

Role of Oxidative Stress in Polycystic Ovary Syndrome

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Abstract: Polycystic ovary syndrome (PCOS) is a multifactorial disorder affecting many women of reproductive age, typically due to hyperandrogenemia, hyperinsulinemia, and enigmatic genetic factors. The complex nature of PCOS is reflected in the broad spectrum of the disorder's clinical presentation, including metabolic and reproductive disorders. As a result, while the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) have agreed on a consensus definition of PCOS to help clinical investigators, the condition is recognized to have multiple clinical phenotypes.

Oxidative stress (OS) occurs when destructive reactive oxygen species (ROS) outbalance antioxidants, causing DNA damage and/or cell apoptosis. Moreover, reactive nitrogen species (RNS), such as nitrogen oxide (NO) with an unpaired electron also are highly reactive and toxic. In a quest to delineate the role of OS in the pathogenesis of PCOS, investigators have examined patients with the disorder for a wide array of OS biomarkers, including malondialdehyde (MDA), protein carbonyl, total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione (GSH).

Keywords: Polycystic ovary syndrome (PCOS), oxidative stress (OS), insulin resistance, hyperandrogenism, reactive oxygen species (ROS), nitric oxide synthase (NOS).

INTRODUCTION

PCOS is one of the most common endocrinological pathologies in women during their reproductive years exhibiting a wide spectrum of clinical manifestations. PCOS women commonly have features of hyperandrogenism and the primary cause of PCOS is probably multifactorial in origin [1]. Increased insulin resistance is viewed as a central feature of PCOS irrespective of the body mass index (BMI). The resulting hyperinsulinemia together with central obesity, which is frequently encountered in PCOS patients, are components of metabolic syndrome. Metabolic syndrome, which affects one in five people, increases the risk of developing cardiovascular disease and type II diabetes, and its prevalence increases with age. PCOS patients have been reported to have markers of cardiovascular and endothelial disorders in addition to the familiar features of hirsutisms, acne, and anovulatory infertility [2].

Oxidative stress is commonly referred as the imbalance between oxidants and antioxidants. When the imbalance favors oxidants, generation of excessive amounts of reactive oxygen species harm our body in various ways [3] through the generation of excessive amounts of reactive oxygen species. In other words, reproductive cells and tissues will remain stable only when antioxidant and oxidant status is in balance. Oxidative stress, which is generally known to be present in women with PCOS regardless of whether they are lean or have metabolic abnormalities, has been documented in infertile women [4]. The present review study provides an

overview of current knowledge in PCOS and ROS' roles in women during their reproductive years, exhibiting a wide spectrum of clinical manifestations in PCOS women, which have been investigated more actively in recent years.

DEFINITION AND DIAGNOSIS OF POLYCYSTIC OVARY SYNDROME

The definition of PCOS has been controversial and still remains unclear due to the syndrome's heterotrophic nature. Following the first report on women with polycystic ovaries in 1935, the term "polycystic ovarian syndrome" was established as more clinicians noticed the correlations between hyperinsulinemia, androstenedione, testosterone levels, and PCOS [5]. However, a wide spectrum of clinical manifestations, including impaired glucose tolerance [6], prevalence of type II diabetes [7], increased risk of hypertension and dyslipidemia, and elevated endothelial dysfunction [8] further complicated the debate on defining PCOS. The presence of clinical or biochemical hyperandrogenism or polycystic ovaries with regular cycles was broadly interpreted as PCOS [9, 10]. As a result, there were no widely accepted diagnostic criteria available until the National Institute of Health (NIH) criteria were introduced in 1990.

In 1990, the NIH established diagnostic criteria that characterize PCOS as the combination of oligomenorrhea or amenorrhea and hyperandrogenemia in the absence of non-classical adrenal hyperplasia, hyperprolactinemia, and thyroid dysfunction [11]. These criteria, however, did not include ultrasound morphology of polycystic ovaries in the belief that broader clinical diagnostic criteria were in need for clinicians to accurately diagnose multi-etiological PCOS. In Europe, clinicians maintained that the ultrasound appearance of polycystic ovary was an essential criterion to diag-

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nose PCOS. As a result of the continued dialogue between the ESHRE and the ASRM, a consensus document was produced, commonly referred to as the Rotterdam 2003 criteria for defining PCOS.

The new definition of PCOS suggested that the diagnosis of PCOS must be based on the presence of two of the three following criteria: (i) oligo- and/or anovulation, (ii) clinical and/or biochemical signs of hyperandrogenism, and (iii) polycystic ovaries on ultrasonography and exclusion of related disorders [12, 13]. The ultrasound criteria for polycystic ovaries is defined as the presence of 12 or more follicles measuring 2 to 9 mm in diameter and /or an increased ovarian volume > 10 cm³ on transvaginal ultrasound scanning. PCOS is diagnosed even when only one polycystic ovary is present [14]. However, these criteria do not apply to women taking oral contraceptive pills since their use modifies ovarian morphology that slightly different biochemical findings were included in Rotterdam criteria (Table 1) [14]. While NIH criteria considered total testosterone, free testosterone, androstenedione, and DHEA as biochemical markers, the 2003 Rotterdam criteria now consider free androgen index, total testosterone, and DHEA as diagnostic biochemical markers. Moreover, the Rotterdam criteria recognize the role of genetics in PCOS and encourage clinicians to take family histories to identify PCOS individuals more effectively [12, 13]. Compared with the NIH definition, the new definition introduced two new phenotypes: (i) ovulatory women with polycystic ovaries and hyperandrogenism, and (ii) oligo-anovulatory women with polycystic ovaries without hyperandrogenism. This has stimulated more debate as to where the boundaries should be set in diagnosing PCOS [1].

Table 1. Comparison of Two Established Diagnostic Criteria of PCOS

NIH Criteria (1990)	Rotterdam Criteria (2003)
(i) Oligomenorrhea or amenorrhea (ii) Hyperandrogenemia in the absence of related disorders	(i) Oligo- and/or anovulation (ii) Clinical and/or biochemical signs of hyperandrogenism (iii) Polycystic ovaries on ultrasonography and exclusion of related disorders
Both (i) and (ii) must be present	Two of three criteria must be satisfied
Diagnostic markers: total testosterone, free testosterone, androstenedione and DHEA	Diagnostic markers: free androgen index, total testosterone, and DHEA

CLINICAL MANIFESTATIONS AND EPIDEMIOLOGY OF POLYCYSTIC OVARY SYNDROME

PCOS occurs in 4-8% of women during their reproductive years, and it is the most frequent endocrine disease in women [8]. Approximately one in 15 women experiences PCOS [1], and an enlarged ovary is observed on ultrasound in 22% of women [14] during their reproductive years. Other common clinical manifestations include oligomenorrhea,

hyperandrogenism, acanthosis nigricans, insulin resistance, reproductive aberration and obesity (Fig. 1).

Oligomenorrhea

PCOS is an ovarian dysfunction caused by androgens, which inhibit folliculogenesis and lead to polyfollicular morphology, which then disturbs the menstrual cycle and leads to anovulation [15]. Among women experiencing oligoanovulation, 65-87% have PCOS [16]. The wide range may be attributed to the heterogeneous and complex nature of PCOS as well as the variation in criteria used for diagnosis. According to Balen *et al.* (1995), among women with PCOS, 47% experience oligomenorrhea [17], defined as six or fewer menses annually. A majority of women with PCOS experience irregular menstrual cycles, of which the most common manifestation is infrequent menstruation related to anovulation [14]. In a study of 173 women, polycystic ovaries were observed on pelvic ultrasound scan in 87% of women who also suffered from oligomenorrhea [9]. Moreover, some evidence indicates the presence of oligomenorrhea to be highly suggestive of PCOS in adolescents [18].

Hyperandrogenism (Hirsutism, Acne, and Male Pattern Alopecia)

Hypersecretion of androgens is the most widespread biochemical feature in PCOS women [19]. PCOS accounts for 70-80% of hyperandrogenism and is associated with elevated serum total or free testosterone concentrations [20]. Hyperandrogenism can manifest as hirsutism, acne, and male pattern alopecia. Whether hyperandrogenemia affects oxidant and antioxidant status in women with PCOS is unknown. However, in a human study, ROS generation was demonstrated to directly correlate with testosterone and androstenedione [21], suggesting that ROS induces OS, which may consequently contribute to hyperandrogenism in PCOS women. Plasma testosterone or androstenedione and ROS generation are associated, suggesting that OS may directly stimulate hyperandrogenism. *In vitro* studies have demonstrated that OS stimulates the androgen-producing ovarian steroidogenic enzymes, while antioxidants such as statins suppress these enzymes [22].

PCOS is present in 60-90% of women with hirsutism [16, 17, 23, 24] as increased androgen production leads to hirsutism and acne. Among women with PCOS, 35% have acne, and 6% express alopecia [17]. An inflammatory disorder of the hair follicle, acne is associated mainly with elevated levels of sebaceous secretion [25, 26]. Among women of mixed ethnicities with androgenic alopecia, 67% had polycystic ovaries compared with the 27% expressed in the BMI-, waist-hip-ratio- and age-matched control group [27]. Also, 21% of the women with androgenic alopecia demonstrated hirsutism, while this is true for only 4% of the BMI-, waist-hip-ratio- and age-matched control group.

Ovarian invasion by macrophages has been observed in PCOS women [28]. Moreover, mononuclear cells in the polycystic ovary activated by glucose can generate OS that could stimulate a local inflammatory response, which could in turn induce the generation of ovarian androgen in PCOS women [29]. More specifically, theca cells in the ovarian

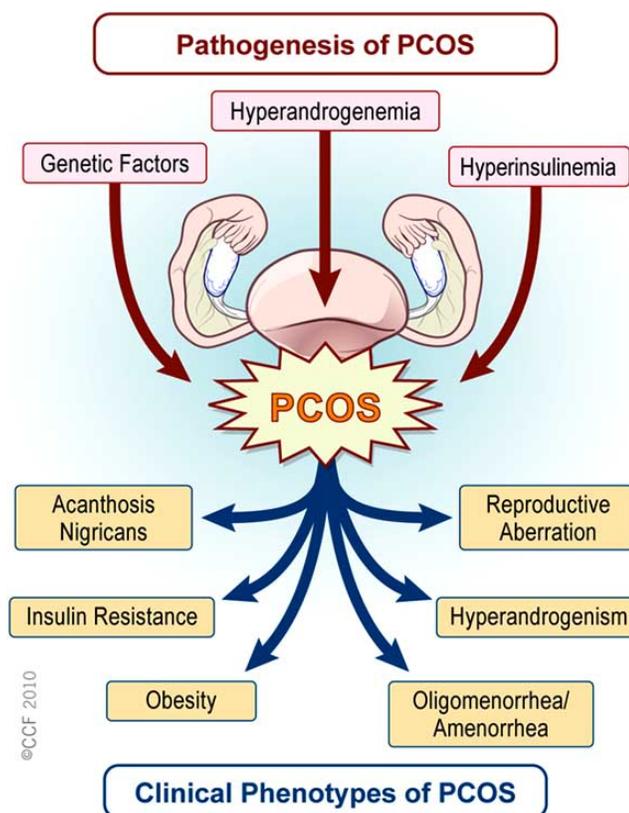


Fig. (1). Schematic description of various pathogenic factors that manifest PCOS in women of reproductive age. Such patients are at risk of developing a spectrum of disease phenotypes.

tissue overproduce androgens and insulin receptors and lead to hyperandrogenism [31]. Moreover, some researchers have investigated genetically programmed androgen secretion by the ovary during early childhood or puberty, which may contribute to pathophysiology in PCOS women [30].

Acanthosis Nigricans

Acanthosis nigricans, a disorder seen as dark and velvety skin with hyperpigmentation and papillomatosis, manifests itself normally in the axillae, skin flexures, and nape of the neck. Among women with PCOS, only 3% express acanthosis nigricans [17], which is associated with insulin resistance and, consequently, hyperinsulinemia [32].

Insulin Resistance

Increased oxidant status has been shown to correlate with insulin resistance. Insulin resistance can be found in 25-60% of women with PCOS [33]. The wide range may be due to varying diagnostic criteria, the heterogeneous nature of PCOS, and ethnic variations. Insulin resistance (IR) and hyperglycemia both can increase OS levels, although higher levels of total oxidant and antioxidant status have been demonstrated in non-obese PCOS patients without IR [34]. Hyperglycemia has been demonstrated to increase lipid peroxidation and lower antioxidant levels [35]. A significantly negative correlation between MDA levels, a marker of OS, and insulin sensitivity, as well as MDA levels and GSH (antioxidant) levels has been demonstrated [4]. This may imply that insulin resistance decreases antioxidant levels and increases lipid hydroperoxide (LPO).

Insulin resistance encourages oxidative stress because hyperglycemia and higher levels of free fatty acids lead to ROS production. An increase in ROS generation resulting from hyperglycemia has been observed in women with PCOS [36], and insulin infusion in obese individuals has been shown to inhibit ROS production [37]. Thus, insulin may defend against pro-inflammatory responses to hyperglycemia by acting as an anti-inflammatory agent.

Approximately 75% of obese PCOS women have IR and hyperinsulinemia [38]. However, insulin resistance independent of obesity can play a role in PCOS. Young, non-obese PCOS patients with high triglyceride levels as the only dyslipidemic feature have demonstrated high oxidative levels [2]. Furthermore, 20-40% of women with PCOS have impaired glucose tolerance [6], and women with PCOS exhibit higher levels of type II diabetes (T2DM) than non-PCOS controls (15% vs 2-3% in normal women) [7]. Insulin resistance and hyperinsulinemia also are features of metabolic syndrome, and women with PCOS exhibit an increased risk of hypertension, dyslipidemia, elevated plasminogen inhibitor type 1, elevated endothelin, endothelial dysfunction, and cardiovascular disease similar to the risks associated with metabolic syndrome [8].

Reproductive Aberration (Irregular Menses, Infertility, Miscarriage)

PCOS is a known cause of menstrual irregularity as well as infertility. Most commonly, irregular menstruation is as-

sociated with anovulation. Between 30-40% of women with amenorrhea are found to have PCOS [39].

Among PCOS women, more than 60% manifest infertility (primary/secondary), and 19% experience amenorrhea [17]. Moreover, pregnancy in PCOS women is more likely to be complicated by gestational diabetes, preeclampsia, pregnancy hypertension, and preterm labor leading to miscarriage [40]. Obesity in PCOS further increases resistance to ovulation induction treatment since obesity is associated with a disturbed pattern of gonadotrophin-releasing hormone production resulting in chronic elevation of tonic LH level with negative consequences on follicular development in the ovary [40].

Obesity

Obesity is more common in women with PCOS, and it can lead to severe hyperandrogenism. According to Franks (1989), 35% of women with PCOS are obese [10]. Increasing visceral adipose tissue and/or its activity may contribute to androgenic modulation [41]. Androgen excess is a known contributor to visceral adiposity in women, which provides high metabolically active tissue that stimulates the ovaries and adrenal to proceed with androgenization [15]. Both androgens and IR seem to have a combined effect on upper body adipose distribution. Obesity contributes to PCOS, as it affects hyperandrogenism and IR. In fact, the most influential factor in endocrinologic and metabolic disturbances in women with PCOS has been shown to be an elevated BMI > 25 [42].

Central obesity is also related to increased oxidant status [4]. Obesity has been shown to play an important role in elevated oxidative stress, which contributes to IR [43]. PCOS patients who are obese express higher levels of insulin resistance than lean PCOS patients [8]. The study by González *et al.* (2006) shows that compared with lean controls, PCOS women express higher p47^{phox} levels independent of obesity. In oxidative stress, p47^{phox} plays a role as part of enzymes that produce the superoxide radical. Increase in p47^{phox} expression decreases insulin sensitivity. It also has been shown to be greater in PCOS women versus controls and in obese PCOS and non-PCOS women versus lean PCOS women and non-PCOS women [29]. Thus, increased obesity may determine ROS-induced OS in obese PCOS women. PCOS also affects insulin performance, as increase in abdominal fat is associated with insulin resistance [1]. Slightly reducing the body weight of anovulatory, obese women was demonstrated to restore ovulation and increase insulin sensitivity by 71% [44]. Weight loss also reduces testosterone concentration, improves menstrual function and conception rates, decreases the likelihood of miscarriage, and increases sex hormone-binding globulin (SHBG) concentration [45-48].

Aside from obese PCOS women, lean PCOS women can express increased levels of abdominal adiposity [21]. In a study of 16 women with PCOS and 15 women without PCOS, mononuclear cells produced elevated levels of ROS in response to hyperglycemia in PCOS women, independent of obesity [29].

ETIOLOGY OF POLYCYSTIC OVARY SYNDROME

Pathogenesis of PCOS

Women with PCOS manifest a wide spectrum of symptoms and clinical features, including hyperandrogenism, ovulatory disturbances and polycystic ovaries and metabolic syndromes (Fig. 1). The latter is linked to insulin resistance and obesity that are often associated with PCOS [6, 49, 50]. In other words, the heterogeneity of PCOS is reflected in the multiplicity of factors such as insulin resistance, hyperandrogenism, and dysfunctional gonadotrophin dynamics that must come into play to manifest the disorder, and no single mechanism accounts for all clinical and biochemical forms of this syndrome. Moreover, environmental factors such as diet or stress also can trigger underlying risk factors and cause the development of PCOS. The most commonly discussed causes of PCOS can be categorized into three mechanisms: (i) insulin resistance and hyperinsulinemia, (ii) hyperandrogenemia and (iii) genetic factors.

(i) Insulin Resistance and Hyperinsulinemia

Insulin resistance, in which an abnormally high amount of insulin (hyperinsulinemia) is required to initiate a cellular response, is the most commonly encountered clinical disorder in both obese and non-obese PCOS women [51]. An oral glucose tolerance test is recommended for PCOS patients with BMI greater than 27 kg/m³ [14] because of the high risk for developing impaired glucose tolerance and diabetes in obese PCOS women (31% of obese PCOS patients vs. 10.3% of lean PCOS patients and 7.5% of obese PCOS patients vs. 1.5% of lean PCOS patients, respectively) [6]. Women with IR display increased fasting insulin level compare with controls of similar age and body weight. As a result, clinical and molecular research has focused on insulin receptor and post-receptor defects [19]. Some studies have correlated severity of hyperinsulinemia to the degree of clinical manifestation.

Insulin signaling, mediated through a protein tyrosine kinase receptor, has been investigated in PCOS patients. Dunaif *et al.* (1997) reported excessive serine phosphorylation, which inhibits insulin receptor tyrosine kinase activity, of insulin receptors in insulin-resistant PCOS patients. Moreover, adverse roles of serine phosphorylation in insulin signaling were further supported by the mechanism of tumor necrosis factor (TNF)- α -mediated insulin resistance in obese women [53] and P450c17 enzyme activity leading to hyperandrogenism in PCOS women [54]. Hyperinsulinemia also potentiates the effects of LH on theca interstitial cells, resulting in increased androgen production [19] while arresting the follicular maturation process [55, 56].

(ii) Hyperandrogenemia

The ovary is the primary source of hyperandrogenism in PCOS, driven by increased levels of LH hormone as ovarian dysfunction causes LH insensitivity [57]. The increase in basal LH level is the result of a disrupted hypothalamic-pituitary-gonadal axis [58]. Moreover, hyperandrogenemia impairs progesterone's ability to slow down the gonadotrophin-releasing hormone (GnRH) pulse [58]. As a result, elevated GnRH pulses further increase LH level and reduce

FSH, which converts excess androgen into estrogens *via* aromatase activity in normal women [15]. The elevated LH level arrests follicular cells and stimulates theca-cell-mediated androgen synthesis. Consequently, the increased androgenic environment in the ovary impairs follicular maturation [8].

Some studies have shown hyperandrogenemia and hypoenestrogenemia in PCOS-like conditions as the result of ovarian steroidogenic enzyme deficiencies such as 3 β -hydroxysteroid dehydrogenase type II and aromatase [59]. In other words, follicles that are unable to change their surroundings from androgen-dominant to estrogen-dominant environments will not acquire normal follicular growth and manifest as a polycystic ovary, a characteristic feature of PCOS. Adrenal steroidogenesis dysfunction also has been implicated in establishing a state of hyperandrogenemia in PCOS. When adrenal steroidogenesis dysfunction results in reduced cortisol production, adrenocorticotropic hormone (ACTH) production is increased to maintain normal serum cortisol level [8]. Increased ACTH production consequently stimulates adrenal androgen excess [8]. Thus, hyperandrogenism in PCOS women is caused by synergic aberration in steroidogenesis of both ovary and adrenal glands.

As stated above, hyperinsulinemia drives increased androgen production by theca cells [60]. Studies have shown that bilateral oophorectomy, the surgical removal of both ovaries [61, 62], the administration of GnRH-agonists to mimic an increased GnRH pulse [63], or antiandrogenic compounds [64] did not alter hyperinsulinemia and IR in PCOS women. Evidence supports disordered insulin action as a predecessor to development of hyperandrogenemia in PCOS patients.

(iii) *Genetic Factors*

Given that the incidence of PCOS is 6-8% in the general population [65], 35% of premenopausal mothers and 40% of sisters of PCOS women [65] suggests a probable role for genetics in PCOS. However, no conclusive role for any gene has been defined. This may be due to the limited selection of candidate genes, PCOS's heterogeneous nature, or lack of knowledge of disease pathophysiology and the role of environmental and lifestyle factors such as diet and obesity in modifying gene expressions [66]. Moreover, lack of universal male patterns and reliable markers for PCOS in women further challenge investigations of the syndrome's genetic origin. The proposed male phenotypes, such as increased serum dehydroepiandrosterone sulfate concentrations in brothers of PCOS women and insulin resistance in fathers and brothers of PCOS women still require further investigation for their practical uses [67]. However, more than 100 candidate gene approaches have selected genes based on their hypothetical roles in PCOS and target four general areas: (i) steroid biosynthesis and action; (ii) gonadotrophin synthesis and action; (iii) weight and energy regulation; and (iv) insulin secretion and action, as well as several areas added recently such as cardiovascular disease *via* inflammation, hypercoagulation, and blood pressure [66]. Of those, genes involved in steroidogenic abnormalities and insulin metabolism aberrations have been investigated the most due to their importance in PCOS' clinical manifestations.

Hyperandrogenemia in PCOS women is due partially to intrinsic defects in metabolic pathways. Because hyperandrogenism is prevalent among PCOS patients, genes involved in steroidogenesis such as cytochrome P450 17-hydroxylase/17,20-desmolase (CYP17) and the aromatase gene (CYP19) have been investigated. Upregulations of 3 α -hydroxysteroid dehydrogenase and 17-hydroxylase/17,20-lyase activities in PCOS women [68] are reflected in increased mRNA expression and an enhanced promoter region of CYP17 genes of the theca cells in young girls compared with controls [69]. On the other hand, a functional mutation of the CYP19 aromatase gene leads to excess circulating androgens in PCOS women [70-72]. However, family studies have not yet shown a correlation between CYP19 and PCOS [73], and more evidence is needed to confirm this hypothesis.

Genes involved in insulin signal transduction have been investigated. Variable number tandem repeat (VNTR) polymorphism in the promoter region of the insulin gene at 11p15.5 has shown quite confusing results. While Waterworth *et al.* (1997) [74] found strong correlations between class III variable number tandem repeats of the insulin gene allele and PCOS, Urbanek *et al.* (1999) [75] did not find evidence to link the class III allele and PCOS. The insulin receptor gene is another probable candidate gene since it seems to be silenced in molecular studies [19]. However, defective insulin receptor function is observed in the presence of serine phosphorylation instead of tyrosine phosphorylation in insulin receptors [52], suggesting more studies are required on downstream targets of the insulin receptor gene [19].

Hormonal Markers in PCOS Patients

Hormonal markers in PCOS women are viewed as a way to evaluate steroidogenesis. The most commonly encountered markers include, but are not limited to LH, FSH, estrogen, sex hormone-binding globulins (SHBG), insulin-like growth factor -1 (IGF-1), total/free testosterone, androstenedione, dehydroepiandrosterone (DHEA) and DHEA metabolite DHEAS, anti-Mullerian hormone (AMH), and 17-hydroxyprogesterone [8, 19, 60, 76].

Testosterone production and high insulin level in PCOS women directly down-regulate SHBG synthesis by the liver, which makes a low SHBG level a good indicator of insulin resistance [77]. SHBG has strong binding affinity to testosterone and dihydrotestosterone thus controlling androgen bioavailability in serum [78]. Reduced SHBG results in increased levels of bioavailable testosterone. Since serum-bound testosterone (T) is the most frequent androgen measured to diagnose hyperandrogenemia, the reduction in the proportion bound to SHBG makes the assessment somewhat unreliable [76]. As a result, the free androgen index (FAI=T/SHBG * 100%) or the association constant for testosterone binding to SHBG and albumin are utilized to account for these metabolic changes [79]. Free T also may be measured directly *via* equilibrium dialysis [76]. Although other androgens such as androstenedione (A4) or total testosterone may also be utilized to diagnose hyperandrogenemia, no studies have indicated their superiority as surrogate markers. For example, Knochenhauer *et al.* (1998) showed that only 2 out of 11 (18%) PCOS women had abnormally higher

thyroxine (T4) level, which is blunted by high testosterone level.

Insulin binds to IGF-1 receptors on theca cells with significantly higher affinities than IGF-1 [81]. Hepatic IGF-1 binding protein secretion also is inhibited in PCOS women, leading to excessive free IGF-1, which is suspected to play a role in the abnormal androgenesis of theca cells along with high LH [82]. IGF-1 and insulin further increase mRNA of P450c17, leading to increased androgen biosynthesis in ovary and adrenal glands [8]. The use of insulin-sensitizing agents such as metformin has been demonstrated not only to reduce circulating insulin concentration but also to reduce ovarian androgen biosynthesis [83].

DHEA secreted from the adrenal zona reticularis is another actively investigated hormonal marker in PCOS women. However, DHEA has several shortcomings as a surrogate marker due to its diurnal variation, intra-subject variation and low serum concentration [84]. On the other hand, DHEAS, DHEA's sulfate ester, is not subject to these variations, making it a more preferred marker to assess increased adrenal androgen production [85]. In clinical studies, approximately 20-70% of PCOS women manifest excess DHEAS serum levels [86-88]. However, DHEAS levels decrease with age [88], and levels are controlled by the activity of DHEA sulfotransferase [89]. Moreover, ethnicity also may affect circulating DHEAS levels with lower circulating levels of DHEAS is reported in Mexican American group compared with Caucasian American controls [90]. Consequently, in PCOS patients with high DHEAS measurements, only 10% will actually have hyperandrogenaemia [76]. Thus, DHEAS measurements should be interpreted with caution [76].

AMH is secreted from the Sertoli cells of the fetal testis to inhibit female Mullerian ducts development in a male embryo. It also is produced by the granulosa cells of small antral and pre-antral follicles to disrupt FSH's aromatase induction in the ovary [91], compromising normal ovulation in PCOS women. Studies have shown that AMH levels are significantly higher in PCOS women [92] and confirmed that granulosa cells release more AMH when cultured *in vitro* [93]. Moreover, AMH level was positively correlated with antral follicle counts [94], suggesting that serum AMH measurements may serve as an alternative diagnostic tool when ultrasonography is not an option in patients younger than 35 (1). While AMH's role in folliculogenesis is generally established, its association with circulating androgens is more controversial. Pigny *et al.* (2003) [95] found a correlation between AMH and testosterone and androstenedione only in PCOS women, while Piltonen *et al.* (2005) [96] reported AMH levels in both PCOS women and controls were correlated with both testosterone and androstenedione.

In conclusion, no hormonal marker can be used as the sole criterion to diagnose PCOS. Hormonal assays may serve as supplementary diagnostic tools for clinicians and scientists.

INTRODUCTION TO OXIDATIVE STRESS

Unstable and highly reactive, free radicals achieve stability by stealing electrons from nucleic acids, proteins, lipids,

carbohydrates, and other nearby molecules [97], thus inducing cellular damage. The two major forms of free radicals are ROS and RNS. Free electrons typically form reactive oxygen species during oxygen reduction as a by-product of natural metabolic pathways [98]. Most of the mitochondrial generation of ROS occurs at complexes I (where NADH dehydrogenase acts), and III (where the ubiquinol to ubiquinone to ubiquinone conversion occurs) of the electron transport chain (ETC) [99].

Of inspired oxygen, 98% is reduced during lipolysis and chemical energy generation, and 2% is incompletely reduced, leading to three major forms of ROS [97]. The three main forms of reactive oxygen species are the superoxide radical [O_2^-], hydrogen peroxide [H_2O_2], and hydroxyl [$HO\cdot$]. Superoxide is formed through electron leakage at the electron transport train. At complex IV, molecular oxygen normally is converted to water, but it may gain an extra electron as they are being passed down the ETC during ATP generation [100]. Hydrogen peroxide is formed from either superoxide dismutation or oxidase enzymes. The most reactive form is the hydroxyl ion, as it has three extra electrons. Through alteration of purines and pyrimidines it can cause strand breaks and damage DNA. When the balance between antioxidants and oxidants does not exist, modification of key transcription factors can occur, which can alter gene expression (Fig. 2). The superoxide radical can be converted to hydrogen peroxide by mitochondrial superoxide dismutase 2, preceding further modification by GSH peroxidase to form water. Thus, the presence of antioxidants is vital to maintaining redox homeostasis. Decreased amounts of antioxidants to counteract the production of ROS can lead to cell damage [99].

ROLE OF ROS IN PCOS

ROS are free radicals with oxygen centers. An unpaired electron in the outermost shell is an extremely unstable configuration, and free radicals quickly react with other molecules or radicals to achieve the stable configuration of pairs of electrons in their outermost shells [101]. Several basic cellular processes lead to the production of ROS within a cell. Cellular respiration involves the reduction of molecular oxygen (O_2) to water in the electron transport chain. This reduction occurs through a series of reactions: (i) $O_2 + e^- \rightarrow O_2^-$, (ii) $O_2^- + 2H_2O \rightarrow 2H_2O_2$, (iii) $O_2^- + H_2O_2 \rightarrow OH\cdot + OH^- + O_2$. As mentioned earlier, the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($HO\cdot$) are three major species of ROS [97].

Role of MDA in PCOS

Unsaturated fatty acid peroxidation is a radical chain reaction initiated by the abstraction of a hydrogen atom from a methylene group of the fatty acid chain. The carbon radical formed by this reaction tends to be stabilized by molecular rearrangement, leading to conjugated double bonds. By reaction with oxygen, a reactive peroxy radical is generated that can abstract a hydrogen atom from lipids [102].

Products of lipid peroxidation reactions have been widely employed as biomarkers for OS. MDA, produced during the decomposition of polyunsaturated fatty acids, is one of the stable end products of lipid peroxidation that can serve as a

good biomarker [102]. Several methods are available for quantification of lipid hydroperoxides and secondary lipid peroxidation products. MDA is most commonly measured by a thiobarbituric acid-reactive substances (TBARS) assay with a simple spectrophotometric method. The amount of MDA corresponds to the chromogen found from MDA and thiobarbituric acid (TBA) with a maximum absorption at 532-535 nm. While the assay for MDA is non-specific, HPLC is a more accurate tool for MDA estimation.

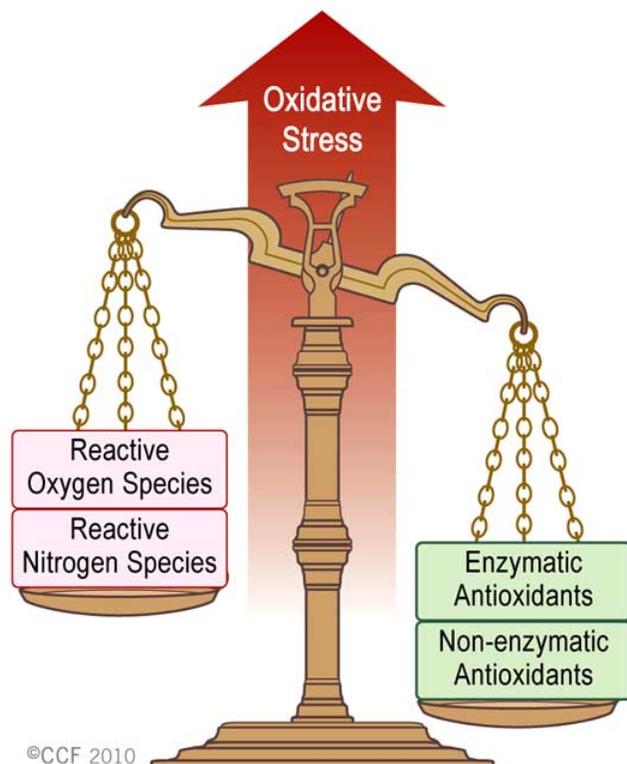


Fig. (2). Oxidative stress occurs when the balance between highly reactive radicals (oxidants) and antioxidants tips towards the oxidants; it negatively contributes to reproductive processes.

Kuşçu *et al.* (2009) compared PCOS patients ($n=31$, mean age 23.8 years and mean BMI 21.8) with healthy controls. Blood MDA level, not specified as measured from serum or erythrocyte, was found to be significantly higher in the PCOS group (0.12 ± 0.03 vs 0.10 ± 0.03 , $p=0.01$). This study demonstrated that PCOS subjects had significantly elevated concentration of plasma MDA independent of obesity. PCOS patients in this study were further divided into two subgroups in terms of insulin resistance, IR- and IR+. The results showed that MDA level is significantly higher in young, non-obese PCOS patients even in the absence of IR when compared with controls (0.125 ± 0.03 vs 0.101 ± 0.03 , $p=0.03$) (2).

Sabuncu *et al.* (2001) compared PCOS patients ($n=27$, mean BMI 31.4 and mean age 26.7 years) with BMI- and age-matched controls. They demonstrated that higher levels of erythrocyte MDA were seen in PCOS patients (mean=70.9 $\mu\text{mol/mol Hb}$) compared with controls ($p=0.009$). Significantly higher levels of MDA in PCOS patients compared

with controls also were also found by Palacio *et al.* (2006) [103].

Zhang *et al.* (2008) 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 $\mu\text{mol/L}$, $P<0.05$) (104). A negative point of this study was that some of the important patient characteristics, such as BMI and age, were not recorded.

However, Karadeniz *et al.* (2008) [105] found MDA levels in PCOS patients ($n=58$) were similar to those of controls (5.38 ± 2.47 vs 4.475 ± 2.06 , $p>0.05$) (105). Furthermore, 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. This observation suggests that the presence of insulin resistance in PCOS patients has no effect on MDA levels. In addition, Dursun *et al.* studied PCOS patients ($n=23$, mean BMI 23.0 and mean age 24.4 years) and found serum MDA levels in PCOS patients were similar to those of BMI- and smoking status-matched controls (3.60 ± 1.22 vs 3.53 ± 1.0 $\mu\text{mol/l}$) [106].

Role of Protein Carbonyl

Protein oxidation status often is assessed with a colorimetric assay that measures protein carbonyl (PC) content, after reacting the serum with dinitrophenylhydrazine. Fenkci *et al.* (2007) demonstrated 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 , $p=0.001$). This observation of higher protein oxidation suggested that free radicals damage proteins in PCOS patients [107]. Furthermore, protein carbonyls were shown to have a positive correlation with fasting insulin, suggesting a strong association between insulin resistance and protein oxidation in PCOS [107].

Role of NOS in PCOS

RNS are free radicals with nitrogen centers. The two major examples of RNS are nitric oxide (NO) and nitrogen dioxide (NO_2). NO is specifically synthesized by NOS during the conversion of L-arginine to L-citrulline [97]. Under normal physiological process, NO acts in a variety of tissues to regulate normal cell functions, but excess of NO can be toxic [101]. NO, with an unpaired electron, is highly reactive and can damage proteins, carbohydrates, nucleotides and lipids. RNS have been associated with asthma, ischemic/reperfusion injury, septic shock, and atherosclerosis [108].

Role of NO

Measuring plasma concentration of NO_3^- and NO_2^- assesses NO concentration. The sum of NO_3^- and NO_2^- is assumed as the best index of total NO. NO contents are assessed by a two-step process consisting of the conversion of nitrate to nitrite first, followed by spectrophotometric detection of total nitrite at 540 nm [109].

Nácul *et al.* (2007) reported 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 $\mu\text{mol/L}$, $p>0.05$). Moreover, a significantly negative correlation was observed

between NO and fasting insulin levels ($r=-0.39$, $p=0.03$) and HOMA ($r=-0.41$, $p=0.02$) (84). These data suggested that NO was related to the presence of insulin resistance in PCOS patients, although further studies are needed to clarify the role of NO in PCOS.

ROLE OF ANTIOXIDANTS IN PCOS

Antioxidants scavenge excess ROS to counteract potential for significant cell damage by excess ROS. Antioxidants help create a balance between beneficial oxidant generation (frequently act as cell signaling molecules) and damaging oxidative stress. There are two categories of antioxidants: enzymatic and non-enzymatic. Enzymatic antioxidants include SOD, catalase, and GPx. Non-enzymatic antioxidants include GSH, α -tocopherol (vitamin E), β -carotene, ascorbate (vitamin C), taurine, L-carnitine, coenzyme Q10, etc (97). There are three SOD isoforms in eukaryotes: manganese SOD (Mn-SOD), copper/zinc SOD (Cu/Zn-SOD), and extracellular SOD (EC-SOD)¹.

Antioxidants that prevent or limit the damaging effects of oxygen radicals have been reported to have important roles in the female reproductive system and in the pathogenesis of female infertility.² Changes in antioxidant concentrations in serum and peritoneal fluid have been studied in idiopathic infertility, tubal infertility, and endometriosis patients [111, 112]. Results indicate that investigation of antioxidant concentrations in PCOS patients is promising. Various studies have measured antioxidant markers to correlate OS and PCOS and the diverse clinical manifestations of metabolic syndrome including diabetes, obesity, and cardiovascular diseases.

Role of TAC in PCOS

Total antioxidant capacity is the ability of serum to quench free radical production, protecting the cell structure from molecular damage. Various detection assays for TAC have been described, one of which is the spectrophotometric assay in which long-lived 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) radical cation is measured. ABTS radical is formed by the incubation of ABTS with a peroxidase (metmyoglobin) and hydrogen peroxide. The principle of the assay is to measure the ability of aqueous and lipid antioxidants to inhibit the oxidation of ABTS to ABTS⁺ [113]. The capacity of the antioxidants to prevent ABTS oxidation was compared with that of standard Trolox, a brand name for 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble derivative of vitamin E. This

assay measures the combined antioxidant capacity of all its components including vitamins, proteins, lipids, glutathione, uric acid, etc. [113].

Another method to measure TAC is through the production of hydroxyl radical *via* Fenton reaction. It is initiated by the hydroxyl radical, and the brown-colored dianisidiny radical cations are produced in the reaction medium of the assay [114]. Antioxidant capacity of the added sample against these colored potent free-radical reactions is measured as a whole to represent TAC. The results also were expressed as millimoles of Trolox equivalent per liter [34].

Fencki *et al.* (2003) demonstrated that TAC was significantly lower in PCOS patients ($n=30$ mean age 25.80 ± 0.63 years and mean BMI 24.3 ± 1.1) compared with the age-, BMI-, and smoking status-matched controls (1.15 ± 0.01 vs 1.30 ± 0.02 mmol/L, $p=0.001$) [115]. This observation suggested that the oxidative status imbalance in PCOS women might contribute to their increased risk of cardiovascular diseases. Moreover, there was a negative correlation between fasting insulin level and TAC, suggesting that that IR may have a detrimental effect on antioxidant defense system in PCOS.

However, Verit *et al.* (2008) reported that TAC levels were significantly higher in PCOS patients ($n=63$ mean age 24.4 ± 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 Trolox Eq/L, $p<0.0001$). This study demonstrated that TAC was increased in non-obese, normoinsulinemic PCOS patients (fasting insulin 10.7 ± 5.0 μ IU/mL, no significant difference compared with controls). High levels of antioxidants in PCOS are thus suggested to have detrimental effects. This result was inconsistent with other studies in the literature. Although the complete mechanism of this elevation is unknown, it is proposed that TAC was increased as to compensate for the increase in total oxidative stress (19.1 ± 7.6 vs 12.3 ± 4.8 μ mol H₂O₂ Eq/L, $p<0.0001$) [34].

Although results of studies about antioxidant levels are conflicting, it is possible to conclude that an imbalance between oxidants and antioxidants occurs in PCOS. Further studies of oxidative stress defenses in PCOS are needed to clarify the association between antioxidants and PCOS.

Role of SOD in PCOS

SOD induces the conversion of superoxide to H₂O₂, a toxic substance that is converted to water by GPx. High SOD levels may explain the absence of endothelial dysfunction markers. Generation of an adequate antioxidant response against such an intrinsic oxidative load may provide proper functioning of vascular system.

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 the PCOS patients were further divided into two subgroups: IR- and IR+. SOD levels were 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). This elevation may have been due to the body's defense mechanisms. Subjects used in this study were relatively young (mean age

¹ Mn-SOD, which contains a manganese prosthetic group, resides in the mitochondria. It is thought to protect mitochondrial membranes, proteins, and DNA from O₂⁻ generated as a result of the electron transport chain. The Cu/Zn-SOD, which contains copper and zinc prosthetic groups, often resides in cytosol. EC-SOD, is secreted and binds to the elements of the extracellular matrix. All forms of SODs are thought to reduce O₂⁻ to form O₂ via the oxidation of the prosthetic group [110].

² The production of H₂O₂ within cells may lead to the production of HO[•] and subsequent cellular damage. Thus, it is important to remove H₂O₂. Catalase functions to rapidly transform H₂O₂ to water and oxygen via the redox reactions achieved by its manganese or heme group. Catalase resides mainly in peroxisomes, mitochondria and the cytosol [110].

23.8±4.37 years) with greater ability to cope with higher levels of ROS production.

Sabuncu *et al.* (2001) demonstrated elevated SOD levels (mean value 94.62 MU/mol Hb) in a group of PCOS patients with mean BMI 31.4 ($p < 0.05$). They proposed that the increase in SOD levels might be due to a compensatory response to OS.

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 $\mu\text{U/mL}$, $P < 0.05$) [104]. However, the study did not capture other patients' characteristics, making it difficult to comment as to why SOD level was lower in this selected PCOS group.

Role of GPx

Sabuncu *et al.* (2001) demonstrated 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). In an environment with increased H_2O_2 , an increase in GPx is to be expected. However, 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 [4].

Role of GSH

GSH was 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 [116, 117].

Sabuncu *et al.* (2001) demonstrated 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, $p=0.03$). Low levels of GSH may have been partly related to IR. Increased ROS and peroxides may also have led to GSH depletion.

In accordance with the findings of Sabuncu *et al.* (2001), Dincer *et al.* (2005) also found GSH levels to be significantly lower in women with PCOS than in the control group (5.03±0.96 vs 5.59±0.82 $\mu\text{mol/gHb}$, $p < 0.05$) [118]. They proposed that GSH depletion might have resulted from increased production of ROS in PCOS patients.

CONCLUSION

In this review we documented the burgeoning interest in the relationship between OS and PCOS, evidenced by a rapidly increasing body of literature. 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 OS and PCOS. Measurement of biomarkers of OS also is known to be a controversial issue. Units of measurement in published studies are not consistent. Standardized measurement units of each biomarker should be used in the future to facilitate comparison across studies. Additional studies are recommended to examine the association and mechanism of OS on PCOS.

KEY POINTS

- PCOS is the most common female endocrinological abnormality, affecting 4-8% of women in their reproductive years.
- Clinical PCOS is diagnosed in women based on presence of at least two of the following criteria a) oligo- or anovulation, b) biochemical and/or clinical features of hyperandrogenism, c) polycystic ovary appearance on ultrasound scanning.
- The condition is multifactorial, but insulin resistance appears to be a central feature that explains many of the manifestations of the syndrome and the increased risk of developing type II diabetes.
- Components of metabolic syndrome, particularly hyperinsulinemia and central obesity (visceral adiposity), are frequently encountered in PCOS.
- Risk markers for cardiovascular disease, endothelial dysfunction, and dyslipidemia are increased in PCOS
- Oxidative stress seems to be involved in altered steroidogenesis in the ovaries, thus contributing to increased androgen production, disturbed follicular development, and, ultimately, infertility.

EXPERT COMMENTARY

There is mounting evidence to substantiate the etiological relationship between PCOS and metabolic syndrome. However, epidemiological research thus far has failed to demonstrate that the markers of cardiovascular disease, endothelial dysfunction, and dyslipidemia in PCOS are associated with increased mortality. The role of oxidative stress in the pathogenesis of PCOS is not fully understood, and the evidence is conflicting. The current evidence merely points towards an association between the oxidative microenvironment of the ovarian tissue and ovarian steroidogenesis and follicular development. Whether oxidative stress is the cause or the result of the metabolic disturbances encountered in PCOS remains to be elucidated. However, a strong relationship among hyperinsulinemia, hyperlipaemia, and oxidative stress is recognized.

FIVE-YEAR VIEW

Research is underway to determine whether reducing visceral adiposity in PCOS patient is associated with reduced markers of cardiovascular risk, improved insulin resistance, and the amelioration of the clinical symptoms of PCOS. Health economic constraints mean that issues associated with PCOS should be addressed in a radical way to modify the associated health risks. A prominent example of this is the increased adoption of systems in which the availability of fertility treatment is restricted for overweight PCOS patients because of poor treatment outcome. In the next few years clinical trials will determine the role of exercise, diet, and other life style modifications, as well as pharmacological intervention, on improving fertility outcomes and reducing health risks in these patients.

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