the activity of SOD both in the serum and follicular fluid (FF) from women with PCOS undergoing intra cytoplasmic sperm injection . They showed SOD activity highly significant decrease both in the mean serum and FF, in PCOS than the control group, and suggested serum SOD activity could be a clinical parameter for determining systemic oxidative stress in PCOS.[64] Further studies are needed to examine the mechanism of SOD as the antioxidant defense in PCOS.

Glutathione peroxidase

GPx is an enzyme family that protects the organism from oxidative damage by reducing lipid hydroperoxides to their corresponding alcohols and reduce H_2O_2 to water. The GPx activity evaluation for anti-oxidant defense assessment in PCOS was reported in several studies. Sabuncu *et al*. determined oxidant and antioxidant status in women with PCOS. They demonstrated that GPx did not differ between a PCOS group and a healthy control group.[44]

Baskol *et al*. investigated relation between oxidative stress and the antioxidant system in the development of PCOS by measuring GPx activity in PCOS patients and age and sex matched healthy control. Results showed that no significant difference was found between GPx activities in PCOS patients and the control women.[59] One meta-analysis showed that the mean GPx activity had no statistically significant difference in women with PCOS compared with controls.[1] However, Savic-Radojevic *et al*. showed that GPx activity of PCOS women significantly decreases compared to control.[65] Further studies are needed to examine the mechanism of GPx as the antioxidant defense in PCOS.

Reduced glutathione

GSH, as an important antioxidant, is synthesized in the cytosol in two steps that require ATP. First, glutamate and cysteine by γ-glutamylcysteine synthetase create γ-glutamylcysteine and second, γ-glutamylcysteine and glycine by the activity of GSH synthetase create GSH that is distributed in the endoplasmic reticulum, nucleus, and mitochondria.[66]

GSH is essential in the regulation of the disulfide bonds of proteins and in the disposal of electrophiles and oxidants.[67] The antioxidant function of GSH is mediated by this redox-active thiol group that becomes oxidized when GSH reduces target molecules.

GSH evaluation for its antioxidant effect in PCOS was reported in several studies. One meta-analysis showed that the mean GSH levels were 50% lower in women with PCOS than in controls.[1] Sabuncu *et al*. demonstrated that GSH was significantly lower in the PCOS patient group than in the control group.[44] In accordance with the findings of Sabuncu *et al*., Dincer *et al*. also found GSH levels to be significantly lower in women with PCOS than in the control group. They proposed that GSH depletion might have resulted from increased production of ROS in PCOS patients.[36]

Vitamin C and E

Several vitamins with antioxidant capacities derived from their scavenging of oxidant molecules have been studied in PCOS. Studies on vitamin C show that this vitamin concentrations are lower in PCOS patients than in controls. Kurdoglu *et al*. reported vitamin C concentrations in serum and Mohan and Vishnu reported in erythrocyte are lower in PCOS patients and the same applies to circulating vitamin E concentrations.[68,69]

Conclusion

The association between oxidative stress and PCOS is an important issue in animal and human reproductive medicine. In this review, we have summarized the current knowledge in the relationship between oxidative stress and PCOS and encompass the most important oxidative stress roles in the pathogenesis of PCOS.

Cumulative studies to date yield an association between oxidative stress and PCOS. Moreover, oxidant and antioxidant status varied between individuals because of differences in diet, lifestyle, and enzymatic and dietary antioxidants.

The measurement of multiple biomarkers of oxidant and antioxidant may be liable indicator of oxidative stress.

Further studies are needed to standardize measurement units of each biomarker to facilitate comparison across studies and also examine the mechanism of oxidative stress on PCOS.

Acknowledgments

This study was supported by Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences. We are grateful to all individuals who participated in this project.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Received: 02 Jan 18 **Accepted:** 03 Dec 18
Published: 17 May 19

References

1. 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 Reprod Update 2013;19:268-88.
2. Zhang J, Fan P, Liu H, Bai H, Wang Y, Zhang F. Apolipoprotein AI and B levels, dyslipidemia and metabolic syndrome in south-west Chinese women with PCOS. Human Reprod 2012;27:2484-93.
3. Moran C, Tena G, Moran S, Ruiz P, Reyna R, Duque X. Prevalence of polycystic ovary syndrome and related disorders in Mexican women. GynecolObstet Invest 2010;69:274-80.
4. Lennon SV, Martin SJ, Cotter TG. Dose-dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli. Cell Prolif 1991;24:203-14.
5. Allen RG, Tresini M. Oxidative stress and gene regulation. Free Radic Biol Med 2000;28:463-99.
6. Droge, W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47-95.
7. Seaver LC, Imlay JA. Are respiratory enzymes the primary sources of intracellular hydrogen peroxide? J Biol Chem 2004;279:48742-50.
8. Geiszt M, Kapus A, Németh K, Farkas L, Ligeti E. Regulation of capacitative Ca2+ influx in human neutrophil granulocytes alterations in chronic granulomatous disease. JBiolChem 1997;272:26471-8.
9. Dvorakova M, Höhler B, Vollerthun R, Fischbach T, Kummer W. Macrophages: A major source of cytochrome b558 in the rat carotid body. Brain Res 2000;852:349-54.
10. Cheng G, Cao Z, Xu X, Van Meir EG, Lambeth JD. Homologs of gp91phox: Cloning and tissue expression of Nox3, Nox4, and Nox5. Gene 2001;269:131-40.
11. Dorsam G, Taher MM, Valerie KC, Kuemmerle NB, Chan JC, Franson RC. Diphenyleneiodium chloride blocks inflammatory cytokine-induced up-regulation of group IIA phospholipase A2 in rat mesangial cells. JPharmacolExp Ther 2000;292:271-9.
12. Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, *et al*. Oxidative stress, prooxidants, and antioxidants: The interplay. Biomed Res Int 2014;2014:761264.
13. Bouayed J, Bohn T. Exogenous antioxidants—Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxid Med Cell Longevity 2010;3:228-37.
14. Al-Gubory KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. Int J Biochem Cell Biol 2010;42:1634-50.
15. Herrera E, Barbas C. Vitamin E: Action, metabolism and perspectives. J Physiol Biochem 2001;57:43-56.
16. Johnson LJ, Meacham SL, Kruskall LJ. The antioxidants--vitamin C, vitamin E, selenium, and carotenoids. J Agromedicine 2003;9:65-82.
17. Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: Antioxidants and beyond. Am J Clin Nutr 2005;81 (1 Suppl):215S-7S.
18. Amato G, Conte M, Mazziotti G, Lalli E, Vitolo G, Tucker AT, *et al*. Serum and follicular fluid cytokines in polycystic ovary syndrome during stimulated cycles. Obstet Gynecol 2003;101:1177-82.
19. Kelly CC, Lyall H, Petrie JR, Gould GW, Connell JM, SattarN. Low grade chronic inflammation in women with polycystic ovarian syndrome. J Clin Endocrinol Metab 2001;86:2453-5.
20. Wang X, Martindale JL, Liu Y, HolbrookNJ. The cellular response to oxidative stress: Influences of mitogen-activated protein kinase signalling pathways on cell survival. Biochem J 1998;333:291-300.
21. Runchel C, Matsuzawa A, IchijoH. Mitogen-activated protein kinases in mammalian oxidative stress responses. Antioxid Redox Signal 2011;15:205-18.
22. Brown MD, Sacks DB. Protein scaffolds in MAP kinase signalling. Cell Signal 2009;21:462-9.
23. Boutros T, Chevet E, Metrakos P. Mitogen-activated protein (MAP) kinase/MAP kinase phosphatase regulation: Roles in cell growth, death, and cancer. Pharmacol Rev 2008;60:261-310.
24. Pollak M. The insulin and insulin-like growth factor receptor family in neoplasia: An update. Nat Rev Cancer 2012;12:159-69.
25. Diamanti-Kandarakis E, Dunaif A.Insulin resistance and the polycystic ovary syndrome revisited: An update on mechanisms and implications. Endocr Rev 2012;33:981-1030.
26. Akbarali HI. Oxidative stress and ion channels. In: Laher I, editor. Systems Biology of Free Radicals and Antioxidants. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014. p. 355-73.
27. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: A review. Reprod Biol Endocrinol 2012;10:49.
28. Rashidi B, Haghollahi F, Shariat M, Zayerii F. The effects of calcium-vitamin D and metformin on polycystic ovary syndrome: A pilot study. Taiwan J Obstet Gynecol 2009;48:142-7.
29. Cabiscol E, Ros J. Oxidative damage to proteins: structural modifications and consequences in cell function. Redox proteomics: From protein modification to cellular dysfunction and disease, 2006: p. 399-471.
30. Dalle-Donne I, Aldini G, Carini M, Colombo R, Rossi R, Milzani A. Protein carbonylation, cellular dysfunction, and disease progression. J Cell Mol Med 2006;10:389-406.
31. Kaya C, Erkan AF, Cengiz SD, Dünder I, Demirel ÖE, Bilgihan A. Advanced oxidation protein products are increased in women with polycystic ovary syndrome: Relationship with traditional and nontraditional cardiovascular risk factors in patients with polycystic ovary syndrome. Fertil Steril 2009;92:1372-7.
32. Abuja PM, Albertini R. Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. Clin Chim Acta 2001;306:1-17.
33. Gonzalez F, Rote NS, Minium J, Kirwan JP. Reactive oxygen species-induced oxidative stress in the development of insulin

resistance and hyperandrogenism in polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:336-40.
34. Torun AN, Vural M, Cece H, Camuzcuoglu H, Toy H, Aksoy N. Paraoxonase-1 is not affected in polycystic ovary syndrome without metabolic syndrome and insulin resistance, but oxidative stress is altered. Gynecol Endocrinol 2011;27:988-92.
35. Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: Mechanisms, mutation, and disease. FASEB J 2003;17:1195-214.
36. Dinger Y, Akcay T, Erdem T, Ilker Saygili E, Gundogdu S. DNA damage, DNA susceptibility to oxidation and glutathione level in women with polycystic ovary syndrome. Scand J Clin Lab Invest 2005;65:721-8.
37. Dib M, Garrel C, Favier A, Robin V, Desnuelle C. Can malondialdehyde be used as a biological marker of progression in neurodegenerative disease? JNeurol 2002;249:367-74.
38. Northrop-Clewes CA, Thurnham DI. Monitoring micronutrients in cigarette smokers. Clinica Chimica Acta 2007;377:14-38.
39. Davey MW, Stals E, Panis B, Keulemans J, Swennen RL. High-throughput determination of malondialdehyde in plant tissues. Anal Biochem 2005;347:201-7.
40. Kuscu NK, Var A. Oxidative stress but not endothelial dysfunction exists in non-obese, young group of patients with polycystic ovary syndrome. Acta Obstet Gynecol Scand 2009;88:612-7.
41. Zhang D, Luo W, Liao H, Wang C, Sun Y. The effects of oxidative stress to PCOS. Sichuan da xue xue bao. Yi xue ban= Journal of Sichuan University. Medical Science edition, 2008;39:421-3.
42. Dursun P, Demirtaş E, Bayrak A, Yarali H. Decreased serum paraoxonase 1 (PON1) activity: An additional risk factor for atherosclerotic heart disease in patients with PCOS? Human Reprod 2006;21:104-8.
43. Palacio J, Iborra A, Ulcova-Gallova Z, Badia R, Martinez P. The presence of antibodies to oxidative modified proteins in serum from polycystic ovary syndrome patients. ClinExp Immunol 2006;144:217-22.
44. Sabuncu T, Vural H, Harma M. Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. Clin Biochem 2001;34:407-13.
45. Tuso P, Stoll SR, Li WW. A plant-based diet, atherogenesis, and coronary artery disease prevention. Perm J 2015;19:62-7.
46. Hassani F, Karami M, Jalali Nadoushan MR, Yazdi PE. Nitric Oxide-Induced Polycystic Ovaries in The Wistar Rat. Int J Fertil Steril 2012;6:111-6.
47. Karabulut AB, Cakmak M, Kiran RT, Sahin I. Oxidative stress status, metabolic profile and cardiovascular risk factors in patients with polycystic ovary syndrome. Med-Science, 2012;1:27-34.
48. Willis G, Hocking R, Udiawar M, Rees A, James P. Oxidative stress and nitric oxide in polycystic ovary syndrome. Nitric Oxide, 2012;27:S28.
49. Nacul AP, Andrade CD, Schwarz P, de Bittencourt PI Jr, Spritzer PM. Nitric oxide and fibrinogen in polycystic ovary syndrome: Associations with insulin resistance and obesity. Eur J Obstet Gynecol Reprod Biol 2007;133:191-6.
50. Unoki H, Yamagishi S. Advanced glycation end products and insulin resistance. CurrPharm Des 2008;14:987-9.
51. Stadtman ER, Berlett BS. Reactive oxygen-mediated protein oxidation in aging and disease. Chemical Res Toxicology 1997;10:485-94.
52. Thorpe SR, Baynes JW. Role of the Maillard reaction in diabetes mellitus and diseases of aging. Drugs Aging 1996;9:69-77.
53. Garg D, Merhi Z. Relationship between advanced glycation end products and steroidogenesis in PCOS. Reprod Biol Endocrinol 2016;14:71.
54. Diamanti-Kandarakis, E. Polycystic ovarian syndrome: Pathophysiology, molecular aspects and clinical implications. Expert Rev Mol Med 2008;10:e3.
55. Diamanti-Kandarakis E, Piperi C, Kalofoutis A, Creatsas G. Increased levels of serum advanced glycation end-products in women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 2005;62:37-43.
56. Diamanti-Kandarakis E, Piperi C, Korkolopoulou P, Kandaraki E, Levidou G, Papalois A, *et al*. Accumulation of dietary glycotoxins in the reproductive system of normal female rats. J Mol Med (Berl) 2007;85:1413-20.
57. Tantalaki E, Piperi C, Livadas S, Kollias A, Adamopoulos C, Koulouri A, *et al*. Impact of dietary modification of advanced glycation end products (AGEs) on the hormonal and metabolic profile of women with polycystic ovary syndrome (PCOS). Hormones (Athens) 2014;13:65-73.
58. Puddu P, Puddu GM, Cravero E, Vizioli L, Muscari A, Relationships among hyperuricemia, endothelial dysfunction and cardiovascular disease: Molecular mechanisms and clinical implications. J Cardiol 2012;59:235-42.
59. Baskol G, Aygen E, Erdem F, Caniklioglu A, Narin F, Sahin Y, *et al*. Assessment of paraoxonase 1, xanthine oxidase and glutathione peroxidase activities, nitric oxide and thiol levels in women with polycystic ovary syndrome. Acta Obstet Gynecol Scand 2012;91:326-30.
60. Isık H, Aynıoglu O, Tımur H, Sahbaz A, Harma M, Can M, *et al*. Is Xanthine oxidase activity in polycystic ovary syndrome associated with inflammatory and cardiovascular risk factors? J Reprod Immunol 2016;116:98-103.
61. Fenkci V, Fenkci S, Yilmazer M, Serteser M. Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. FertilSteril 2003;80:123-7.
62. Verit FF, Erel O. Oxidative stress in nonobese women with polycystic ovary syndrome: Correlations with endocrine and screening parameters. GynecolObstetInvest 2008;65:233-9.
63. Barondeau DP, Kassmann CJ, Bruns CK, Tainer JA, Getzoff ED. Nickel superoxide dismutase structure and mechanism. Biochemistry 2004;43:8038-47.
64. Seleem AK, El Refaeey AA, Shaalan D, Sherbiny Y, Badawy A. Superoxide dismutase in polycystic ovary syndrome patients undergoing intracytoplasmic sperm injection. JAssistReprodGenet 2014;31:499-504.
65. Savic-Radojevic A, Bozic Antic I, Coric V, Bjekic-Macut J, Radic T, Zarkovic M, *et al*. Effect of hyperglycemia and hyperinsulinemia on glutathione peroxidase activity in non-obese women with polycystic ovary syndrome. Hormones (Athens) 2015;14:101-8.
66. Marí M, Morales A, Colell A, García-Ruiz C, Fernández-Checa JC. Mitochondrial glutathione, a key survival antioxidant. Antioxid Redox Signal 2009;11:2685-700.
67. Hammond CL, Lee TK, Ballatori N. Novel roles for glutathione in gene expression, cell death, and membrane transport of organic solutes. J Hepatol 2001;34:946-54.
68. Kurdoglu Z, Ozkol H, Tuluce Y, Koyuncu I. Oxidative status and its relation with insulin resistance in young non-obese women with polycystic ovary syndrome. J Endocrinol Invest 2012;35:317-21.
69. Mohan S, Priya V. Lipid peroxidation, glutathione, ascorbic acid, vitamin E, antioxidant enzyme and serum homocysteine status in patients with polycystic ovary syndrome. Biol Med 2009;1:44-9.