OXIDATIVE STRESS BIOMARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS)

REVIEW

HIGHLIGHTS

- PCOS is associated with endocrine/metabolic/reproductive complications and moreover exhibits oxidative stress. Oxidative stress is implicated in obesity, dyslipidemia, insulin resistance, type 2 diabetes, CVD, malignancies and inflammation cascades.
- Oxidative stress impairs insulin action and promotes insulin resistance as seen in type 2 diabetes mellitus.
- Increased protein carbonyl, lipid peroxidation, increased TNF-α and decreased insulin mediated signaling through ROS mediated serine phosphorylation of IRS-1 promotes insulin resistance.
- Obesity and hyperglycemia contribute to oxidative stress in PCOS women creating an inflammatory environment.
- Oxidative stress markers in various studies are positively correlated with testosterone and androstenedione levels in PCOS women.
- Oxidative stress enhances ovarian steroidogenesis which is reported to be inhibited by antioxidants.

ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the most common and heterogeneous endocrine disorders among women of reproductive age. The exact pathophysiology of PCOS is complex and still remains largely unclear. Both insulin resistance and hyperinsulinemia are considered to play important role in the pathogenesis of PCOS. These two factors produce hyperandrogenism which leads to anovulation, amenorrhea and infertility. PCOS is associated with an increased risk of metabolic complications and exhibit oxidative stress. Oxidative stress means an imbalance between the production of reactive oxygen species and the antioxidant defence system which results in oxidative damage. Oxidative stress has been implicated in a number of diseases such as obesity, dyslipidemia, insulin resistance, diabetes, cardiovascular disease, malignancies, and inflammatory cascades. A lot of investigations have revealed that oxidative stress is significantly increased in women with PCOS. In a quest to delineate the role of oxidative stress in the pathogenesis of PCOS, investigators have examined patients with this disorder for a wide array of oxidative stress biomarkers, including malondialdehyde (MDA), protein carbonyl, nitric oxide (NO), total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (GSH), catalase (CAT) and paraoxonase1 (PON1). This review summarizes PCOS pathophysiology in context of oxidative stress.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathy in women of reproductive age. It is a disorder that affects endocrine, metabolic and reproductive functions and is considered as the leading cause of chronic anovulation which leads to infertility [1]. It is a heterogenous disorder that typically has its onset early in life with a wide spectrum of clinical manifestations which includes symptoms of hyperandrogenism like chronic anovulution, hirsuitism, acne, alopecia and USG features of Polycystic ovaries [2]. Insulin resistance is viewed as a central feature of PCOS which promotes hyperinsulinemia. The resulting hyperinsulinemia together with central obesity, commonly seen in PCOS patients, are components of metabolic syndrome which is a risk factor for developing type II diabetes and cardiovascular disease. In addition to the familiar features of hirsutism, acne, and anovulatory infertility, elevated markers of cardiovascular and endothelial dysfunction have been reported in PCOS women [3]. It is therefore regarded as a chronic systemic disease instead of the simple local disease, and is also associated with chronic inflammation and oxidative stress [4]. Oxidative stress means an imbalance between pro-oxidants and antioxidants, and is produced by increased levels of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) or a decrease in antioxidant defense mechanisms. A lot of investigations have revealed that oxidative stress level is significantly increased in patients with PCOS compared with the normal, when oxidative status is evaluated by circulating markers, such as malondialdehyde, superoxide dismutase, and glutathione peroxidase [4]. Although the mechanisms underlying PCOS are not fully understood but recent studies strongly suggest that oxidative stress plays a crucial role in the pathogenesis of PCOS besides other superimposing factors [5]. The present review provides an overview of current knowledge of oxidative stress in PCOS women, which have been investigated more actively in recent years.

OXIDATIVE STRESS – A BRIEF OVERWIEW

The biological systems living in aerobic conditions are exposed to oxidants, which occur in two categories: reactive oxygen species (ROS) and reactive nitrogen species (RNS). Chemical species that are formed upon incomplete reduction of oxygen include superoxide anion (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO•) constitute ROS. In contrast, the collective term used to describe all the oxidation states and reactive adducts of nitrogenous nitric oxide synthase (NOS) products, from nitric oxide (NO) to nitroxyl (NO−), S-nitrosothiol (RSNO), and peroxynitrite (OONO−) constitute RNS. ROS and RNS are toxic agents, capable of damaging molecules that have indeed critical biological functions essential for normal physiology. In both physiological and pathophysiological processes these reactive species are able to initiate or mediate many enzyme- and gene-dependent reactions, and an overproduction of ROS and/or RNS may result in impaired homeostasis and associated pathology. Under normal conditions, scavenging molecules commonly known as antioxidants prevent an overproduction of ROS by converting it to H_2O. These antioxidants in the human body are of two types: enzymatic antioxidants and non-enzymatic antioxidants [6]. Enzymatic antioxidants also known as natural antioxidants neutralize excessive ROS and prevent it from damaging the cellular structure. Superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, which also causes reduction of hydrogen peroxide to water and alcohol collectively constitute enzymatic antioxidants. Non-enzymatic antioxidants are also known as synthetic antioxidants or dietary supplements and the body's complex antioxidant system is influenced by dietary intake of antioxidant vitamins and minerals such as vitamin C, vitamin E, selenium, zinc, taurine, hypotaurine, glutathione, beta carotene, and carotene [6]. Iron-binding proteins, transferrin and ferritin also play a role in antioxidant defense by preventing the catalyzation of free radicals through chelation [7]. Although some nutrients such as Se, Cu, and Zn have no antioxidant action themselves but these nutrients are required for the activity of some antioxidant enzymes. Thus oxidative stress in human body occurs as a consequence of a general increase in reactive oxygen species (ROS) generation, a depression of the antioxidant systems, or both.

OXIDANT/ANTIOXIDANT SYSTEM: IN RELATION TO PCOS PATHOGENESIS

PCOS is one of the heterogenous disorders, pathogenesis of which is complex and still not clear. It is characterized by hyperandrogenemia, hirsutism, oligo- or amenorrhea, and is associated with a high frequency of cardiovascular risk factors such as obesity, hyperinsulinemia, impaired glucose tolerance, dyslipidemia, hypertension, chronic low grade inflammation and diabetes, which suggests that this illness leads to endocrinological, metabolic and cardiovascular disorders [8]. The mechanisms underlying the pathophysiology of PCOS are not fully understood, but recent studies strongly suggest that oxidative stress has a tremendous role in induction of PCOS and its complications [5]. Oxidative stress impairs insulin action and promotes insulin resistance as seen in type 2 diabetes mellitus. This is evidenced by increased concentration of circulating protein carbonyls, lipid peroxidation and tumor necrosis factor-a (TNF-α) and decreased insulin mediated signaling as a result of increased ROS mediated serine phosphorylation of insulin receptor substrate-1 (IRS-1), which results in suppression of insulin-stimulated

tyrosine phosphorylaton and activation of IRS-1 and consequently insulin resistance [9].Factors such as obesity and hyperglycemia commonly found in PCOS women are likely to contribute to an increase oxidative stress in these women [10]. Physiological hyperglycemia generates increased levels of ROS from mononuclear cells in PCOS women, which then promotes the release of TNF-alpha and inflammatory transcription factor NF-kappa β, both of which are known mediators of insulin resistance. The increased oxidative stress creates an inflammatory environment that further increases insulin resistance and contributes to hyperandrogenism in PCOS women [11]. In multi investigations, serum oxidative stress markers are discovered to be positively correlated with testosterone and androstenedione levels in PCOS women [12]. In vitro studies reported that oxidative stress enhance the activities of ovarian steroidogenesis enzymes, which further stimulate androgen production, and antioxidative chemicals such as statins inhibit the activities of these enzymes, suggesting oxidative stress contributes to hyperandrogenism in PCOS women [13]. In addition to the role of oxidative stress in induction of insulin resistance and hyperandrogenism, it also predispose these women to various cardiovascular risk factors including obesity, dyslipidemia, presence of metabolic syndrome and is also known to independently contribute to endothelial dysfunction [14]. The role of ROS and oxidative damage in the pathogenesis of cardiovascular disease is firmly established, and evidence is accumulating which suggests their involvement in the process of atherogenesis [15]. In atherosclerotic patients, a positive correlation exists between elevations in malondialdehyde levels (index of lipid peroxidation) in plasma and in arterial walls, both of these paralleling the severity of coronary atherosclerosis [15]. Paraoxonase1 (PON1) which is an HDL-associated antioxidant enzyme is capable of hydrolyzing lipid peroxides and acts as an independent risk factor for coronary artery disease. Human PON1 contributes to the antiatherogenic effects of HDL, and its activity has been shown to be inversely associated with oxidative stress in serum of PCOS women [16]. Oxidative stress also causes an increase in lipid peroxides in the cell membrane, thereby induces intracellular expression of specific genes such as VCAM-1, ICAM-1, and also of those located in the upstream regions of cytokines and growth factors including monocyte chemoattractant protein-1 and Plattelet derived growth factor, which are the markers of low-grade inflammation, and a predictor of diseases related to PCOS [17]. Therefore, the study of oxidative stress in PCOS is crucial since cardiometabolic risk factors along with insulin resistance seen in these women are associated with an increased risk of cardiovascular disease [18]. Though oxidative stress is considered as a potential inducement of PCOS pathogenesis, but it is still unknown whether the abnormal levels of oxidative stress markers in patients with PCOS derive from PCOS itself or they are related to the potential complications associated with PCOS. The schematic representation of oxidative stress involvement in the pathophysiology of PCOS is shown in Figure-1.
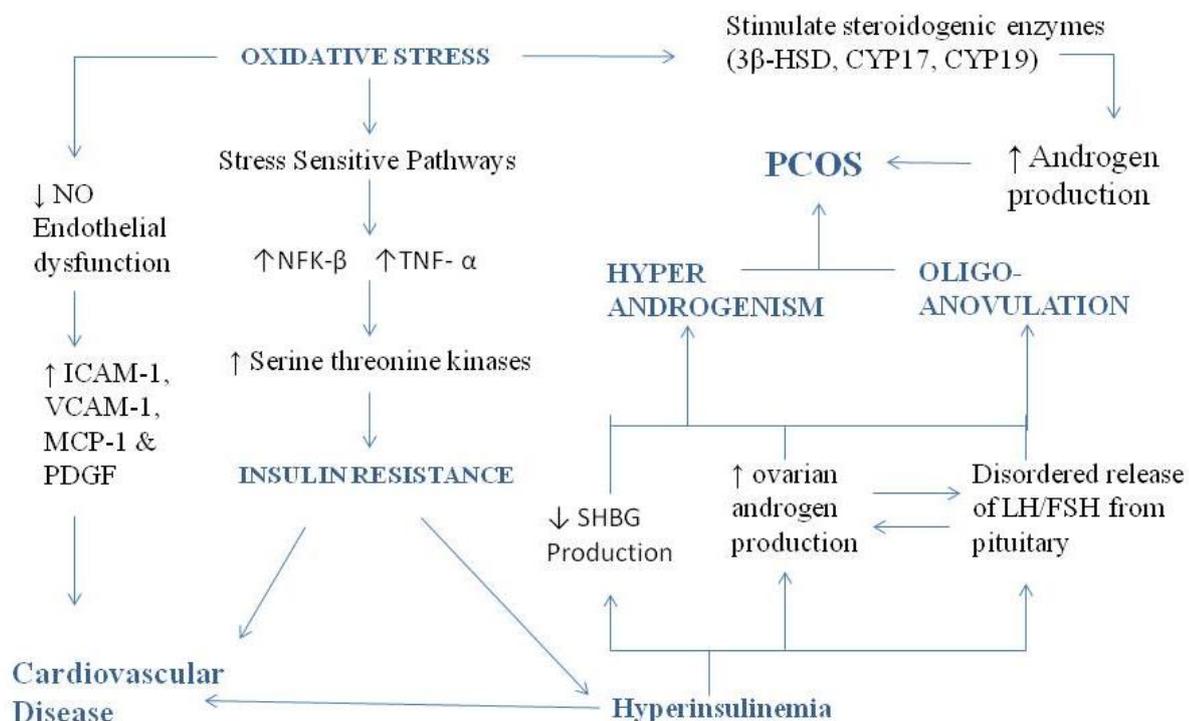

Figure 1. Oxidative stress in relation to PCOS pathophysiology

Several investigations indicated that PCOS increases the risk of developing endometrial cancer, and factors such as abnormal hormone level, IR, hyperinsulinemia, and even obesity were suggested as the potential inducements in the pathogenesis of endometrial cancer in PCOS women [19]. Studies have

reported high concentrations of malondialdehyde (MDA), proinflammatory cytokines (IL-6, TNF-alpha, and IL-beta), angiogenic factors (IL-8 and VEGF), monocyte chemoattractant protein-1, and oxidized LDL (ox-LDL) in the peritoneal fluid of patients with endometriosis [20]. Oxidative stress in PCOS is discovered to play an important role in cancer pathogenesis, as it causes genetic changes by attacking DNA, leading to DNA damages, such as DNA strand breaks, point mutations, aberrant DNA cross-linking, and DNA-protein cross-linking [21]. As a result, the disruption of normal DNA repair mechanisms due to oxidative stress and mutations in protooncogenes and tumor suppressor genes cause cell proliferation out of control [22]. Oxidative stress also causes epigenetic changes by DNA methylation and silencing tumor suppressor genes [23]. Therefore, oxidative stress could be considered as one of the major underlying inducements of the increasing risk of gynecological cancers in PCOS women.

In a quest to delineate the role of oxidative stress in the pathogenesis of PCOS, investigators have examined patients with this disorder for a wide array of oxidative stress biomarkers, including malondialdehyde (MDA), protein carbonyl, nitric oxide (NO), total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (GSH), catalase (CAT) and paraoxonase1 (PON1). The data obtained is summarized below:

Malondialdehyde (MDA)
Products generated from lipid peroxidation reactions have been widely employed as biomarkers for oxidative stress. One of the stable end products of lipid peroxidation reactions is MDA. MDA, produced during the decomposition of polyunsaturated fatty acids serve as a good biomarker of lipid peroxidation [24]. Numbers of methods are available for quantification of lipid hydroperoxides and secondary lipid peroxidation products. Most commonly employed method for measurement of MDA is TRABS (thiobarbituric acid-reactive substances) assay. The amount of MDA corresponds to the chromogen found from MDA and thiobarbituric acid (TBA) with a maximum absorption at 532-535 nm. The TRABS assay for MDA is non-specific while HPLC is considered as a more accurate tool for MDA estimation. Kusçu et al. (2009) when compared PCOS patients (n=31, mean age 23.8 years and mean BMI 21.8) with healthy control found blood MDA level, not specified as measured from serum or erythrocyte, was significantly higher in the PCOS group (0.12±0.03 vs 0.10±0.03, p=0.01) independent of obesity than healthy controls. In this study PCOS patients were divided into two subgroups in terms of insulin resistance, IR- and IR+. The results showed that even in the absence of insulin resistance MDA level was significantly higher in young, non-obese PCOS patients compared with controls (0.125±0.03 vs 0.101±0.03, p=0.03) [3]. Zhang et al. (2008) also demonstrated that serum MDA levels in PCOS patients (n=30) were significantly higher than those of controls (12.313±2.512 vs 6.932±1.663 µmol/L, P<0.05) [25], but some of the important patient characteristics such as BMI and age, were not recorded. However, Karadeniz et al. (2008) found MDA levels in PCOS patients (n=58) were similar to those of controls (5.38±2.47 vs 4.475±2.06, p>0.05) [26]. Also, MDA levels were found to be similar in a PCOS patient group where the homeostatic model assessment (HOMA)-IR was below and above the cutoff value of 1.75, suggesting the presence of insulin resistance in PCOS patients has no effect on MDA levels. Karabulut AB et al. (2012) found significant differences between PCOS patients (n=32) and healthy controls (n=32) in terms of MDA levels. The MDA levels were found to be significantly higher in PCOS patients than those of controls (15, 93 ± 2.1 vs. 9,41 ± 3,1 nmol/ml respectively) (p< 0.001) [27]. Azzawie HF et al. (2010) found a statistically significant increase in erythrocyte MDA levels in patients with PCOS (n=50) compared to controls (n=50), (37.31± 2.46 vs 14.32 ±2.43 nmol/gm of Hb respectively) (p< 0.001) [28]. Thus a number of studies have reported increased MDA levels in patients with PCOS compared to healthy controls and it was proposed that rise in MDA could be due to increased generation of reactive oxygen species (ROS) which in turn oxidizes many important bimolecules including membrane lipids [29].

Protein carbonyl content
Protein oxidation status which is often assessed with a colorimetric assay measures protein carbonyl (PC) content, after reacting the serum with dinitrophenylhydrazine. Fenkci et al. (2007) found that the PC level was significantly higher in PCOS patients with normal BMI compared with controls (18.01±0.80 vs 14.19±0.40 nmol/L, p=0.001). Furthermore, it was found that protein carbonyls were positively correlated with fasting insulin, indicating a strong association between insulin resistance and protein oxidation in PCOS women [30]. In a study conducted by S. Kandasamy et al.(2010) higher plasma protein corbonylation content was found in obese PCOS patients(n=104, BMI ≥23 Kg/m2) than controls (n=95), indicating the presence of increased oxidative stress in obese PCOS patients (4.95 ±1.61 vs 3.21 ±0.79 µmol/ml respectively, p <0.05) [31]. Higher protein oxidation status in PCOS women suggests that free radicals damage proteins in these women [30].

Nitric Oxide (NO)
NO is specifically synthesized during the conversion of L-arginine to L-citrulline by Nitric oxide synthase [32]. NO acts in a variety of tissues to regulate normal cell functions under normal physiological conditions, but in excess it can become toxic [33]. NO, with an unpaired electron, becomes highly reactive and cause damage by reacting with proteins, carbohydrates, nucleotides and lipids.

Excess of NO has been found to be associated with number of diseases including asthma, ischemic/ reperfusion injury, septic shock, and atherosclerosis [34]. NO concentration can be assessed by measuring plasma concentration of NO3 - and NO2-, which is assumed as the best index of total NO. NO contents are assessed by a two-step process firstly involving conversion of nitrate to nitrite then followed by spectrophotometric detection of total nitrite at 540 nm [35]. Nácul et al. (2007) demonstrated that NO levels in PCOS patients (n=31, mean age 22.4±7.1 years and mean BMI 26.7±10.1) were similar to that of age- and BMI-matched controls (NO mean value 11.5 vs 10.2 umol/L,p>0.05) [35]. Moreover, a significantly negative correlation was observed between NO and fasting insulin levels (r = -0.39, p=0.03) and HOMA (r = -0.41, p=0.02) [36], suggesting that NO was related to the presence of insulin resistance in PCOS patients. Karabulut AB et al. (2012) also found a significant difference in terms of NO levels in PCOS women (n=32) and healthy controls (n=32). NO levels were found to be higher in study group (43.36 ±3.54 vs 27.65 ±4.7 µmol/mg respectively, p <0.001) [27]. However, Gulden Baskol et al. (2012) found an increased but not significantly different NO levels in PCOS women (n=30) than those of control women (n=20), (8.1 ±3.0 vs 7.7 ±2.5 µmol/L respectively, p =0.892) [37].

Total antioxidant capacity (TAC)

Total antioxidant capacity is defined as the ability of serum to quench free radical production and thus protecting the cell structure from molecular damage. Among Various detection assays for TAC, most commonly used one is the spectrophotometric assay in which long-lived 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) radical cation is measured. It is based on the principle to measure the ability of aqueous and lipid antioxidants to inhibit the oxidation of ABTS to ABTS+. Verit et al. (2008) demonstrated that TAC levels were significantly higher in PCOS patients (n=63 mean age24.4±4.1 years and mean BMI 21.2±1.8) compared with age and BMI-matched controls (1.8±0.5 vs 1.1±0.2 mmol TroloxEq/L, p<0.0001). This study also reported that TAC was increased in non-obese, normoinsulinemic PCOS patients (fasting insulin 10.7±5.0 µIU/mL, no significant difference compared with controls). Although the mechanism lying underneath for the elevation of TAC level was not clear, it was proposed that TAC was increased to compensate for the increase in total oxidative stress (19.1±7.6 vs 12.3±4.8µ mol H_2O_2 Eq/L, p<0.0001) [38]. However, Mohamadin AM et al. (2009) observed significantly (p < 0.001) decreased levels of TAC in PCOS group (n=35) compared with the control group (0.88±0.10 mmol Trolox/l vs. 1.63±0.17 mmol Trolox/l), suggesting an increased oxidative potential and decreased antioxidant status in PCOS women [39].

Superoxide Dismutase (SOD)

A major cellular defense system against superoxide in all vascular cells is SOD. It is involved in the conversion of superoxide to H_2O_2, a toxic substance that is converted by GPx to water. SOD activity can be determined in serum by the method of Beauchamp and Fridovich which is based on the amount of enzyme required to inhibit the reduction of NBT by 50% [40]. Kuşçu et al. (2009) demonstrated that SOD levels were significantly higher in a PCOS group compared with a control group (8.0±0.7 vs 7.28±0.8, p=0.001). In this study PCOS patients were divided into two subgroups: IR- and IR+, and SOD levels were found to be significantly higher in both subgroups compared with the control (7.99±0.7 vs 8.22±0.8 vs 7.28±0.8, p=0.009 and 0.03, respectively) [3]. Further, Azzawie HF et al. (2010) also found increased SOD levels in PCOS patients (n=50) compared with the control group (825.56 ± 69.82 vs 733.59 ±35.54 U/gm of Hb respectively) (p< 0.001) [28]. The over expression of SOD observed in PCOS patients was proposed to be an adaptive response which results in increased dismutation of superoxide to hydrogen peroxide [41]. On the other hand Zhang et al. (2008) demonstrated that the serum SOD level in PCOS patients (n=30) was significantly lower than that in the control group (67.316±12.463 vs 113.815± 13.003 µU/mL, P<0.05) [25]. However, this study did not capture other patient's characteristics, making it difficult to explain properly why SOD level was lower in this selected PCOS group.

Glutathione Peroxidase (GPx)

Glutathione peroxidase (GPX), selenium-containing antioxidant enzyme effectively reduces hydrogen peroxide and lipid peroxides to water and lipid alcohols respectively and in turn oxidizes glutathione to glutathione disulfide. In the absence of adequate GPX activity or glutathione levels, hydrogen peroxide and lipid peroxides are not detoxified and may be converted to hydroxyl radicals and lipid peroxyl radicals, respectively, by transition metals (eg, Fe2-). The GPX/glutathione system is thought to be a major defense in low-level oxidative stress. GPx activity can be determined by minor modification of the method of Paglia and Valentine [42]. Sabuncu et al. (2001) observed that GPx did not differ between a PCOS group and a healthy control group (2.88±0.52 vs 2.98±0.54 MU/mol Hb). An increase in GPx was to be expected with increased H_2O_2, the fact that GPx activity did not increase in PCOS women might result from the low amount of GSH, which is the substrate of GPx [43]. However, Azzawie HF et al. (2010) reported an increased GPx activity in PCOS patients (n=50) than that in control group (63.42 ± 2.78 vs 54.32 ±1.50U/gm of Hb respectively) (p< 0.001). The rise in the activity of GPX could be in order to counter the effect of increased oxidative stress [28]. Gulden Baskol et al. (2012) found an increased but not significantly different GPx activity in PCOS women (n=30) than those of control women (n=20), (168.9 ±58.5 vs 154.3 ± 58.5 U/ml respectively, p =0.748) [37].

Glutathione

Glutathione, a tripeptide is an important antioxidant present in millimolar concentrations in all the cells and plays the role of an intracellular radical scavenger. It is the substrate of many xenobiotic elimination reactions and is often determined by adding 5, 5'-dithiobis (2- nitro-benzoic acid), which is a disulfide chromogen that is readily reduced by sulfhydryl compounds, to an intensely yellow compound. Reduced chromogen absorbance is measured at 412 nm and is directly proportional to GSH concentration [44]. Sabuncu et al. (2001) reported that GSH was significantly lower in the PCOS patient group than in the control group (0.39 ± 0.07 vs 0.44 ± 0.07 mol/mol Hb, $p=0.03$), and proposed that the lower levels of GSH may have been partly related to insulin resistance [43]. In consistent with the findings of Sabuncu et al. (2001), Dincer et al. (2005) also found significantly decreased GSH levels in women with PCOS than in the control group (5.03 ± 0.96 vs 5.59 ± 0.82 μmol/gHb, $p<0.05$) [45]. Azzawie HF et al. (2010) also found significantly decreased GSH levels in PCOS patients (n=50) compared with the control group (10.41 ± 1.63 vs 23.22 ± 1.33mg/gm of Hb respectively) ($p< 0.001$) [28]. In addition to this Karabulut AB et al. (2012) also observed significant difference in terms of GSH levels. GSH levels were found to be lower in PCOS group (n=32) compared to that of controls (n=32) (6.7 ± 0.6 vs 7.7 ± 2.1 nmol/ml respectively, p <0.001) [27]. The decrease in GSH levels could be because of an increased utilization of glucose under hyperglycemic conditions in polyol pathway that consumes NADPH necessary for GSH regeneration by the GSH-reductase enzyme. Hyperglycemia associated with insulin resistance generally found in PCOS patients is therefore indirectly the cause of GSH depletion, which in turn leads to increased oxidative stress.

Catalase

Catalase, an intracellular antioxidant enzyme that is mainly located in cellular peroxisomes and to some extent in the cytosol, where it catalyzes the reduction of hydrogen peroxide to water and molecular oxygen. In case of limited glutathione content or reduced GPX activity this enzyme plays an important role in the development of tolerance to oxidative stress. Catalase activity can be determined by the method of Claiborne in which activity is calculated in terms of nmoles of H_2O_2 consumed/min/mg of protein. It can also be determined by the method of Beers and Sizer [46]. Azzawie HF et al. (2010) in his study observed a significant decrease in the activity of catalase in PCOS patients (n=50) compared to control group (7.05 ± 1.36 vs 9.89 ± 1.13U/gm of Hb respectively) ($p< 0.05$) [28]. In accordance with the findings of Azzawie HF et al. (2010), S. Kandasamy et al.(2010) also found significantly decreased catalase activity in PCOS patients (n=104) compared to control group (15.31 ± 4.3 vs 23.44 ± 6.71) [31]. The decrease in catalase activity supports the presence of oxidative stress in PCOS patients.

Paraoxonase1 (PON1)

Paraoxonase1, an antioxidant enzyme located on high-density lipoprotein hydrolyses lipid peroxides in oxidized lipoproteins [47]. PON1 activity can be determined spectrophotometrically by the method using p-nitrophenylacetate as a substrate and the increase in the absorbance at 412 nm due to formation of p-nitrophenol is measured [48]. Mohamadin AM et al. (2009) conducted a study with the aim to analyse serum PON1 activity in PCOS women (n=35) and healthy controls (n=30). PON1 activity was found to be significantly ($p < 0.001$) lower in patients with PCOS (161.2 ± 6.1 U/l) compared with the control group (217.6 ± 9.3 U/l) [39]. Dursun P et al. (2006) also observed a significant decrease in mean serum PON1 activity in PCOS group (n= 23) compared to the controls (151.2 ± 90.8 versus 217.7 ± 101.6, respectively; $P = 0.027$). In accordance with the findings of Mohamadin AM et al. (2009) and Dursun P et al. (2006), Gulden Baskol et al. (2012) also observed significantly decreased PON1 activity in PCOS women (n=30) than those of control women (133.3 ± 69.3 vs 192.1 ± 82.6 U/L respectively, $p =0.011$) [37].The mechanism of the observed decrease in serum PON1 activity in PCOS patients remains unclear. This decrease in PON1 activity could be related to enhanced lipid peroxidation, as oxidized lipids are known to inhibit PON1 activity thus contributing to increased inactivation of PON1 and generation of ROS in PCOS women. All the above mentioned data can be summarized in the Table-1.

CONCLUSION

PCOS, a common complex condition associated with psychological, reproductive and metabolic features with manifestations across the lifespan represents a major health and economic burden. Both hyperandrogenism and insulin resistance contribute to pathophysiology of PCOS, but Insulin resistance occurs in the majority of women with PCOS especially those who are overweight. PCOS encompasses many long-term health problems such as the development of metabolic syndrome, type2 diabetes mellitus and cardiovascular disease. Further prolonged exposure to unopposed estrogen in PCOS, can lead to endometrial hyperplasia and endometrial carcinoma. There is a growing literature on the involment of oxidative stress in the pathophysiology of PCOS. It has been found that PCOS is associated with excess oxidative stress and decreased antioxidant reserves. Increased oxidative stress is associated with defective pattern of insulin receptor phosphorylation, namely increased serine phosphorylation and reduced tyrosine phosphorylation, which promotes insulin resistance and consequently results in hyperinsulinemia. Hyperinsulinemia further increases their risk to cardiovascular

disease and contributes to altered steroidogenesis in the ovaries as observed in PCOS.

Oxidant/Anti oxidant markers	Scenario in PCOS	Reported by	References
1. MDA (Marker of lipid peroxidation)	Increased in PCOS women	Kusçu et al. (2009)	[3]
	Increased in PCOS women	Zhang et al. (2008)	[25]
	Increased in PCOS women	Karadeniz et al. (2008)	[26]
	Similar in PCOS women and controls	Karabulut AB et al.(2012)	[27]
	Increased in PCOS women	Azzawie HF et al. (2010)	[28]
2. Protein carbonyl content (marker of protein oxidation status)	Increased in PCOS women	Fenkci et al. (2007)	[30]
	Increased in PCOS women	S. Kandasamy et al.(2010)	[31]
3. Nitric Oxide (oxidant marker)	Similar in PCOS women and controls	Nácul et al. (2007)	[35]
	Increased in PCOS women	Karabulut AB et al. (2012)	[27]
	Increased in PCOS women but not statistically significant	Gulden Baskol et al. (2012)	[37]
4. Super oxide dismutase (Antioxidant enzyme)	Increased in PCOS women	Kusçu et al. (2009)	[3]
	Increased in PCOS women	Azzawie HF et al. (2010)	[28]
	Decreased in PCOS women	Zhang et al. (2008)	[25]
5. Catalase (Antioxidant enzyme)	Decreased in PCOS women	Azzawie HF et al. (2010)	[28]
	Decreased in PCOS women	S. Kandasamy et al.(2010)	[31]
6. Glutathione (antioxidant marker)	Decreased in PCOS women	Sabuncu et al. (2001)	[43]
	Decreased in PCOS women	Dincer et al. (2005)	[45]
	Decreased in PCOS women	Azzawie HF et al. (2010)	[28]
	Decreased in PCOS women	Karabulut AB et al. (2012)	[27]
7. Glutathione Peroxidase (Antioxidant enzyme)	Similar in PCOS women and controls	Sabuncu et al. (2001)	[43]
	Increased in PCOS women	Azzawie HF et al. (2010)	[28]
	Increased in PCOS women but not statistically significant	Gulden Baskol et al. (2012)	[37]
8. Paraoxonase1 (Antioxidant enzyme)	Decreased in PCOS women	Mohamadin AM et al. (2009)	[39]
	Decreased in PCOS women	Gulden Baskol et al. (2012)	[37]
9. Total antioxidant capacity	Increased in PCOS women	Verit et al. (2008)	[38]
	Decreased in PCOS women	Mohamadin AM et al. (2009)	[39]

Table 1. Present studies related to oxidant/ antioxidant status in PCOS women

This review clearly shows a link between oxidative stress and metabolic abnormalities on both theoretical backgrounds and epidemiological evidences. The discussion has included multiple biomarkers of both ROS and antioxidants in various PCOS patient groups. Cumulative studies to date do not yield a definitive conclusion regarding the association between oxidative stress and PCOS. Measurement of biomarkers of oxidative stress also is known to be a controversial issue, as units of measurement in published studies are not consistent. In future, standardized measurement units of each biomarker should be used to facilitate accurate comparison across studies. Additional studies are recommended to examine the mechanism and association of oxidative stress in PCOS. In addition, treatment strategies of antioxidant supplementation need to be investigated in randomized controlled trials to reduce oxidative stress. Antioxidant supplementation may be effective in controlling the production of ROS and may be explored as a potential strategy to overcome metabolic as well as reproductive disorders associated with infertility.

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