Determination of Oxidative Stress Markers in Women with Polycystic Ovarian Syndrome

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Abstract: Polycystic ovarian syndrome (PCOS) is a condition that increases the risk of metabolic diseases, such as insulin resistance, diabetes mellitus, and obesity, among others. Oxidative stress is a state of elevated oxidants and reduced antioxidants, which can be detrimental to the body. Reduced glutathione (GSH) is an important material with antioxidant properties. This study aimed to investigate the status of oxidative stress in PCOS women by measuring total oxidant status (TOS) and GSH in their serum, and explore the possibility of using oxidative stress biomarkers in the diagnosis of the disease. The study included 60 women with PCOS and 30 women without PCOS as controls. PCOS women had significantly higher values of body mass index, testosterone, and TOS, while GSH levels were significantly reduced. There was also a significant negative correlation between TOS and GSH levels in PCOS women. These findings indicate that PCOS women exhibit systemic oxidative stress, and suggest the use of anti-oxidative drugs to manage the health consequences of PCOS. The study demonstrates the excellent sensitivity of TOS and GSH as prognostic biomarkers for PCOS.

Keywords: PCOS, testosterone, TOS, GSH, oxidative stress.

1. Introduction

PCOS (polycystic ovarian syndrome) is a very common condition that affects 4% to 12% of women of reproductive age [1, 2]. Despite the high frequency of PCOS, diagnosing it and distinguishing it from other conditions can be difficult. This is due in part to the fact that there is no specific diagnostic test for the illness [3]. PCOS is a diverse disorder with a wide range of phenotypic expression, which has led to much debate about diagnostic criteria. Depending on the diagnostic criteria used, the prevalence of this illness ranges from 6% to 15% [4, 5]. The severity of the components of PCOS varies from person to person, and management must be adjusted to the patient's preferences [6].

The diagnostic criteria for PCOS can be grouped into four categories: 1) the presence of hyperandrogenism, chronic anovulation, and polycystic ovarian morphology; 2) the presence of polycystic ovarian morphology, chronic anovulation, and no clinical or biochemical evidence of hyperandrogenism; 3) the presence of hyperandrogenism, chronic anovulation, and normal ovaries; and 4) the presence of hyperandrogenism and polycystic ovaries.

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[7]. PCOS is linked to serious metabolic effects, which are mediated by obesity and insulin resistance, as well as other mechanisms. Increased risk of impaired glucose tolerance and type 2 diabetes, atherogenic dyslipidemia, systemic inflammation owing to enhanced adipose tissue secretion of pro-inflammatory mediators, nonalcoholic fatty liver disease, hypertension, as well as possible coagulation issues are among the consequences. It remains to be shown if this apparent elevated cardiovascular risk throughout reproductive ages translates to greater cardiovascular disease morbidity and also mortality later in life. Data from several retrospective as well as prospective cohort studies, based on markers of subclinical atherosclerosis, suggest a probable elevated cardiovascular disease risk, primarily for coronary heart disease [8].

Oxidative stress is a scientific expression referred to a state of elevated oxidants, reduced antioxidants, or both [9]. The major oxidants in humans are free radicals and reactive oxygen species (ROS), while ROS can be divided into radical and non-radical materials [10]. ROS is produced in normal levels and act as redox signaling materials within cells [11], but on elevated levels they can react oxidatively with the components of cells causing oxidative damage to the lipids and proteins [12]. The oxidative damage of lipids proceeds in a process known as lipid peroxidation. When the lipid of the cellular membrane undergo lipid peroxidation, the properties of the membrane is then distorted, most importantly the permeability, leading to apoptosis and pathological conditions [13].

The antioxidants are materials act to neutralize free radicals and ROS and maintain the beneficial levels required for healthy body [14]. Wide spectrum of materials have antioxidant properties in nature [15]. The antioxidant materials that found in humans can be organized as enzymatic and non-enzymatic [14]. Reduced glutathione (GSH) is one of the most important materials with antioxidant properties [16]. GSH acts to detoxify peroxides in the human cells by donating an electron and converted to the oxidized form (GSSG) [17]. Our study was aimed to investigate the status of oxidative stress in PCOS women by measuring the total oxidant status (TOS) and GSH in their serum, and exploring the possibility of using oxidative stress biomarkers in the diagnosis of the disease.

2. Materials and Methods

2.1. Patients

Kamal Al-Samarai's clinic in Baghdad, Iraq, recorded details of female patients with PCOS. The women were briefed on the study's typical requirements and expressed their willingness to participate as volunteers. From April 2021 to January 2022, 60 women with PCOS were chosen for the research, while 30 healthy women volunteered to serve as a control group.

2.2. Methods

The PCOS women patients as well as healthy control women were gave a vein blood. The blood then centrifuged in a medical centrifuge (4000 rpm for 10 minutes), and the serum is stored in a deep freezer at -20 °C to be examined for TOS, then GSH in spectrophotometric methods (Apel PD-303, Japan). The level of testosterone was determined by using cobas e411 analyzer (Roche, Germany). TOS levels were determined by using Erel's method that have been reported in Abod *et al.* study [18], while the GSH was determined according to the method of Ellman [19].

2.3. Statistics

The statistical analysis of the data was performed using the IBM program SPSS version 26.0 on a computer. An independent sample t-test was used to compare the means, while the Pearson correlation was used to calculate the connection between TOS and GSH. Finally, the

diagnostic potential of TOS and GSH for PCOS was evaluated using the receiver operating characteristic (ROC) curve by measuring the area under the curve (AUC) for each variable.

3. Results

Table 1 contains the details of the women who volunteered for the study. The ages of the PCOS patients (30.30 ± 5.48 years) and the control group (29.00 ± 5.19 years) were not significantly different (P>0.05). However, the body mass index (BMI) of the PCOS patients (27.66 ± 1.54 kg/m2) was significantly higher (P<0.05) than that of the control group (23.00 ± 1.32 kg/m2).

Table 1: Volunteered women characteristics.

Parameter	**PCOS**	**Control**	***P*-value**
N	60	30	-
Age (year)	29.00±5.19	30.30±5.48	0.276
BMI ($kg.m^{-2}$)	23.00±1.32	27.66±1.54	<0.001
Testosterone (ng/mL)	0.56±0.28	2.71±0.78	<0.001
TOS (μmol H_2O_2 Eq/L)	1.29±0.20	2.63±0.59	<0.001
GSH (μmol/L)	1987.47±288.57	1193.61±204.22	<0.001

The level of testosterone was significantly (P<0.05) elevated in the serum of PCOS women patients (2.71±0.78 ng/mL) compared to the serum of control women (0.56±0.28 ng/mL). Furthermore, TOS level was increased significantly (P<0.05) in the serum of PCOS women (2.63±0.59 μmol H_2O_2 Eq/L) compare to control women (1.29±0.20 μmol H_2O_2 Eq/L). On the other hand, GSH level was decreased significantly (P<0.05) in the serum of PCOS women (1193.61±204.22 μmol/L) compared to control women (1987.47±288.57 μmol/L).
The results have shown significant negative association between TOS and GSH in the blood of PCOS women patients, as shown in Table 2.

Table 2: Correlation of TOS and GSH in PCOS women patients.

Parameter	**TOS**		**GSH**	
	r	*p*-value	r	*p*-value
GSH	-0.289	0.025	-	-
Age	0.112	0.394	-0.142	0.280
BMI	0.077	0.560	0.063	0.633
Testosterone	0.074	0.572	-0.061	0.641

The ROC curve analysis demonstrated that TOS is a valuable biomarker for the detection of PCOS. The AUC of TOS was 0.974 (P<0.0001), indicating that it has excellent sensitivity in identifying PCOS patients compared to healthy control women, as depicted in Figure 1.

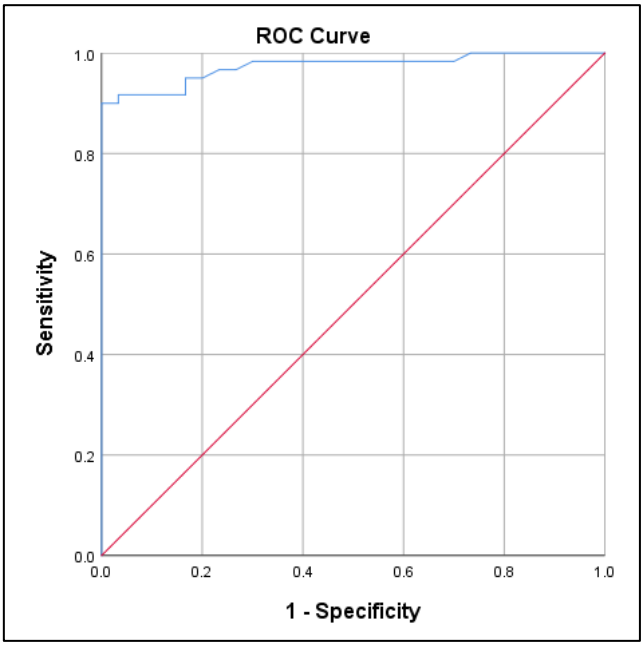

Fig. 1: The ROC curve of TOS in the diagnosis of PCOS.

Figure 2 shows that the ROC curve analysis of GSH confirms its usefulness as a diagnostic biomarker for PCOS. The AUC of GSH was 0.992 (P<0.0001), indicating excellent sensitivity in identifying PCOS patients compared to healthy control women.

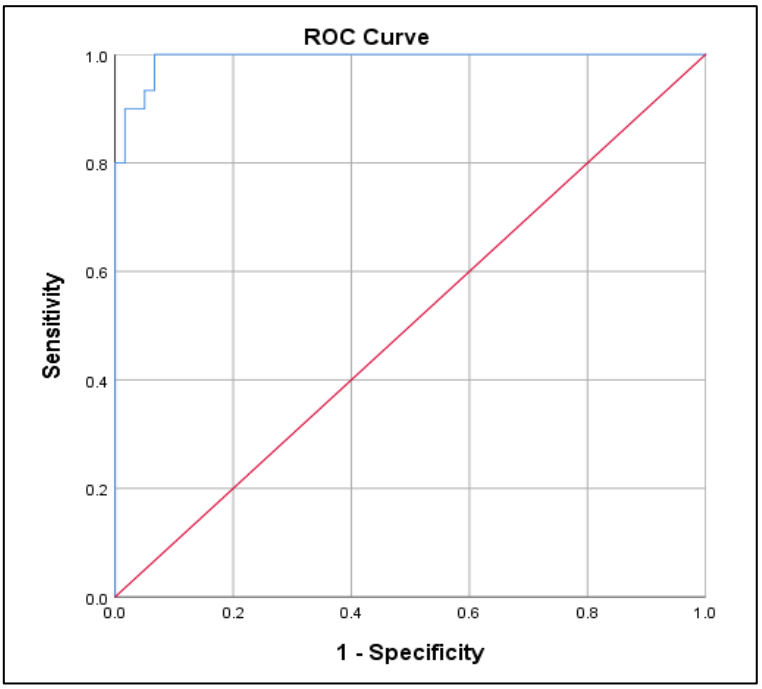

Fig. 2: The ROC curve of GSH in the diagnosis of PCOS.

4. Discussion

The present study has indicated the increase of BMI in women with PCOS. A previous study have indicated that women with PCOS are either overweight or obese [20], in this study the majority of PCOS women were overweight. Women with PCOS experience not only peripheral tissue obesity but also a significant increase in intra-abdominal fat, which is not linked to overall obesity [21]. Besides the body weight, PCOS women were had significant elevated levels of testosterone in this study. Winters *et al.* have reported that serum testosterone level was increased significantly in PCOS women compared to those without PCOS, and they have found that testosterone level has correlated negatively with age [22]. Naeemah *et al.* have reported that not only testosterone level was elevated in PCOS women, but also luteinizing hormone was elevated as well [23].

PCOS women have shown elevated status of oxidative stress in the circulation. The systemic oxidative stress was found previous in PCOS women in the study of Mohammadi, as the author has reported that lipid peroxidation, nitric oxide and xanthine oxidase are elevated in PCOS women, while glutathione and total antioxidant capacity are reduced [24]. In this study, we have found similar results, in which TOS level was increased but GSH level was reduced. Furthermore, Naeemah *et al.* have reported a significant increase in the level of TOS in PCOS women, and attributed this systemic oxidative stress to the onset of insulin resistance [23]. Kanafchian *et al.* have reported a significant reduction in the total antioxidant capacity of PCOS women, which suggested a systemic oxidative stress [25]. According to Sulaiman et al., oxidative stress may have a role in the etiology of PCOS, suggesting that oxidative stress indicators could be used as diagnostic markers for high-risk individuals [26]. Additionally, we have found that TOS and GSH can be used as very excellent sensitive biomarkers in the diagnosis of PCOS as shown in the ROC curve test.

5. Conclusions

The women with PCOS in the present study have shown that PCOS is linked directly to increasing the body weight, as all of the participants were overweight. Additionally, they were have elevated levels of testosterone in the circulation, indicating a disturbance of the gonadotropic system. Furthermore, PCOS women exhibited a significant higher serum levels of TOS, indicating the presence of oxidative stress systemically. Oxidative stress was also indicated from the significant reduction of GSH. Both TOS and GSH have proven their excellent sensitivity in the prognosis of PCOS. Based on these findings, we suggest the use of anti-oxidative drugs to control the oxidative stress and health consequences of PCOS in women.

6. Highlights

The study analyzed oxidative stress in women with PCOS by measuring TOS and GSH in their serum. PCOS women showed systemic oxidative stress, with elevated TOS and reduced GSH levels. Anti-oxidative drugs are recommended to manage the health risks associated with PCOS.

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