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RESEARCH ARTICLE

EVALUATION OF OXIDATIVE STRESS PARAMETERS IN RELATION TO ANTHROPOMETRIC STATUS AND METABOLIC INDICATORS IN WOMEN WITH PCOS

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Abstract

Introduction: Among women of reproductive age, PCOS (polycystic ovarian syndrome) is a prevalent endocrine condition marked by metabolic abnormalities such as insulin resistance and hyperandrogenism. PCOS is linked to oxidative stress and chronic inflammation, which add to the disease's complicated pathophysiology and long-term health hazards. This study aims to assess oxidative stress markers in women with PCOS compared to healthy controls and to evaluate the impact of obesity, hyperandrogenism, and insulin resistance on these markers.

Methods: This study analyzed a cohort of 170 women aged 18 to 46 at a medical facility in Odisha, India, from 2018 to 2020, following the Rotterdam criteria for diagnosing PCOS. Participants included 26 women with PCOS and 21 healthy controls, with assessments involving hormonal, metabolic, and oxidative stress evaluations through blood samples and pelvic ultrasounds. Insulin resistance and free androgen index were calculated using standardized formulas.

Results: In this study, women with PCOS exhibited substantially higher androgen levels and oxidative stress markers, including malondialdehyde and catalase activity, compared to the control group. Hirsutism and acne were observed exclusively in the PCOS cohort, with 29.27% undergoing infertility treatment. Additionally, 48.42% of the PCOS group showed elevated androgen levels or hirsutism, and 43.16% were classified as overweight or obese. Insulin resistance was present in 21.05% of participants, highlighting a complex interplay between hormonal and oxidative stress parameters in PCOS.

Conclusion: The study discovered that women with PCOS had significantly greater levels of androgen and oxidative stress indicators compared to healthy controls. This implies that in this population, oxidative stress and hyperandrogenism might be connected.

Keywords: Polycystic Ovary Syndrome (PCOS), Hyperandrogenism, Oxidative Stress, Malondialdehyde (MDA), Testosterone

BACKGROUND/INTRODUCTION

Endocrine diseases, which impact 5-14% of women of reproductive age, are frequent and include polycystic ovarian syndrome (PCOS). Because of its complicated causes, research is currently being done on it. Women with PCOS are more likely to experience metabolic issues, including insulin resistance (IR), elevated insulin levels, type 2 diabetes, abdominal obesity, and components of the metabolic syndrome [1-3]. One of the main features of PCOS is hyperandrogenism, which primarily presents as hirsutism but can also cause severe and challenging acne. The overproduction of androgens, which can begin during pregnancy, may be the reason for the distribution of fat like an android. This hyperandrogenism is made worse by decreased levels of sex hormone-binding globulin (SHBG), which raises free physiologically active androgens [5, 6]. Women with PCOS may experience infertility, recurrent miscarriages, irregular menstruation, and a variety of chronic effects from metabolic issues, including hypertension, cardiovascular disease, and estrogen-influenced cancers [7-9].

Insulin resistance, which affects 50–70% of PCOS patients, is thought to be a major pathophysiological mechanism. Insulin resistance (IR) measures, like the homeostatic model assessment of insulin resistance (HOMA-IR), are substantially greater in PCOS-affected women than in non-PCOS-affected women [10,11]. About 90% of obese PCOS women and 75% of lean PCOS women are thought to have blood system dysfunctions linked to atherosclerosis, ageing, obesity, type 2 diabetes, cardiovascular disease, and cancer [12]. Obesity and excess weight aggravate IR and hyperandrogenism and interfere with proper ovulation. Decreased fat breakdown and increased creation of fat cells are the results of several obesity-related illnesses. The interplay between ovarian function and adipose tissue may give rise to problems with follicle growth. Furthermore, pro-inflammatory adipokines released in response to obesity affect the ovaries' ability to produce testosterone [13, 14]. A relative deficit of follicle-stimulating hormone (FSH) causes a persistent absence of ovulation, while luteinizing hormone (LH) is assumed to be the driving force behind the excessive production of androgens in ovarian thecal cells [9]. Due to problems with follicular development, selection, and ovulation, ovarian hyperandrogenism increases the risk of estrogen-related malignancies and causes irregular menstruation and infertility as a result of a relative excess of oestrogen and a lack of progesterone [7].

Empirical evidence suggests that PCOS is significantly influenced by chronic inflammation. Fat cell expansion and consequent tissue hypoxia may result from this syndrome. Obesity and high insulin levels are frequently associated with inflammation,

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which raises androgen levels [15]. Increased oxidative stress (OS) and inflammation are caused by free radicals, which are produced by both hereditary and environmental causes and are linked to metabolic abnormalities in PCOS. It has been observed that in PCOS, inflammation and IR are correlated [16].

Obesity, elevated insulin levels, and oxidative stress all have a negative impact on glucose metabolism, particularly the uptake of glucose by muscle and fat tissue. Protein oxidation may be accelerated by elevated insulin brought on by IR, even in the absence of elevated blood sugar. Antioxidant treatment may improve tissue sensitivity to insulin, according to certain studies [17]. Reactive oxygen species (ROS) are produced in response to higher free fatty acid levels and high blood sugar, both of which are associated with oxidative stress indicators [18]. An imbalance between the body's oxidants and antioxidants is what defines oxidative stress. Reactive oxygen and nitrogen species can efficiently fight infections at low concentrations, but when they get too high, they can damage proteins, lipids, and DNA [19]. There are several ways to counteract these oxidative agents, such as low-molecular-weight antioxidants like ferritin, albumin, vitamin E, and ascorbic acid, as well as macromolecular antioxidants like malondialdehyde (MDA) and enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). As a main antioxidant defence, ascorbic acid shields additional antioxidants such as vitamin E.

Catalases degrade hydrogen peroxide, whereas peroxidases aid in its reduction. Peroxide accumulation can result in the production of free radicals, which harm cell membranes and are linked to diseases like cancer and atherosclerosis [20]. Vitamin E and peroxidases are essential components of the defence mechanism against lipid oxidation. Reduced CAT activity may be a sign of ongoing oxidative stress because CAT is essential for removing free radicals [21, 22]. Polyunsaturated fatty acid lipid peroxidation produces MDA, another indicator of persistent oxidative stress. It is a valuable indication for assessing the effectiveness of antioxidant therapy since its concentration tends to increase with increased ROS generation [23]. Diets low in calories and glycaemic index, or high in antioxidants, can help people lose weight while reducing oxidative stress markers. Studies have revealed that women with PCOS had higher levels of oxidative stress indicators than women without the condition, although the precise causes of this differential are still unknown [24].

Hyperandrogenism, insulin resistance resulting from genetics, environmental factors, and abdominal obesity are thought to be the main causes of oxidative stress in PCOS. Infections may also contribute, however this is less well known. It remains unclear how much of an impact these factors have on oxidative stress. Assessing the risk of oxidative damage and associated disorders by the evaluation of antioxidant biomarkers and oxidative stress may be helpful in managing and preventing oxidative

conditions. There has been conflicting research on oxidative stress parameters. These markers have not been concurrently analysed in studies looking at SOD, MDA, CAT, and GPx [24, 25]. There are no studies that compare these markers of oxidative stress to insulin resistance and body weight. This study aimed to measure oxidative stress indicators in PCOS patients and compare them to women with normal body weight who did not have menstruation problems (control group). The study evaluated the levels of MDA, SOD, CAT, and GPx in the PCOS group according to their levels of obesity, hyperandrogenism, and insulin resistance. The aim was to determine the primary mechanisms responsible for antioxidant protection and their impact on the advancement of this pathological disease.

MATERIALS AND METHODS

Study Design

This research involved a cohort of 170 women aged 18 to 46 who were admitted to the Department of Endocrinology, Gynecology, and Gynecological Oncology at a medical facility in Odisha, India, from 2018 to 2020.

Inclusion and exclusion criteria

There were 26 participants in the study, including a group of women with infrequent menstruation and a verified diagnosis of PCOS based on the Rotterdam criteria. By means of ultrasound exams, however, it was confirmed that the twenty-one women in the control group had neither polycystic ovaries nor menstruation abnormalities.

To ensure the integrity of the study, certain exclusion criteria were applied to both groups. Participants with a history of diabetes, ischemic heart disease, or hypertension were excluded. Additionally, women who had undergone current or previous hormonal therapies, those taking lipid-lowering or insulin-sensitizing medications, and those using antioxidant supplements were not considered for inclusion in the study.

Diagnostic Procedures

All participants underwent a pelvic ultrasound examination using the General Electric Voluson E8 Expert device equipped with a 7.5 MHz vaginal probe. PCOS diagnoses were made during the 2nd to 5th day of the menstrual cycle.

Assessments and Measurements

Each participant completed a comprehensive medical history assessment and physical examination. Additionally, hirsutism was assessed using the Ferriman-Gallwey scale, with a diagnosis made for scores exceeding eight points.

Anthropometric Measurements

Body mass index (BMI) and waist-to-hip ratio (WHR) were recorded for all participants. A WHR below 0.8 was considered normal. According to the 2016 guidelines from the American College of Endocrinology and the American Association of

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Clinical Endocrinologists, a BMI between 18.5 and 24.99 kg/m² was classified as normal, while values above 25 kg/m² indicated overweight or obesity. Blood pressure readings were also taken for each individual.

Blood Sample Analysis

Fasting blood samples were drawn via ulnar vein puncture to analyze various parameters, including total cholesterol, triglycerides, insulin (fasting and post-oral glucose load), glucose, and hormone levels (FSH, LH, estradiol, testosterone, and androstenedione). Blood was collected into two EDTA tubes, which were then centrifuged at 1000× g for 15 minutes at 4 °C. The resulting plasma was stored at -80 °C for later analysis.

IR was calculated using the HOMA formula: HOMA-IR = (fasting glucose concentration [mmol/L] × fasting insulin concentration [μIU/mL]) / 22.5.

A HOMA-IR value of 3.8 or higher indicated IR. The free androgen index (FAI) was determined using the formula:

FAI = (testosterone [nmol/L] / SHBG [nmol/L]) × 100.

Assessment of Antioxidant Activity

Hemoglobin levels in hemolysate samples were measured using the Drabkin method. Enzyme activity for catalase CAT, SOD, and GPx was assessed via spectrophotometry. GPx activity in erythrocytes was evaluated with the Wendel method, CAT activity was measured using the Aebi method, and SOD activity was analyzed with the Misra and Fridovich technique. MDA levels were measured using high-performance liquid chromatography (HPLC) with an Agilent Technologies 1200 Series system.

Statistical Analysis

SPSS 13.0 was used for statistical analysis. Participant characteristics were described using mean, standard deviation, and range. The Shapiro-Wilk test determined data distribution normality. Spearman's rho assessed quantitative variable correlations, while the Mann-Whitney U test assessed group differences. A p-value under 0.05 was significant.

RESULTS

While the control group (n = 75) had a mean age of 33.12 ± 8.59 years, the study group (n = 95) had a mean age of 28.35 ± 6.92 years. In the study group, 8.42% (n = 8) of the PCOS patients had hirsutism (score > 8), and 62.11% (n = 59) had acne; the control group did not show any signs of these diseases. Notably, among the women in the study group, 29.27% (n = 24) received infertility treatment. There were no discernible changes between the two groups' anthropometric test results, which are displayed in Table 1.

Table no.1: Anthropometric Test Results

Parameter	Control Group (n = 75)	Study Group (n = 95)	p-value
Height (cm)	165.80 ± 5.85	166.02 ± 5.91	0.855
Body Weight (kg)	68.50 ± 11.25	72.85 ± 14.73	0.210
Hip Circumference (cm)	102.56 ± 9.88	106.12 ± 10.95	0.182
Waist Circumference (cm)	85.25 ± 11.09	89.55 ± 15.44	0.647
Waist-to-Hip Ratio	0.915 ± 0.054	0.904 ± 0.072	0.391
BMI (kg/m²)	25.88 ± 4.50	27.45 ± 6.35	0.294

Hormonal Concentrations

Table 2 reveals a notable difference in androgen (A) levels between the PCOS group (3.55 ± 1.15 ng/mL) and the control group (2.42 ± 1.10 ng/mL) ($p < 0.0001$). Testosterone (T) levels were also significantly elevated in the PCOS group (0.495 ± 0.223 ng/mL) compared to the controls (0.332 ± 0.175 ng/mL) ($p < 0.001$). Additionally, the free androgen index (FAI) was higher in the PCOS group (1.10 ± 0.61) than in the control group (0.64 ± 0.42) ($p = 0.003$). On the other hand, sex hormone-binding globulin (SHBG) levels were lower in the PCOS group (50.20 ± 24.50 nmol/L) than in the control group (81.25 ± 75.33 nmol/L), although this difference was not statistically significant ($p = 0.092$). Lipid profile measures, including triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and cholesterol (Chol), showed no significant differences between the two groups.

Table no.2: Hormonal Concentration Values

Parameter	Control Group	Study Group	p-value
Testosterone (T) [ng/mL]	0.332 ± 0.175	0.495 ± 0.223	0.001
Sex Hormone-Binding Globulin (SHBG) [nmol/L]	81.25 ± 75.33	50.20 ± 24.50	0.092
Androgens (A) [ng/mL]	2.42 ± 1.10	3.55 ± 1.15	0.0001
Free Androgen Index (FAI)	0.64 ± 0.42	1.10 ± 0.61	0.003
Low-Density Lipoprotein (LDL) [mg/dL]	107.50 ± 32.40	110.80 ± 38.00	0.674
High-Density Lipoprotein (HDL) [mg/dL]	66.30 ± 14.80	57.25 ± 15.75	0.091

Triglycerides (TG) [mg/dL]	97.80 ± 73.00	106.50 ± 92.25	0.842
Total Cholesterol (TChol) [mg/dL]	182.50 ± 38.70	171.80 ± 41.90	0.289

Oxidative Stress Parameters

Patients diagnosed with PCOS had significantly greater levels of MDA, measuring 0.09 ± 0.02 μM, than the control group, which had a mean of 0.07 ± 0.03 μM ($p = 0.002$). Additionally, there was a significant increase in the PCOS group's average CAT activity, measuring 362.47 ± 68.25 k/gHb, compared to 179.90 ± 31.70 k/gHb in the control group ($p < 0.00001$). However, there were no appreciable variations in the levels of other oxidative stress markers, such as GPx and SOD, between the groups.

Table no.3: Oxidative Stress Parameters

Parameter	**Control Group**	**Study Group**	**p-value**
Malondialdehyde (MDA) [μM]	0.07 ± 0.03	0.09 ± 0.02	0.002
Catalase (CAT) [k/gHb]	179.90 ± 31.70	362.47 ± 68.25	<0.00001
Superoxide Dismutase (SOD) [A/gHb]	1680.5 ± 130.4	1710.3 ± 250.7	0.540
Glutathione Peroxidase (GPx) [U/gHb]	5.10 ± 0.80	5.00 ± 0.70	0.650

In the PCOS cohort, 48.42% exhibited elevated androgen levels (A levels >3.5) or hirsutism (score >8), while 43.16% were classified as overweight or obese. Among the subjects, six participants (21.05%) had IR, with three of them also categorized as overweight or obese. The comparison of oxidative stress parameters based on IR, obesity, and hyperandrogenism is detailed in Tables 4 and 5.

Table no.4: Oxidative Stress Parameters Based on Insulin Resistance, Obesity, and Hyperandrogenism

Condition	**Malondialdehyde (MDA) [μM]**	**Catalase (CAT) [k/gHb]**	**p-value**
Without IR (n = 19)	0.09 ± 0.01	360.15 ± 72.25	0.410

With IR (n = 6)	0.12 ± 0.02	405.30 ± 75.45	0.018

Table no. 5: Oxidative Stress Parameters in Control Group Based on Obesity, Insulin Resistance, and Hyperandrogenism

Condition	Glutathione Peroxidase (GPx) [U/gHb]	p-value
Without Obesity (n = 38)	4.85 ± 0.65	0.220
With Obesity (n = 37)	5.40 ± 0.80	0.220

DISCUSSION

Women who have higher testosterone levels are more likely to develop vascular diseases. Increased OS indicators have been observed in PCOS patients, indicating a potential role for OS and free radicals in the development of atherosclerosis. Research on OS parameters in PCOS is still pending, nevertheless. While some studies revealed higher levels of MDA, SOD, CAT, and GPx, others reported lower activity of these enzymes [26–34].

When compared to the healthy control group, the PCOS group in our study exhibited significantly higher levels of MDA, a key marker of OS and lipid peroxidation. Notably, MDA levels were elevated in PCOS patients with insulin resistance (IR) compared to those without, regardless of their weight. Some researchers argue that obesity primarily contributes to OS through IR, while others suggest that obesity has minimal influence on the IR-OS relationship [34–39]. Our findings align with Kuscu et al., who also reported higher MDA levels in PCOS patients regardless of weight [36], and with Zhang et al., who found higher serum MDA levels in PCOS patients compared to controls [34]. Olusi et al. observed significantly elevated MDA levels in individuals with a BMI over 40 kg/m², attributing the increase to reduced enzyme activity as a factor in OS among obese women [39]. However, due to the lack of severely obese participants in the study, notable OS differences was not found between overweight and obese PCOS patients. This suggests that IR, rather than obesity, may be the primary driver of OS in PCOS. Importantly, non-obese women with PCOS can also develop IR, indicating that all PCOS patients should undergo IR evaluation and potentially consider antioxidant treatments.

The antioxidant enzymes CAT, SOD, and GPx play crucial roles in neutralizing OS [40]. GPx, which reduces lipid hydroperoxides and hydrogen peroxide (H_2O_2), showed similar activity levels in both PCOS patients and healthy controls in our study. This

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finding is consistent with previous research by Sabuncu et al., who found no significant difference in GPx activity between these groups [33]. However, other studies have reported lower GPx levels in PCOS patients. We suggest that GPx activity may fluctuate throughout the course of PCOS, possibly depending on selenium availability, which could be insufficient in certain diets [32].

We also observed significantly higher MDA levels in PCOS patients with IR compared to those without, indicating distinct OS mechanisms within these groups. MDA has been linked to increased levels of TC, particularly LDL, implying that elevated LDL and/or IR may trigger MDA production.

CAT, which converts H_2O_2 into water and molecular oxygen without generating free radicals, protects cells from H_2O_2 toxicity. Increased CAT activity is often associated with inflammatory conditions due to cellular permeability [41, 42]. Our findings revealed significantly higher CAT activity in PCOS patients compared to controls, and this activity was correlated with total cholesterol levels, though not with specific lipid profile elements, suggesting that CAT sensitivity may be diminished in PCOS patients.

SOD, another critical antioxidant, converts superoxide radicals into H_2O_2, which is then reduced to water by GPx. Although our data indicated a trend of rising SOD levels with increasing IR and obesity, there was no significant difference between groups. While Seleem et al. found opposite results, previous research has shown elevated SOD levels in PCOS patients compared to controls. These discrepancies could be due to differences in disease severity or treatment methods across studies [43–47].

It is commonly known that metabolic syndrome and cardiovascular problems are related. According to Wang et al., PCOS patients with metabolic syndrome have lower antioxidant activity and worse OS [48]. In a similar vein, our research found that MDA and CAT in the PCOS group had inverse relationships with TC and LDL levels. Significant relationships were seen in controls between TC, LDL, triglyceride levels, and peroxidase activity. This highlights the possibility that OS in PCOS may make people more vulnerable to heart problems. Despite the fact that obesity is a known cardiovascular risk factor, we did not find any association between OS indicators and obesity parameters in the PCOS group, which lends credence to the idea that IR is the main issue with PCOS.

Therefore, therapeutic strategies for PCOS should address not only reproductive and menstrual issues but also focus on improving antioxidant mechanisms to manage metabolic complications, including hyperinsulinemia and OS. Approaches such as diet, exercise, and antioxidant pharmacotherapy could be beneficial. The varied results regarding OS parameters indicate potential additional factors influencing the body's antioxidant capacity, warranting further investigation beyond the MDA marker to explore enzymatic activities of GPx, CAT, and SOD.

CONCLUSION

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This study emphasises the strong correlation, especially when considered in the setting of IR, between oxidative stress markers and metabolic problems in women with PCOS. Patients with PCOS, particularly those with IR, had elevated levels of MDA, which may indicate a unique oxidative stress mechanism in this population. Notwithstanding the inconsistent results of antioxidant enzyme activities, such as GPx and CAT, the results highlight the necessity of a thorough evaluation of metabolic parameters and oxidative stress in PCOS patients, irrespective of body mass index. This emphasises how crucial it is to create focused treatment plans, such as dietary changes and antioxidant supplements, to lower the risk of cardiovascular issues and enhance the general metabolic health of PCOS-affected women. It is necessary to do more research to investigate the possible advantages of antioxidant therapy in this population as well as other factors influencing oxidative stress.

LIMITATION

Small cohort was the limitation of the study and to confirm the findings of the study more such studies on large cohorts are required.

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CONFLICT OF INTEREST

There are no conflicts of interests.

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