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The Role of oxidative stress in obese and non-obese polycystic ovary syndrome among women attending infertility clinics in Karbala

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is one of the most significant endocrine disorders that affects female fertility with serious physiological complications.
Objectives: This study examined the association between oxidant-antioxidant status, lipid profile, and the risk of developing metabolic syndrome in women with polycystic ovarian syndrome (PCOS).
Methods: A case-control study approach was used to gather data on 160 patients from November 2022 to April 2023; two groups were formed from them. The first group is 80 obese (BMI >30 kg/m2) women, 40 were determined to have PCOS (the case group) & 40 healthy women as a control group. The second group is also 80 women, just like the first group, but they were non-obese (BMI <30 kg/m2). The Karbala Health Directorate, University, and College of Medicine validated the study's ethical approval. Additionally, the administration of the teaching hospital for gynecology and obstetrics gave their approval.
By using spectrophotometry, the antioxidant activity of the catalase enzyme (CAT) and serum malondialdehyde (MDA) levels were established.
Results: In the group of obese women with PCOS, a statistically significant variation was seen in the average serum MDA concentration. which was observed to be higher when compared to the obese control group ($p<0.05$). When the antioxidant measure, CAT, was compared to those who are not obese, obese PCOS, and control groups, there was a statistically significant decrease in the difference between the groups ($p<0.05$). MDA levels were found to be positively correlated with those of the PCOS patient group's BMI, WHR, triglyceride, total cholesterol, HOMA-IR, and LH levels.
Conclusion: Hyperinsulinemia and dyslipidemia were found to relate to reduced antioxidant measurements and oxidative stress in PCOS patients. We believe that this oxidative stress situation may have a role in metabolic syndrome and cardiovascular illnesses in PCOS patients.

Keywords Oxidative stress, PCOS, insulin resistance, MDA, catalase

NOMENCLATURE

BMI:	Body mass index
CAT:	Catalase
FBS:	Fasting blood sugar
FSH:	Follicle-stimulating hormone
GSH-PX:	Glutathione peroxidase
HDL:	High-density lipoprotein
HOMA-IR:	Homeostasis model assessment-estimated insulin resistance
LDL	Low-density lipoprotein
LH:	Luteinizing hormone
MDA:	Malondialdehyde
NO:	Nitric oxide
PCOS:	Polycystic ovary syndrome
PUFA:	Polyunsaturated fatty acid
ROS:	Reactive oxygen species
SDS:	Sodium dodecyl sulfate
SOD:	Superoxide dismutase
TBA:	Thai barbituric acid
TC:	Total cholesterol
TCA:	Trichloroacetic acid
TG:	Triglyceride
WHR:	Waist hip ratio

INTRODUCTION

An endocrine disorder known as polycystic ovarian syndrome (PCOS) affects women who are fertile and is caused by hyperandrogenism and oligo-anovulation [1]. PCOS is currently recognized as a metabolic condition in which the patient experiences complex symptoms such as insulin resistance, impaired glucose tolerance, dyslipidemia, and an increase in several cardiovascular risk factors [2]. Numerous investigations discovered a strong relationship between the emergence of PCOS and insulin resistance with compensatory hyperinsulinemia [3]. Additionally, PCOS patients have larger body mass [4].

Glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) are two of the most important antioxidant defense enzymes found in cells. By reactivating oxygen species (ROS) that are created during the metabolism of cells, they can regulate ROS levels in physiological circumstances [5]. Sources of PUFAs, or polyunsaturated fats, rapidly assault the cell membranes when oxidizing radicals are present. A consequence of this process is malondialdehyde (MDA), a crucial last product of the lipid peroxidation process. Given that it is correlated with how much lipid peroxidation there is, it is among the often-used indicators for evaluating oxidant [6][7][8].

In response to MDA levels, insulin resistance, dyslipidemia, hyperandrogenism, and obesity associated with PCOS are likely to increase, but antioxidant enzyme levels are also likely to decrease [9]. Overproduction of reactive oxygen species (ROS) and an imbalance between oxidants and antioxidants characterize oxidative stress. Many investigations have demonstrated that oxidative stress contributes significantly to PCOS development and that individuals with PCOS have elevated levels of oxidative circulation markers [10]. Relative insulin resistance is thought to be the source of chronic hyperinsulinemia, which results in deviations in the metabolism of ovarian androgen, reduced follicle development, and modified gonadotrophin response [11].

Oxidative stress and the production of species of reactive oxygen species have both been connected to insulin resistance. Oxidative stress prevents muscle and fat tissue from absorbing glucose and reduces the amount of insulin produced by the pancreatic beta cells [12]. Additionally, PCOS patients' hyperinsulinemia prevents the vascular endothelium from secreting nitric oxide (NO). Endothelial dysfunction comes from the drop in membrane fluid and consequent rise in calcium levels inside cells. Therefore, endothelial dysfunction is an early indicator of atherosclerosis [13]. In these patients, many PCOS-related factors and many conditions, such as obesity, insulin resistance, androgen excess, can raise oxidative stress. As a result of PCOS, ROS production increases [14].
In both obese and nonobese subjects, the aim of this study is to clarify the relationship between antioxidants and oxidative stress and polycystic ovarian syndrome (PCOS).

Materials and Methods

The study population had to be between the ages of 18 and 40, and PCOS also applying the standards of Rotterdam [15]. Based on these diagnoses' standards, polycystic ovaries were present if there were at least 12 follicles with a diameter between 2 and 9 mm, secondary amenorrhea (menstrual periods at least six months apart), oligomenorrhea (when menstrual cycles occur more than 35 days apart), and/or an ovarian volume larger than 10 cm3 per ovary. Furthermore, the Ferriman-Gallwey grading system was employed to assess the existence of hirsutism [16].
Body Mass Index (BMI) was computed in kg/m2. 40 patients with a BMI of less than 30 kg/m2 were included in the group of obese people. To measure the non-stretchable measuring tape's waist circumference (WC), expressed in millimeters (cm) was inserted approximately midway between the top of the iliac crest and the lower border of the last palpable rib. Using the greater trochanter of the femur as a reference point, the hip circumference (HC) was measured in centimeters. The measuring tape that is not stretchable was also wrapped around the widest area of the buttocks. The hip circumference (HC) was measured in cm utilizing the femur's greater trochanter as a point of reference. Additionally, the broadest part of the buttocks was wrapped with non-stretchable measuring tape [17].
Using the formula (fasting glucose (mg/dl) x fasting insulin (μU/ml)/405), the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. A cut-off of 2 was seen as typical [18].
Exclusion criteria for the study: The study excluded those with endometriosis, diabetes mellitus, chronic hypertension, thyroid dysfunction in cardiovascular patients, and those taking medications that decrease cholesterol or make people more sensitive to insulin. The socioeconomic status of the sick and control groups could not have been distinguished. The trial was carried out following the guidelines of the Helsinki Declaration as it was received in 2000, and all patients who volunteered were made aware of it. The local ethics committee gave its approval to the study.
Blood Collection: Gel tubes were used to collect blood samples. Centrifugation was used to separate the serums, which were then kept at 85°C until use.
In the early follicular phase of the menstrual cycle [days 2-4], venous blood samples were collected using the chemiluminescent automated immunoassay system (ECL) (Cobas e 411, Roche Diagnostic, Germany) to assess LH, FSH, free testosterone, FBS, and insulin. The lipid profile (total cholesterol, triglycerides, HDL, and LDL) was assessed using an automatic analyzer (SMART-120) and enzymatic end-point colorimetric techniques.
Using a spectrophotometer, the plasma antioxidant (catalase) was measured in accordance with previously described procedures (Table 1) [19].

Table 1: Method used to determine the CAT level

Reagents	Test µl	Standard µl	Blank µl
Sample	100	------	-----
Phosphate buffer	900	1000	3000
Hydrogen peroxide	2000	2000	----
After mixing with a vortex and incubating for two minutes at 37 °C, add:			
Vanadium reagent	2000	2000	2000
The tubes were then held at 25 °C for 10 minutes. At 452 nm, the variations in absorbance were measured in comparison to the reagent blank.			

To quantify the MDA content, 100 L of the sample was added to a test tube holding two milliliters of the subsequent working solution: A total volume of 200 ml was created by combining with the exception that the sample was replaced with distilled water.

Statistical Analysis

The collected data was aggregated and represented as mean + standard deviation (mean + SD). When a normal distribution between the groups was present, the Pearson correlation coefficient was calculated to assess the relationship between continuous variables. When a normal distribution was absent, the correlation coefficient of Spearman's rank was computed. In addition, the effectiveness of the patient group in differentiating itself from the control group was assessed using the Receiver Operating Characteristic Curve (ROC Curve) study. The computations were performed using SPSS Statistics software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA), with a statistical significance level of $p < 0.05$.

Results

The research on population's demographic information is shown in the Table. 2.

Table. 2: Demographic characteristics studied in control and women with polycystic ovary syndrome.

Variables	Non-obese		P-value	Obese		P-value
	case	control		case	control	
Anthropometric measurements						
Age (year)	23.60±4.65	28.03±4.70	<0.001*	24.83±4.53	27.6±4.82	0.009*
BMI (kg/m2)	24.77±2.34	23.71±1.69	0.023*	33.40±3.71	33.77±3.52	0.648
WHR	0.82±0.05	0.79±0.04	0.019*	0.86±0.03	0.85±0.04	0.413

Data expressed as mean±SD; Pcos polycystic ovary syndrome; BMI= body mass index; WHR= waist to hip ratio. Statistical significance was defined as $p<0.05$.

Table 2 displays mean age was 23.60±4.65 for non-obese PCOS and 28.03±4.70 for the control group, 24.83±4.53 for obese PCOS, and 27.6±4.82 for obese control group.

Statistically different results were observed between non-obese groups (p<0.001) and obese groups (p=0.009). There was a slight difference in the mean of body mass index (BMI) between the non-obese case and the non-obese control group (24.77±2.34 and 23.71±1.69, p=0.023). In the obese group, both cases and controls were matched. A statistically significant difference was observed between the non-obese case and non-obese control in WHR(p=0.019).

The outcomes of the hormonal and biochemical studies are displayed in the Table. 3

Table 3: Comparison results of biochemical parameters studied in control and women with polycystic ovary syndrome

Data expressed as mean±SD; Pcos= polycystic ovary syndrome; TC= total cholesterol; TG= triglyceride; HDL= high-density lipoprotein; LDL= low-density lipoprotein; LH= luteinizing hormone; FSH= follicle stimulating hormone; FBS= fasting blood sugar; HOMA-IR= homeostasis model assessment-estimated insulin resistance. Statistical significance was defined as *p*<0.05.

Table 3 displays High high-density lipoprotein, LDL, LH levels, FSH, LH/FSH ratio, free

Variables	**Non-obese**		**p-value**	**Obese**		**p-value**
	Case	**Control**		**Case**	**Control**	
Lipid profile						
Serum TC (mg/dl)	151.71±32.20	148.79±17.24	0.614	166.45±33.17	153.56±24.90	0.050*
Serum TG (mg/dl)	75.95±22.26	71.41±15.16	0.290	116.33±56.79	77.37±15.29	<0.001*
Serum HDL-C (mg/dl)	56.95±9.57	69.02±5.67	<0.001*	54.71±8.12	57.03±13.48	0.354
Serum LDL-C (mg/dl)	83.53±23.72	68.90±16.85	0.002*	96.34±34.62	73.23±7.68	0.002*
Hormonal components						
LH (mIU/ml)	8.47±3.97	4.37±1.24	<0.001*	11.58±4.23	5.05±1.02	<0.001*
FSH (mIU/ml)	5.60±1.55	8.63±1.68	<0.001*	5.54±1.33	9.10±1.67	<0.001*
LH/FSH ratio	1.62±0.90	0.50±0.11	0.002*	2.49±0.73	0.56±0.14	<0.001*
Free Testosterone (pg/ml)	2.73±1.21	0.96±0.46	<0.001*	2.77±1.06	1.21±0.56	<0.001*
Insulin sensitivity						
Insulin (µU/ml)	13.21±8.10	7.13±1.62	<0.001*	15.71±4.56	7.29±1.31	<0.001*
FBS (mg/ml)	93.61±10.48	84.68±4.43	<0.001*	93.06±9.51	87.20±4.65	<0.001*
HOMA-IR	2.06±0.76	1.28±0.27	0.002*	3.81±0.94	1.54±0.26	<0.001*

testosterone, insulin, FBS, and HOMA-IR were significantly different in the non-obese PCOS group compared to the non- obese control group (p<0.05), as shown in Table 3. However, total cholesterol and TG levels did not differ between non-obese groups. Total cholesterol, TG, LDL, LH, FSH, LH/FSH ratio, free testosterone, insulin, FBS, and HOMA-IR were

notably different in the obese PCOS subjects than in the obese control ($p<0.05$) as shown in Table 3.
Fig.1: illustrates the mean serum MDA level, which was (4.10±1.42) mmol/l in the non-obese PCOS group, (5.65±1.59) mmol/l in the obese PCOS group, (2.86±1.15) mmol/l in the non-obese control group, and (3.71±1.10) mmol/l in the obese control group. Comparing the obese PCOS group to the obese control group, there was a statistically significant difference in the mean serum MDA level ($p < 0.001$), Fig. 1When the MDA value of the non-obese PCOS group and the non-obese control group were compared, the groups' differences were statistically significant ($p < 0.001$) Fig.1.

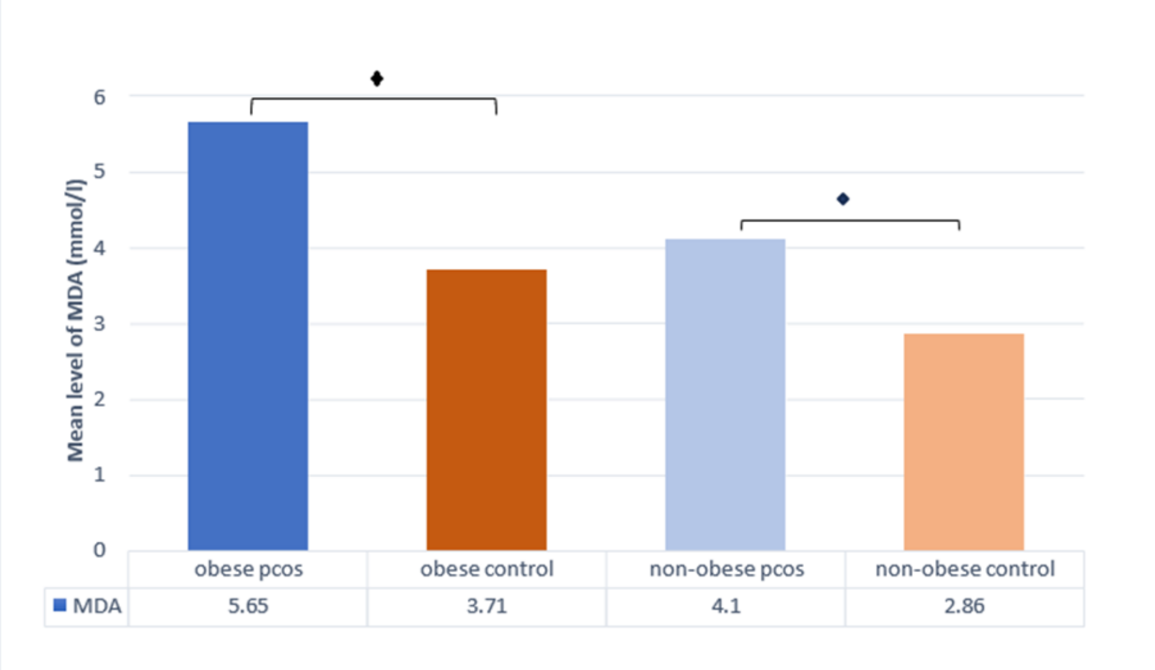

Figure. (1)**:** A comparison results of malondialdehyde studied in control and women with polycystic ovarian syndrome.
Statistical significance was defined as * significant when $p \leq 0.05$

When the antioxidant marker catalase in the non-obese PCOS group was compared to the non-obese control group, there was statistically significant difference between the groups ($p<0.001$) Fig.2. When the antioxidant parameter was looked at, it was discovered that the obese PCOS group's level was much lower than the obese control group's ($p < 0.001$), Fig. 2.

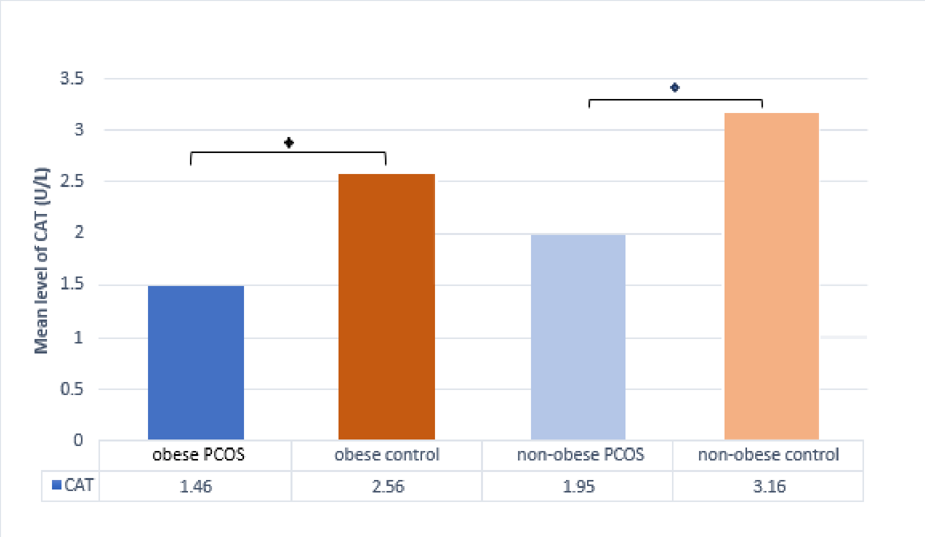

Figure. (2): A comparison results of catalase studied in control and women with polycystic ovarian syndrome.

Statistical significance was defined as * significant when $p \leq 0.05$

The clinical, biochemical, and hormonal evaluations of PCOS-afflicted women are correlated with blood MDA and catalase levels, as shown in Table 4.

Table 4: Association of catalase and MDA with the studied parameters in PCOS women

Parameter	Catalase(U/L)	MDA (mmol/l)
Age (year)	-0.124	0.093
BMI (kg/cm^2)	-0.47 7*	0.461*
WHR	-0.316*	0.356*
HOMA-IR	-0.213*	0.748*
TC (mg/dl)	-0.615	0.251*
TG (mg/dl)	-0.381*	0.349*
HDL-C(mg/dl)	0.093	-0.116
LDL-C(mg/dl)	-0.150	0.210
LH (mIU/ml)	-0.112	0.262*
FSH (mIU/ml)	0.116	0.081
Free testosterone(pg/ml)	0.175	-0.088

MDA=malondialdehyde; BMI= body mass index; WHR= waist to hip ratio; HOMA-IR= homeostasis model assessment-estimated insulin resistance; TC=total cholesterol; TG= triglyceride; HDL= high-density lipoprotein; LDL= low-density lipoprotein; LH= luteinizing hormone; FSH= follicle-stimulating hormone. *Statistical significance was defined as $p \leq 0.05$

MDA was found to positively correlate with BMI, WHR, MOMA-IR, total cholesterol, triglyceride, and LH. The positive correlation of MDA with parameters linked to a metabolic condition that involves insulin resistance and dyslipidemia supports the hypothesis that increased oxidative stress in cardiovascular disorders, such as metabolic syndrome and cardiovascular disease, is linked to oxidative stress in PCOS patients (MS). A negative correlation exists between catalase and triglycerides, HOMA-IR, WHR, and BMI.
The ROC curve for MDA is shown on Fig. 3. In the ROC evaluation, the cut-off point for MDA was 4.305, with a confidence interval of 0.691–0.836, a sensitivity of 60%, and a specificity of 81.2% ($p < 0.001$). The area under the curve for MDA was 76.3%.

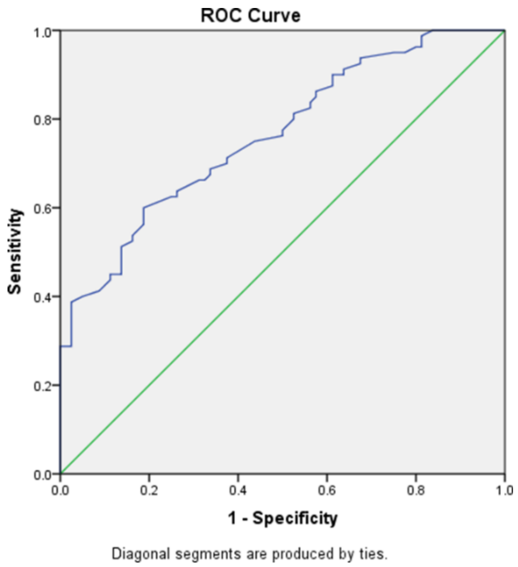

Figure. (3): Receiver operating characteristic curve (ROC curve) for MDA

Discussion

Metabolic diseases like insulin resistance, obesity, and diabetes have been linked to PCOS. Obesity affects fertility in women negatively and is linked to anovulation, miscarriage, and several other pregnancy issues. In PCOS patients, Insulin resistance is linked to abdominal fat persistent anovulation, hyperandrogenism, and inflammation [20].
Obesity and PCOS are strongly associated with one another [21], although it can also happen to non-obese women. In the current study, young women without PCOS were compared to obese and non-obese PCOS cases in terms of their early clinical and biochemical characteristics. In our investigation, a big disparity between the cases and the controls was seen. This was like a study's findings, which showed age disparities between patients and controls to be significantly different in both cases and controls [22].
The anthropometric measurements of the obese and non-obesity groups in the current investigation showed a substantial difference, Table 2. Furthermore, cases considerably outperformed controls in terms of mean BMI and WHR. This was comparable to a study that found that controls and women with PCOS had considerably lower central fat mass and BMI, respectively [23]. According to a different study, abdominal obesity occurs in lean PCOS individuals despite having a low BMI [24].
Increases in TG, TC, and LDL-C and a reduction in HDL-C are characteristics of obese patients' aberrant blood lipid profiles [25]. Our findings indicated that obese PCOS women had greater anomalies in lipid metabolism than the obese control group, including higher

levels of TG, Cholesterol, and LDL-C and no differences in HDL-C levels Table 4 like the result reported by [26]. Hyperinsulinemia and hyperandrogenism may also be linked to elevated blood LDL-C levels in PCOS patients. When IR exists, lipoprotein lipase activity declines, which diminishes the liver's ability to remove LDL-C.

Furthermore, it has been demonstrated that testosterone inhibits the ability of estrogen to cause the liver to produce LDL-C receptors, lowering hepatic LDL-C receptors and raising plasma LDL-C levels [27]. Additionally, testosterone can stimulate the liver to create TG by changing the catecholamine signal in adipocytes and increasing the release of circulating non-esterified fatty acids. Therefore, in PCOS patients, hyperandrogenism may also result in a rise in TG [28]. However, it seems that different research has reported contradicting results because of characteristics like race, genetics, lifestyle, and geographic region. In the current study, BMI showed an effect on lipids that is statistically significant.

In the current study, we discovered that obese patients with PCOS had significantly lower FSH levels and higher LH levels. Additionally, there is a shaky inverse correlation between BMI and FSH levels and a weak direct correlation between BMI and LH levels. These findings are supported by considerably increased LH/FSH ratios in PCOS individuals, Table 3. Literature offers contrasting interpretations of the LH, FSH, and LH/FSH ratio increases associated with PCOS and obesity. There were no appreciable variations in the LH/FSH ratio between the groups of women with and without PCOS, according to Banaszewska et al. [29]. However, Cho et al. discovered that the median LH/FSH ratio did not substantially differ between the PCOS and non-affected group, indicating that the LH/FSH ratio is not very useful in the diagnosis of PCOS [30].

In PCOS, gonadotropin production is abnormal, which causes greater serum LH levels and a higher LH/FSH ratio [31]. In PCOS patients, an increase in gonadotropin-releasing hormone causes the FSH β-subunit to upregulate the transcription of the LH β-subunit, increasing the LH/FSH ratio [32].

When obese PCOS women were compared to an obese control group, our results revealed a substantial difference in free testosterone levels Table 3; this finding was consistent with Pasquali et al. [33]. The concentration of free androgens rises because of obesity's inhibition of SHBG secretion in the liver [34].

IR is the source of lipid and glucose metabolic disorders in PCOS patients. Additionally, obese PCOS patients experienced more severe IR in contrast to obese patients without PCOS, as evidenced by a large rise in FINS, an INS secretion peak that is delayed, and an additional compensatory rise. The HOMA- IR of non-obese PCOS patients was also observed to be considerably greater than that of the non-obese control group Table 3. Therefore, obesity may be the primary contributor to IR in PCOS individuals. However, there may also be mechanisms independent of fat that cause IR, such as the serine on insulin receptors being hyperphosphorylated [35]. So, even though PCOS is a component of IR, it is also claimed that increases in BMI are considerably more noticeable.

According to studies, Ninety-five percent of obese patients and 75 percent of non-obese patients have insulin resistance (IR) in PCOS [36].

In the current study, we discovered that the concentration of malondialdehyde significantly increased at the probability level ($p \leq 0.01$) in the group of obese women with PCOS compared to the obese control group. Based on the findings in Fig. 1, we believe that the source of the elevated oxidative stress was obesity. The findings of this study are in agreement with those of Zimmer and his team's study [37], which showed a rise in malondialdehyde levels due to an uptick in the lipid peroxidation process of unsaturated fatty acids in various cell types, including egg cells, as a result of an uptick in various active forms of oxygen and nitrogen, and this rise gives a hint. Increased ROS production because of excessive oxidative damage in the participants may therefore be the cause of higher MDA concentrations. As a result, these oxygen species can oxidize a variety of other important biomolecules, such as membrane lipids.

Rasool and his team have determined that women with polycystic ovarian syndrome exhibit symptoms of oxidative stress since the MDA value is one of the markers of the occurrence of oxidation within the body caused by the lipid peroxidation of fatty acids [38] agreed with the results as well, indicating that MDA concentrations were higher in PCOS-afflicted women than in control women, and Ukan [39] further demonstrated that MDA concentrations were higher in obese women than in non-obese women.

We used receiver operating characteristic analysis to assess our findings to gauge the diagnostic potency of the expression. We noticed that the AUC of MDA levels was 76.3% Fig.3, and this could aid in distinguishing patients with PCOS from those without PCOS. A study by Rashad et al. supported our findings [40].

An internal antioxidant enzyme called catalase is mostly found in cell peroxisomes and to a lesser extent in the cytoplasm. It catalyzes the conversion of hydrogen peroxide to water and molecular oxygen. According to this study, the activity of catalase in serum samples from PCOS patients was significantly lower than that of controls. Al-Azzawie et al. and Kandasamy et al. both observed that PCOS patients have significantly lower catalase activity compared to the control group. Al-Azzawie et al. and Kandasamy et al. both observed that, in PCOS patients, compared to the control group, there was a substantial decline in catalase activity Fig. 2 [41]. As a result, the decrease in catalase activity may be caused by the formation of ROS since PCOS patients' oxidative stress reduced catalase function. Additionally, we believe that hyperinsulinemia and dyslipidemia factors actively contribute to the reduction in antioxidant levels associated with obesity and an increase in oxidative stress.

In the correlation, there were positive associations between MDA and BMI, WHR, HOMA-IR, total cholesterol, triglycerides, and LH Table 4. Our results are consistent with those of earlier research [42]. Another study that found a negative association between MDA and BMI, WHR, cholesterol, and LH conflicts with our findings [43]. Reviewing the correlation analysis carried out in the patient group revealed that increased oxidative stress exacerbated dyslipidemia and hyperinsulinemia, two indications of PCOS-associated metabolic syndrome. Additionally, a study recommended tracking variables including MDA, SOD, TG, and LDL throughout PCOS patients' recovery periods [42].

Additionally, a negative association between the waist-hip ratio (cm), BMI, HOMA-IR, and triglyceride levels were discovered. These findings were in line with earlier research [44]. Therefore, it can be said that patients who have low catalase levels undergo oxidative stress.

In conclusion, it was found that both PCOS groups reported poor antioxidant levels, particularly the obese PCOS group, and considerably increased MDA values.

Since the findings of hyperinsulinemia and dyslipidemia were linked to oxidative stress and lowered antioxidant markers, we believe that PCOS patients' oxidative stress conditions may play a role in metabolic syndrome and cardiovascular diseases. Elevated oxidative stress, low

antioxidant levels, and insulin resistance, as well as the connections between these variables, lend credence to the theory that oxidative stress contributes to the etiology of PCOS.
Furthermore, increased insulin resistance has given rise to higher HOMA-IR readings in obese PCOS individuals. The HOMA-IR level in PCOS patients who are not obese, in contrast to the control group, has not changed. It most likely results from elevated levels of LDL, triglycerides, and total cholesterol in PCOS groups with obesity.
As a result, in addition to existing risk factors including dyslipidemia, central obesity, and insulin resistance, oxidative stress may further raise the chance of developing PCOS in women. Future studies on obese and non-obese PCOS patients, as well as phenotypes of PCOS, need to pay more attention to oxidative stress.

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