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A Biochemical Study of Infertile Women With and Without Polycystic Ovarian Syndrome in Basra City, Iraq

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***Abstract*—Background: 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, it is considered as one of the main cause of infertility. The present study aimed to evaluate a biochemical hormones (FSH, LH, LH/FSH ratio and TESTO) of infertile women with and without polycystic ovarian syndrome in Basra City - Iraq, in order to know whether they represent biomarkers of polycystic ovarian syndrome.**

Method:The present study included 60 patients infertile women (30 with PCOS and 30 without PCOS) aged between 16 to 40 years, which was admitted to Infertility and IVF Centre in Basra Hospital for Women and Children (IbnGhazwan) in Basra Government-Southern Iraq. A control group of 30 entirely healthy participants with an age range of (16 -40) years were considered. Body mass index was calculatedfor the two groups. There follicle stimulating hormone, luteinizing hormone and testosterone were measured using enzyme-linked immunosorbent assay. Software of SPSS 26 was used for the statistical analysis.

Results: The results of the present study showed a significant increase in each of (LH and LH/FSH) concentration in PCOS compared to the control. Moreover, the results illustrateda significant increase in patients without PCOS(LH and BMI)compared to the controls.

Conclusion: The study concluded that there is a significant increase in LH, LH/FSH levels in addition to BMI among women with PCOS and without PCOS compared to Fertile women.

***Keywords: FSH, LH, LH/FSH ratio, Testosterone, polycystic ovarian syndrome*.**

I. INTRODUCTIONI

Hormonal balance plays a crucial part in thewoman's life and in many diseases caused by hormone imbalances, including early menopause, primary ovarian insufficiency, ovarian cancer, and polycystic ovary syndrome (PCOS) [(1)]. Polycystic ovarian syndrome (PCOS) is the most prevalent endocrine condition in women, manifesting with a variety of different combinations of signs and symptoms and phenotypes, including reproductive, endocrine, and metabolic changes[(2)]. Serious health issues, including hypertension, diabetes type 2, infertility, and uterine cancer, are brought on by this syndrome[(3)]. Several genes, in addition to environmental and nutritional variables, have been linked to polycystic ovary syndrome (PCOS), although its exact pathophysiology remains largely unknown[(4)]. Therefore, it is still unclear what factors, including but not limited to genes, insulin resistance, the environment, inflammation, and androgen concentrations, create polycystic ovary syndrome [(5)].

Menstrual abnormalities and clinical or biochemical symptoms of hyperandrogenism, diminished fertility, polycystic ovaries, and biochemical profile abnormalities including elevated LH, testosterone, and insulin levels while decreasing FSH levels are among the distinguishing markers (6). According to Johamet *al.*,(2015), the prevalence of PCOS varies from 6% to 21% depending on the diagnostic criteria and demographic investigated. Thus, the prevalence of PCOS is 4–8 percent among reproductive-aged women using the diagnostic criteria proposed by the National Institute of Child Health and Human Development (NICHD, USA) and the American National Institute of Health (NIH, USA), while according to the Rotterdam criteria, approximately 18 percent of women have PCOS (7).

Thus, the prevalence of PCOS is 4–8% among reproductive-aged women using the diagnostic criteria proposed by the National Institute of Child Health and Human Development (NICHD, USA) and the American National Institute of Health (NIH, USA), while according to the Rotterdam criteria, approximately 18 percent of women have PCOS[(8)]. About 60–80% of PCOS patients are diagnosed with being overweight or obese (9). Despite the fact that many obese women have healthy fertility, having a high body mass index is associated with an increased risk of fertility problems [(10)].

Since being overweight makes the hormonal and clinical symptoms of polycystic ovary syndrome (PCOS) worse, and since women who have PCOS already have a higher risk of being overweight, the two conditions are linked (11). It isproposed that the pathophysiology of polycystic ovary syndrome (PCOS) can be linked to hormonal imbalance (LH, FSH,LH/FSH ratio, and testosterone), resulting in particular physical characteristics[12].

After a woman has tried unsuccessfully to conceive, she may finally get tested for PCOS. Elevated luteinizing hormone, decreased follicle-stimulating hormone (FSH), and increased androgens and insulin are hallmarks of polycystic ovary syndrome (PCOS) in most women [13]. Both oligomenorrhea and amenorrhea are symptoms of hormonal abnormalities (infrequent or lack of menstruation) [14]. Additional clinical aspects, such as hair and skin complaints, can arise from the ovaries' inability to produce enough estrogen or their overproduction of androgens (testosterone) [15]. To summarize, PCOS Polycystic ovary syndrome (PCOS) is characterized by the accumulation of tiny antral follicles on the periphery of the ovary, along with menstrual irregularity, anovulatory, subfertility, and hyperinsulinemia and hyperandrogenism, both of which can affect ovarian follicle growth (PCOS) [16].

Aim of the study

The study aimed to estimate the hormones concentration among women in Basrah -Iraq with/without PCOS compared with control, and to evaluate the effect of BMI on the level of hormone.

II. MATERIALS AND METHODS

Between November 2021 and march 2022, a case control study was performed at Infertility and IVF centre in Basra Hospital for Women and Children in Al-Basrah Government-Southern Iraq.

Five millilitres of venous blood from the control group and infertile patients after three days from menstruation were collected in gel tubes, allowed to clot at 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 (LH, FSH and Testosterone) by using ELIZA-Monobind, Inc-USA Kits.

Sixty infertile women (30 with PCOS and 30 without PCOS) aged between (16 to 40) years, which were admitted to Infertility and IVF centre in Basra 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% [17]. 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 (17-38) years.

The statistical analysis was conducted using SPSS 26. Biochemical parameter descriptive analysis was expressed as mean standard deviation (SD). Student's test for parametric variables and Mann were used to do comparisons. Using Pearson's correlation coefficient, a regression study of the correlation between parameters among fertile and infertile women was conducted. P-values less than 0.05 were deemed statistically significant. p-values 0.01 were regarded as very significant.

III. RESULTS AND DISCUSSION

Table 1 shows the comparison of the studied parameters between fertile and infertile women with PCOS. There were non-significant changes observed in age (p value =0.605) and BMI (P value= 0.14) between fertile and infertile groups. The results revealed that there is a significant decrease in FSH (p value=0.017) with a significant increase in hormones levels of LH (P value=0,001) and LH/FSH (0,001). Whereas a non-significant deference was found in Testosterone level (p value= 0.018).

TABLE1:COMPARISON OF HORMONAL BIOMARKER BETWEEN FERTILE AND INFERTILE WOMEN WITH PCOS.

Parameter	Fertile (N=30)		PCOS and Infertile (N=30)		P value
	Mean	*SD*	*mean*	*SD*	
Age (year)	26.433	7.426	28.700	6.655	0.605
BMI	24.883	2.630	26.620	3.139	0.14
FSH mlU/ ml	8.406	3.293	6.188	2.745	0.017
LH mlU/ ml	4.731	1.188	18.294	10.945	0.001
LH/FSH Ratio	0.642	0.288	3.287	1.908	0.001
TESTO ng/ml	0.549	0.247	0.762	0.685	0.18

Table 1 shows the age and BMI characteristics of infertile with PCOS women cases and fertile women. In terms of age, it was found that there was no significant differences (26.433±7.426 vs 28.700±6.655; P=0.605) and BMI (24.883±2.630 vs 26.620±3.139; P=0.14) between fertile and infertile women with PCOS respectively. Our findings about BMI was in consistence with what was previously reported by Fatima et al., (2019), they 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),[18].

FSH level shown in Table 1 illustratesa significant decreased in the infertile women group with PCOS compared to fertile group (6.188 ± 2.745 vs 8.406 ± 3.293; P= 0.017). These findingsare consistent with Al-Assadi et al., (2019). In women with polycystic ovary syndrome (PCOS), the levels of follicle stimulating hormone (FSH) appear to be low or within the lower follicular range, and the response to gonadotropin-releasing hormone (GnRH) is somewhat comparable to that of ovulatory controls (6.2 ± 1.8 vs 7.2 ± 1.8; P= 0.001) [19].

The findings of Sugawara &Nikaido, (2014), investigation are supported by our results. They reported that women who were unable to conceive had higher levels of the hormone FSH than those who were able to conceive (8.77 ± 4.65 vs 6.71 ± 4.12; P= 0.002). The researchers found that FSH levels were significantly greater in infertile women than in fertile women. Female somatotropin (FSH) is the most abundant gonadotropin hormone in the female bloodstream [20].

LH concentration in Table 1 shows a statistically significant increase in the proportion of PCOS-affected infertile women compared to PCOS-affected fertile women (18.294 ± 10.945 vs 4.731 ± 1.188; P=0.001). This result

concurred with a previously reported results of Khmil et al., (2020). In women with infertility, the levels of luteinizing hormone was found to be elevated compared to the fertile group (8.35 vs 7.25; P = < 0.001) due to PCOS.

Ovulation dysfunction is a common symptom of polycystic ovary syndrome (PCOS), and it has been found that GnRH release in people with PCOS is rather rapid and does not slow down very well in response to estrogen and progesterone. Increased LH production is a common symptom of PCOS in women [21]. In addition to what was reported by Vander Borght&Wyns, (2018), we found that the LH hormone concentration of infertile women was significantly higher than that of fertile women (4.6689 ± 1.89769 vs 2.354 ± 0.45 ; P ≤ 0.05) .This high concentration of LH hormone may be an indicator of an imbalance in the secretion process of LH hormone, the higher the incidence rate of infertility[22].

Female infertility is linked to polycystic ovarian syndrome (PCOS), a condition in which the LH hormone levels approach (2:1), rendering hormone production in the ovary to be impossible [23]. Consistent with the findings of the study by Inhorn&Patrizio, (24), they conducted that elevated levels of LH hormone reflects pituitary gland malfunction [25]. On the other hand, and in contrast to the findings, who first reported that the concentration of the luteinizing hormone (LH) was much lower in infertile compared with intact [26]. The present findings suggest that an increase in the body mass index (BMI) may have a suppressing effect on luteinizing hormone (LH) secretion, and that hyperinsulinemia and insulin resistance may contribute to abnormal gonadotropin secretion either directly or indirectly. All of these things contribute to elevated amounts of free androgens, which in turn causes ovulation to cease [27].

Women with PCOS have an abnormal gonadotrophin axis, resulting in elevated LH and decreased FSH levels and a reversed LH/FSH ratio. As the frequency of LH pulses rises, androgen production by the theca cells rises along with it, but follicle development and, ultimately, ovulation are hampered by the low amounts of follicle-stimulating hormone [28]Whereas we disagree with the findings of other study of Prasad et al.,2015 There was a statistically significant difference between the mean serum FSH levels of infertile women (8.77 ± 4.65 mIU/ml) and fertile women (6.71 ± 4.12 mIU/ml) (p=0.0022) [29].

LH/FSH Ratio shown in Table 1 appearsa significant increase in the number of infertile women with PCOS compared to fertile women, (3.287 ± 0.288 vs 0.642 ± 0.288; P=0.001). Comparing our findings on the LH/FSH ratio to those of Nath et al.,(2019), it is clear that they are consistent. The most common clinical symptom of PCOS in women is an elevated LH/FSH ratio. 70% of PCOS-diagnosed women had an abnormally high LH/FSH ratio [30].

Consistent with the findings of the study [31], around 70% of women with PCOS have an elevated LH/FSH ratio (LH/FSH ratio > 2) (31). However this finding disagrees with the study Le et al. who reported no statistical significant difference in the predictive value LH/FSH levels (P = 0.340) in the diagnosis of PCOS [32]. Additionally, many studies have found that non hyperandrogenic PCOS patients have the least severe endocrine and metabolic dysfunction and the lowest prevalence of metabolic syndrome compared to healthy control [33].

For Testosterone (TESTO) levels illustrated in Table 1, a non-statistically significant difference is appeared between infertile women with PCOS and fertile women when compared to other women (0.762 ± 0.685 vs 0.549 ± 0.247; P=0.18). This result was concurred with what a previously reported results of Lerhbaum et al., (2014). In which they demonstrate that a high testosterone level is connected with a favourable metabolic profile in PCOS women found that the results of the study were positive. In addition, women who have PCOS and high testosterone levels are at a higher risk of metabolic complications [34].

The results of the present study also agree with the results of the study of Sheehan, (2004), who confirmed that insulin may cause a number of undesirable side effects, including as the suppression of ovarian function and an increase in androgens like testosterone [35]. The finding was also consistent with what was previously reported by Lin et al., (2021). When comparing the level of testosterone concentration for Fertile women with infertile women with PCOS, we notice an increase in the concentration of testosterone level, but this increase is not significant (P=0.188) [36].

Table 2 represents the study comparison of hormonal biomarkers between fertile and infertile women without PCOS. The result revealed that there was a non-significant deference in age (P value= 0.088). While a significant increase in BMI (p value=0.001) with non-significant deference in hormones levels of FSH(p-value= 0.999). On the other hand, the study results found a significant increase in the levels of LH and LH/FSH Ratio (p value = 0.001and 0.001 respectively), with a non- significant relation in the level testosterone (P value= 0.99) .

TABLE 2:COMPARISON OF HORMONAL BIOMARKER BETWEEN FERTILE AND INFERTILE WOMEN WITH PCOS.

Parameter	Fertile (N=30)		No PCOS and infertile (N=30)		P value
	mean	*SD*	*mean*	*SD*	
Age (year)	26.433	7.426	30.333	6.343	0.088
BMI	24.883	2.630	28.087	4.183	0.001
FSH mlU/ ml	8.406	3.293	8.661	3.006	0.999
LH mlU/ ml	4.731	1.188	8.649	1.789	0.001
LH/FSH Ratio	0.642	0.288	1.130	0.486	0.001
TESTO ng/ml	0.549	0.247	0.583	0.216	0.999

In regards to Follicle stimulating hormone (FSH), Table 2 shows that there was no significant relation between infertile women without PCOS and fertile women (8.661 ± 3.006 mlU/ml vs 8.406 ± 3.293 mlU/ml ; P=0.999). The table also shows a significant increase in LH/FSH ratio between infertile women without PCOS and fertile women (1.130 ± 0.486 vs 0.642 ± 0.288 ; P= 0.001).

These results agree with many previous studies: [23], [37] which reported FSH levels between infertile and fertile women (4.81±3.30 and 2.90 ± 1.41 mIU/mL; P= ≥0.05) respectively. In spite of non-emersion of associated symptoms (e.g. irregular menstrual cycle) with a raise in LH and normal FSH suggests PCOS. Therefore, it is possible that some women who appear to be normal and who have regular ovulatory cycles have a poor fertilization rate. It's possible that this is a sign of pre-PCOS [23].

The FSH hormone is one of the most crucial hormones regulating the maturation and development of eggs. Increase or decrease FSH hormone levels can cause imbalance in ovulation process loss mechanism feedback [37].

Luteinizing hormone (LH) concentration showed a significant increase between infertile women without PCOS and fertile women (8.649± 1.789 vs 4.731±1.188 mlU/ml; P= 0.001). This result concurred with a previously reported results of Shanmugham et al., (2018). That revealed LH levels were significantly higher in infertile women than in fertile women (7.64±5.16 vs 5.66±3.17 ; P= < 0.05). In most cases, elevated levels of LH are associated with ovarian dysfunction [38].

The LH levels were considerably higher in infertile women compared to fertile women, as indicated by the Alam et al., (2019) research, which also confirmed the same conclusion (10.05 ± 0.34 vs 5.34 ± 0.32 ; P= <0.001). It's possible that it's owing to the fact that hypersecretion of LH induces early oocyte maturation, which in turn reduces fertilization, lowers cleavage rate an excessive amount of luteinizing hormone (LH) might have negative consequences during the follicular phase, which can make fertilization and pregnancy more difficult [39].

An increase level of LH may have direct or indirect effects on the developing oocyte and endometrium. Because of the interaction between LH and FSH, a rise in estradiol synthesis occurs, which in turn stimulates follicle growth and development [40].

This topic is in agreement with the research of Abdoli et al., (2020), who found that causality must be tested throughout experimental models. Findings in Italian fertile women showed an inverse relationship between obesity and LH and FSH levels. In the present in this research, a positive relation was found between FSH and obesity [41]. In addition, infertility risk rises when there is an imbalance between oxidants and antioxidants, which can be caused by hormonal fluctuations. LH/FSH ratio has been suggested as a biomarker of pregnancy outcome in infertile women, but this has not been proven in the present study [42].

For Testosterone (TESTO) levels, Table 2 shows a non-significant relation between infertile women without PCOS and fertile women (0.583 ± 0.216 vs 0.549 ± 0.247;). This corresponds with Jawad et al., (2015), who reported that Testosterone levels in obese infertile and non-obese fertile women (0.316 ± 0.155 vs 0.259 ± 0.169; P= 0.164). The fat on a person's body is essential for reproduction. Reproductive failure can occur in people with either an excess or a deficit of body fat [43]. According to [44], the majority of the male steroid hormone androgen is produced in the testes. The ovaries of women only generate a tiny fraction of what men do. the adrenal glands were produced Androgen precursors by of both sexes and then transformed to testosterone in the body's periphery. Males are thought to have 15–25 times more levels than females in their blood [44]. Although these findings are consistent with those of a previous study by Abdelazim et al., (2020), both total and free testosterone levels were considerably higher in the PCOS-free group than in the fertile group (P= < 0.0001) [45].

IV. CONCLUSIONS

1.The levels of LH, LH/FSH and BMI increased significantly in PCOS as well as in women without PCOS compared to Fertile women.

2. In addition, the level of FSH was found by the study to be significantly decreased in PCOS comparing to Fertile women.

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