HORMONAL CONCENTRATION AND ANTIOXIDANT ENZYMES ACTIVITIES: A STUDY AMONG INFERTILE WOMEN WITH AND WITHOUT POLYCYSTIC OVARIAN SYNDROME IN BASRAH, IRAQ

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Abstract: Antioxidants are substances within the cells that neutralize free radicals by providing them with an electron. Antioxidant enzymes including catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPX) are important for preventing damage to oocytes and embryos caused by reactive oxygen species. Hormones such as thyroid hormones, prolactin, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) all have a role in regulating the menstrual cycle. Several hormones become out of whack during a case of infertility. Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder that affects body systems and leads to reproductive and metabolic complications among women of reproductive age, is considered as one of the main cause of infertility. After 12 months of frequent coitus. The inability to conceive is known as infertility. Our study aimed to estimate hormonal concentration and antioxidant enzymes activities among women (with and without) PCOS in Basrah -Iraq during (2021-2022). A case control study that included 60 patients' infertile women (30 with PCOS and 30 without PCOS), a control group of 30 entirely healthy participants aged (16 to 40) years. The results of the study showed a significant increase in each of (TSH and prolactin) levels in PCOS comparing to the control (8.228 ± 2.261 vs 2.914 ± 1.649; P value= 0.001) and (32.999 ± 9.789 vs 9.189 ± 5355; P value=0.001) respectively. While a significant decrease in each of (SOD and CAT) in patients without PCOS comparing control (1.877 ± 0.464 vs 2.300 ± 0.852; P value= 0.021) and (0.109 ± 0.186 vs 0.236± 0.241; P value =0.021) respectively.

Keywords: Antioxidant enzymes, polycystic ovarian syndrome, infertile women

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a frequent disorder of the reproductive-aged women (You, Be, and In 2020). Hormonal balance plays an important role a woman's life and in many diseases caused by hormone disorders, such early menopause, primary ovarian insufficiency, ovarian cancer, and polycystic ovary syndrome (PCOS) (Rocha et al. 2019). The pathophysiology of PCOS is largely unknown; evidence suggests that several genes are involved, as well as environmental and nutritional factors(Zuo, Zhu, and Xu 2016). Hence, the causes of PCOS are still unknown and may belong to gene, insulin resistance, environmental factors, and/or inflammation, which are affected by androgen concentrations (Setji and Brown 2014).

In terms of etiology and clinical symptoms, PCOS is quite similar to metabolic syndrome (MetS), which includes abdominal obesity, insulin resistance, and dyslipidemia. MetS prevalence in PCOS varies by population and is about twice as high as maturity level women in the overall population , according to studies (YaqiongHe et al. 2019). It's critical to do research that identifies the root cause of PCOS, as well as its hereditary link and hormonal imbalance (Deswal, Nanda, and Dang 2019). The elements that have an impact on both men and women are: Hypogonadotrophic hypogonadism, hyperprolactinemia, cystic fibrosis, ciliary function disorders, systemic diseases, infections, and lifestyle-related Worries. All these factors contribute to infertility(Sulaiman et al. 2018).

One of most prevalent fertility symptoms in women with hyperthyroidism is menstrual abnormalities, which include hypomenorrhea, oligomenorrhea, and amenorrhea (Anon 2016).

Subclinical and clinical hyperthyroidism was detected in 2.1 percent of infertile women in previous research, although 5.8% of hyperthyroidism patients have primary or secondary infertility in retrospective investigations (Vedal et al. 2018). Hypothyroidism can cause sex steroids to fail by affecting the hypothalamo-pituitary-ovarian axis' activity. Thus, a clinical picture in close relation to menstruation irregularity, infertility, miscarriage, and undesirable pregnancy difficulties may

emerge, following thyroiditis and drug-induced hypothyroidism(Karaca and Akpak 2015). Hyperprolactinemia (hyperPRL) is described as PRL levels in the bloodstream that exceed the usual reference limit (Labad et al. 2020). Hyperprolactinemia can be produced by a number of different reasons, including physiological, pathological, or medication-induced hyperprolactinemia.Secondary hypogonadism can be caused by elevated serum prolactin levels, which inhibit hypothalamic gonadotropin-releasing hormone and pituitary gonadotropins (Martin 2021).

The term "oxidative stress" refers to a state of cellular damage caused by an imbalance between free radicals and antioxidants (Saker, Jewad, and Hussein 2020). Oxidative stress is caused by an increase in free radicals or a reduction in the antioxidant system (Hyderali and Mala 2015). It has an impact on the quality of gametes and how they interact. The mismatch between the pro-oxidant compounds generated by aerobic metabolism and also the protective antioxidants causes oxidative stress (Bhardwaj et al. 2020).

It has been shown that PCOS patients are exhibited to oxidative stress in which increased production of free radicals and inflammatory mediators play important role. Namely, NADPH oxidase activity of mononuclear cells stimulated by hyperglycemia was shown to be major enzymatic source of free radicals, leading to increased lipid peroxidation in PCOS (Adeoye et al. 2018) . The whole reproductive lifetime of men and women is affected by oxidative stress, which is caused by an imbalance between reactive oxygen species (ROS) and protective antioxidants. ROS can influence cellular processes, and oxidative stress can disrupt the intracellular environment, leading to sick cells or putting cell viability in jeopardy (Halliwell and Gutteridge 2015). A number of cellular defence systems, including catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD), and non-enzymatic substances including vitamin C, vitamin E, and glutathione, control the body's normal oxidant/antioxidant imbalance (Bahar, Jewad, and Al-Mansouri 2021). The oxidative metabolism of aerobic organisms produces ROS, which are one of these molecules [Superoxide anion (O2), hydrogen peroxide (H2O2), organic hydroperoxide (ROOH), hydroxyl radical (OH), alkoxy and peroxy radicals (RO and ROO), hypochlorous acid (HOCl), and peroxynitrite (ONOO)], these molecules considered the major ROS having physiological activity (Al Jothery et al. 2016). Antioxidants are cellular molecules that inhibit these interactions by giving an electron to free radicals while remaining stable(Abudawood, Tabassum, and Alanazi 2021).

To prevent overproduction of ROS under normal circumstances, scavenging molecules are known as antioxidants turn ROS to H2O. Endogenous and exogenous molecules are the two forms of antioxidants found in the human(Pisoschi and Pop 2015). Endogenous antioxidant is an enzymatic or non-enzymatic result of the body's metabolism. Antioxidants both non-enzymatic and enzymatic enzymes can be found (Valgimigli, Baschieri, and Amorati 2018). It is consist of the following: Catalase (CAT), Glutathione , Glutathione peroxidase (GPx), Bilirubin, Alpha-lipoic acid (LA), Superoxide dismutase (SOD) , Ferritin and Uric acid (Al-kado 2013).

Catalase, superoxide dismutase (SOD), and glutathione peroxidase are antioxidant enzymes (GPX) can help protect oocytes and embryos by inactivating Reactive oxygen species, increased production ROS or a lack of antioxidants can OS is produced. SOD converts O2 into H_2O_2 (Pisoschi and Pop 2015), which is degraded by CAT into water and oxygen. The production of hydroxyl radicals is avoided. By oxidizing reduced glutathione (GSH) to glutathione disulfide and then decreasing glutathione reductase to GSH, Peroxides and hydroxyl radicals are converted to harmless molecules by GSH-PX (Panti et al. 2018).

AIM OF THE STUDY

To estimate hormonal concentration and antioxidant enzymes activities among women (with and without) PCOS in Basrah - Iraq during (2021-2022)**.**

MATERIALS AND METHODS

The study design & sampling

Between November 2021 and march 2022, a case control study was performed at Infertility and IVF center in Basra Hospital for Women and Children in Al-Basrah Government-Southern Iraq. Sixty infertile women (30 with PCOS and 30 without PCOS) aged between (16 to 40) years, which were admitted to Infertility and IVF center in Basrah Hospital for Women and Children in Al-Basrah Governorate-Southern Iraq. The (infertile women) were classified according to whether a woman has had a previous pregnancy or not : primary infertility (NO PCOS) primary showing a minimal decline of 0.1% and secondary infertility(PCOS) with a slight increase of 0.4% (Aziz, Anwar, and Mahmood 2015). A control group (fertile) of thirty entirely healthy participants, with no chronic illnesses, no history of infertility, heart failure, thyroid or pituitary disease or inflammation or infection in the previous two weeks and an age range of (16-40) years.

The collected sample preparatory procedure was then to collect the serum from the blood samples, five milliliters of blood was taken from the study population in gel tubes, allowed to clot at the room temperature for fifteen minutes and then centrifuged for a period of 5 minutes to obtain their sera at 4000 RPM. The sera were then gathered into Eppendorf tubes and used for measuring the concentration of hormones (TSH and prolactin) by using ELIZA-Monobind (USA) Kits. and enzymatic antioxidant (GPX, CAT and SOD) by using ELIZA- Sun Long (China) kits.

The data was analyzed statistically for the difference between groups of fertile and infertile-partnered women. The comparison of parameters between PCOS and non-PCOS women in a correlation regression analysis. Statistical significance was defined as a p-value less than 0.05. The significance level was set at 0.01, which was deemed to be very high.

RESULTS AND DISCUSSION

Table 1 shows the comparison of the studied parameters between fertile and infertile women with PCOS. The results revealed that there is a significant increase in hormones levels of prolactin (P- value=0,001) and TSH (0,001). A significant decrease of antioxidant enzyme activity of CAT was also found (p- value=0.001). Whereas a non-significant deference was

found in GPX (P-value=0.396) and SOD (P-value=0.246) as well as in BMI (p-value= 0.14).

In terms of BMI, the study found no significant differences (24.883 ± 2.630 **vs** 26.620±3.139; P=0.14) between fertile and infertile women with PCOS respectively (Table 1). Our findings about BMI is in consistence with what was previously reported by Fatima in 2019, who showed that the mean of the obese women tended to be higher in the PCOS group compared to the fertile group (23.24 ± 3.72 **vs** 22.39 ± 2.41; P= 0.078), (Fatima et al. 2019).

Prolactin hormone levels (PRL) was found significantly more among women with PCOS who are unable to conceive than fertile women (32.999±9.789 **vs** 9.189±5.355 ; P = 0.001) (Table 1). This finding is consistent with what Nasser *et al. found in* 2021 who reported that the results of the analysis of prolactin hormone among infertile patients were (26.38 ±1.71), while those in healthy women were (15.46± 0.76), representing a highly significant difference (P 0.01).

Infertile women had elevated serum Prolactin levels. Prolactin's main function is to regulate and orchestrate the breast feeding process in females. Amenorrhea, unexpected lactation, low estrogen levels, and the inability to ovulate are all caused by elevated prolactin levels. The study provided conclusive evidence that hyper prolactinemia is the root cause of female infertility (Nasser, Al-Jumaili, and Alhusni 2021).

Table 1 also shows a significant increase of Thyroid stimulating hormones (TSH) levels in infertile women with PCOS compared to fertile women: (8.228±2.261 **vs** 2.914±1.649 ; P=0.001). This result is in agreement with those reported by AbAdelsalam *et al.,* 2015. His study revealed an increase of TSH level in PCOS patient compared to the fertile group (5.669±1.562 **vs** 2.698±0.5682; P=0.001). In addition, hypothyroidism may be the cause of PCOS in some patients indicating that severe prolonged hypothyroidism contributes to larger ovarian size and/or cyst development, and that recovery in serum hormone levels due to achievement of euthyroidism produces a reduction in ovarian size, resolution of ovarian cysts, and reversal of polycystic ovary syndrome-like symptoms (AbAdelsalam and Ibrahim 2015).

Our results agrees with the authors Malini & George, 2018. In his study, there were no discernible differences in PCOS patients' levels of thyroid hormones or prolactin. Both fertile individuals and those with PCOS had hormone levels that fell within the usual range. In all cases of polycystic ovary syndrome (PCOS), a rise in body weight may be attributable to the patients' elevated insulin levels (Malini and George 2018).

Table 1. Comparison of antioxidant enzymes activity and hormones biomarkers between fertile and infertile women with PCOS

Parameter	Fertile (N=30)		PCOS (N=30)		P value
	Mean	SD	mean	SD	
BMI	24.883	2.630	26.620	3.139	0.14
GPX pmol/ml	8.375	4.778	6.941	3.060	0.396
SOD ng/ml	2.300	0.852	2.030	0.343	0.246
CAT ng/ml	0.236	0.241	0.066	0.036	0.001
PRL ng/ml	9.189	5.355	32.999	9.789	0.001
TSH mlU/ml	2.914	1.649	8.228	2.261	0.001

Catalase (CAT) activity in table 1, indicated a statistically significant decline between PCOS-infertile women and fertile women (0.066±0.036 **vs** 0.236±0.036; P=0.001), was similarly reported by Oyebanji & Asaolu, 2020, shows a significant decrease in the activity of catalase in serum samples of the PCOS patients compared with the fertile women (1.18± 2.01 **vs** 1.48± 0.32 ; P= < 0.001). This enzyme plays a crucial role in increasing resistance to oxidative stress, thus it's possible that the decline in catalase activity in PCOS patients is related to the elevated levels of reactive oxygen species (ROS) that accompany the condition of oxidative stress (Oyebanji and Asaolu 2020).

Table 2 shows the comparison of hormonal biomarkers between fertile and infertile women without PCOS. The result revealed that there is a significant increase in the level of prolactin and TSH (p-value = 0.006 and 0.0001 respectively). In addition to a significant increase in BMI (p-value=0.001) Also significant decrease in antioxidant activity of SOD and CAT (p-value= 0.021and 0.021 respectively) Whereas a non-significant deference was found in GPX(P-value=0.237) in fertile group compared to infertile women without PCOS.

Prolactin hormone levels (PRL) in table 2 shows a significant increase between infertile women without PCOS and fertile women (17.450 ± 13.210 vs 9.189 ± 5.355 ng/ml ; P= 0.006). This result is in agreement with the results reported by Bassey *et al.,* 2015. In his study, he included infertile cases compared to controls and found that the mean serum prolactin levels was significantly higher (67±11 **vs** 29.7± 2.01; P= <0.001). HE also concluded that hyperprolactinemia disrupts hypothalamic-pituitary-ovarian function, leading to a spectrum of menstrual dysfunctions, including but not limited to corpus luteum insufficiency and oligo/amenorrhea (Bassey et al. 2015).

Furthermore, Thyroid stimulating hormones (TSH) concentration in table 2 shows a significant increase between infertile women without PCOS and fertile women(8.255±2.576 **vs** 2.914±1.649 mlU/ml; P=0.0001), This topic is in line with what Ory *et al.,* 2014 found in their earlier research. The comparison of the amounts of the TSH hormone in infertile females with those in intact females showed that there were significant differences, (4.45 ± 1.75 vs 3.66 ± 1.20; P= < 0.05). Infertility in women can have a variety of causes, and hypothyroidism and hyperthyroidism are two of them. The normal activities of egg and ovary development are disrupted when thyroid hormones overlap with reproductive hormones. Therefore, ovulation abnormalities, an irregular menstrual cycle, and low fertility might result from either an overabundance or a deficiency in the secretion of these hormones (Ory et al. 2014).

Table 2. Comparison of antioxidant enzymes activity and hormonal biomarkers between fertile and infertile women without PCOS

Parameter	fertile (N=30)		N0 PCOS (N=30)		P value
	mean	SD	mean	SD	
BMI	24.883	2.630	28.087	4.183	0.001
GPX pmol/ml	8.375	4.778	6.699	2.796	0.237
SOD ng/ml	2.300	0.852	1.877	0.464	0.021
CAT ng/ml	0.236	0.241	0.109	0.186	0.021

PRL ng/ml	9.189	5.355	17.450	13.210	0.006
TSH mlU/ ml	2.914	1.649	8.255	2.576	0.0001

Catalase (CAT) activity and superoxide dismutase (SOD) activity in table 2 showed significant decrease difference that between infertile women without PCOS and fertile women, for both (0.109±0.186 **vs** 0.236±0.241 ng/ml; P=0.021) and (1.877±0.464 **vs** 2.300±0.852 ng/ml; P=0.021) respectively. This subject agrees with previous study Al-Fartosy *et al.,* 2019. There is evidence that oxidative stress contributes to the pro inflammatory changes associated with polycystic ovary syndrome (PCOS) and that reactive oxygen species (ROS) play a detrimental role in both unexplained infertility and infertility caused by endometriosis in the pathogenesis of endometriosis and tubal factor infertility (Al-Fartosy, Awad, and Mahmood 2019).

As for BMI, table 3 shows a positive significant correlation with each of prolactin (r = 0.25), (P-value = 0.02) and TSH (r = 0.21), (p-value = 0.04) hormones in all the studied groups. This result is concurred with those found by Seth *et al.,* 2013; Therkelsen *et al.,* 2016 in which they stated a statistically significant positive correlation between prolactin and both body weight and body mass index, suggesting that prolactin may play a role in obesity (Seth, Arora, and Singh 2013; Therkelsen et al. 2016).

In addition, this result agrees with previous studies of Sachdeva *et al.,* 2019; Rotondi *et al.,* 2009 in which they stated that serum TSH level was found to be positively correlate with both body mass index and weight. Increased TSH levels found in obese people may be attributable to elevated circulating antibodies to the thyroid gland, which is consistent with the fact that obesity is associated with a chronic low inflammatory state (Sachdeva et al. 2019). Thyroid stimulating hormone (TSH) elevations was also seen in a separate research of the health of the thyroids of morbidly obese people (Rotondi et al. 2009).

In regards to Glutathione peroxidase (GPX), the study shows a highly significant negative correlation with age between all studies group (r = - 0.37), (P-value = 0.00). Glutathione peroxidase activity (GPX) in table 3 shows a negative correlation (r= - 0.37), (P-values =0.00) with age between all studies group. The result in in concurrent with those of another study done by Wang *et al.,* 2017.

Table 3. Correlation between BMI, GPX, SOD and CAT and hormonal biomarkers for all groups

Parameter		**BMI**	**GPX**	**SOD**	**CAT**
Age	R*	0.11	-0.37	-0.19	-0.13
	P value	0.29	0.00	0.08	0.21
PRL	R*	0.25	-0.06	-0.10	-0.27
	P value	0.02	0.55	0.36	0.01
TSH	R*	0.21	-0.14	-0.24	-0.27
	P value	0.04	0.19	0.02	0.01

There was a negative correlation between age and GPX activity, antioxidant defense mechanism related to aging. These results show that glutathione system may be a characteristic parameter in aging process. Alterations in GSH and GSSG levels and/or its metabolizing enzymes have been linked to age-related declines in immune function, sensitivity to medicines and free radicals, and susceptibility to certain diseases (Wang et al. 2017).

For Superoxide dismutase (SOD), the study shows a negative significant correlation with TSH (r – 0.24) , (P=0.02), between all studies group. This result is agree with those found by (Hosseini-Zijoud et al. 2016).

Finally in regards to Catalase activity (CAT), table 3 shows a negative correlation with each of PRL (- 0.27) (P values <0.05) and TSH (- 0.27) (P values <0.05) in all studied groups. This result is concurred with the results found by Hussain *et al.,* 2021 in which he reported that in female fertility via enhancing the production of ROS and increasing enzymatic antioxidant activity through alteration of the expression of enzymatic antioxidant (catalase). Consequences of slightly increase in LH level returns in oxidative stress condition which give rise to destroy lipids in the oocyte membrane leading to loss membrane viability and oocyte premature death (Hussain et al. 2021).

CONCLUSION

According to the findings of this study, women with PCOS have higher levels of Prolactin, TSH, and BMI than fertile women. Compared to fertile women, women without PCOS have statistically higher levels of Prolactin, TSH, and BMI.

BMI positively correlated with prolactin and TSH whereas there are negatively correlation between age and GPX as well as a negative correlation among CAT with prolactin and TSH in all studied groups. A future study is recommended to include a larger sample size to obtain better results.

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Consent of Ethics: Administrative approval was taken from all places where samples were collected, as well as written and oral consent was taken for all participants in the research

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