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Article

An Enzymological and hormonal comparative Study Between Obese And Normal Weight In Iraqi Females With PCOS

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Abstract

Background Polycystic Ovary Syndrome (PCOS) is defined as a series of interconnected reproductive disorders, including as irregular gonadotropin secretion, elevated testosterone levels, persistent anovulation, and polycystic ovarian structure. It is often linked to insulin resistance and obesity. These reproductive and metabolic disorders result in significant morbidities throughout life, including anovulatory infertility and type 2 diabetes (T2D)".**Objectives** enzymes activities (aromatase and 3β-Hydroxysteroid Dehydrogenase) and hormones levels (estrogen and DHEA-s) disturbances between obese and non obese (normal weight) polycystic women and highlight the outcomes according to the body weight **Methods** The study design is the case-control, where (60) Blood samples have been taken from polycystic individuals at the Maternity and Children's Hospital. divided into (G1) obese women with PCOS / 30 samples, while group 2 (G2) consists of 30 samples of normal weight PCOS women,. The activities of Aromatase, the 3β-Hydroxysteroid Dehydrogenase enzymes, and others hormones were determined and the difference were analyzed by analysis of results by one way (ANOVA).**Results** A non significant decrease of Aromatase

activity between obese (G1) and non-obese patients (G2) (3.56±2.40), (5.25±4.88) respectively. Also, a significant decrease in the levels of 3β-Hydroxysteroid Dehydrogenase was reported between G1 and G2 (3.39±1.09),(4.66±1.43**)** at respectively at P-value less than 0.05 was reported.,And There are significant decrease of estrogen and DHEA-s hormones for the obese (G1) and non-obese patients (G2) (82.20±25.10), (169.24±38.25) , (347.13±90.56), (243.06±83.71) at respectively at P-value less than 0.05 was reported. **Conclusion** The present study is the first in Iraq reporting that obese women with PCOS have reduced amounts of the enzyme Aromatase, 3β-Hydroxysteroid Dehydrogenase, and reduced level of estrogen while DHEA-s level was increased in the same patients compared with PCOS patients with normal weight. The clinical biochemistry lab uses the disruptions in hormones and enzymes As a diagnostic tool for this disease by highlighting the obesity.

Keywords: Aromatase , 3β-HSD , DHEA-s, Polycystic ovarian syndrome (PCOS).

Introduction

Polycystic ovarian syndrome (PCOS) is distinguished by anovulation, ovarian cysts, and endocrine variance(Bulsara et al., 2021) . In women between the ages of 17-45, Polycystic ovary syndrome prevalence is estimated to be between 5.5% and 12.6% worldwide(Suri & Kapur, 2021). The most frequent androgen abnormality linked to PCOS is higher levels of free testostarone levels. Progesterone levels are moderate in an-ovulatory PCOS, and estradiol levels are moderate in the mid-follicular phase (Dapas & Dunaif, 2022).

The conventional manifestations of PCOS include amenorrhea, oligomenorrhea , hirsutism , obesity, subfertility, anovulation and insulin resistance Hirsutism has been shown to markedly elevate psychological stress, whereas infertility issues can lead to worry, distorted self-image, and workplace difficulties (Wayadande & Honklas, 2023).

A malfunction of genetic, environmental, and behavioral variables play a key role in the pathogenesis of PCOS. Ovarian enlargement and increased androgen secretion compared to normal theca cells are the most prevalent clinical manifestations of PCOS. The increased enzymatic activity in the steroid production pathway results in heightened androgenic secretion. Hyperandrogenism causes irregular menstrual cycles and contributes to the hirsutism and acne characteristic of PCOS (Dubey et al., 2021).

Polycystic Ovarian Syndrome is commonly associated with overweight and obesity(Barber et al., 2019), Compared to healthy controls, PCOS patients have a four-fold increased risk of obesity. Obese and overweight people are not the only ones with PCOS. It is also diagnosed in people who are underweight or of normal weight (Mohapatra & Samantaray, 2024).

Obesity, insulin resistance, and PCOS are closely related. One of the main causes of the onset and aggravation of PCOS symptoms is insulin resistance, which can be exacerbated by excess adipose tissue (Zhao et al., 2023).

Aromatase is a cytochrome P450 monooxygenase encoded by the CYP19A1 gene. Its expression is modulated by differential promoter activation in a tissue-specific way. "Aromatase predominantly exists in the ovaries, brain cells, and testes of rodents, as well as in human adipocytes and placental cells". This enzyme catalyzes the demethylation of testosterone and androstenedione, leading to the aromatization of the A ring and ultimately resulting in the production of estrone and estradiol. Furthermore, the converting aromatase is situated in the granulosa cells of the ovarian follicles. Aromatase is crucial in glucocorticoid synthesis (Liu et al., 2021).

Ovarian granulosa cells (GCs) are the primary source of estrogan. Aromtase, the principal enzyme in estrogen production, is crucial not just for ovarian developmant but also for estrogen release by Gcs. Disruptions in ovarian estrogen production are more likely to precipitate estrogen-dependent illnesses and reproductive complications in females, including ovarian cancer and polycystic ovary syndrome. Aromatase is a significant pharmacological target; the use of its inhbitors in estrogen-dependant disorders has garnered growing interest (Liu et al., 2021).

3-hydroxysteroid dehydrogenase (3-HSD) is a crucial enzyme in the steroid production process within the adrenal glands and gonads. 3-HSD is a 42-kDa microsomal enzyme that facilitates the transformation of the hydroxyl group to a keto group at carbon 3 and the isomerization of 5-steroid precursors into 4-ketosteroids. 3-HSD catalyzes the transformation of pregnenolone into progesterone, 17-hydroxypregnenolone into 17-hydroxyprogesterone, dehydroepindrosterone (DHEA) into androstendione, and androstendiol into testosterone. 3-HSD is encoded by two closely related genes located on chromosome 1 (1p13.1), HSD3B2 and HSD3B1, which code for type II and I isozymes, respectively. Type I isozyme (HSD3B1) is predominantly expressed in

the placenta and peripheral tissues, whereas type II isozyme (HSD3B2) is primarily found in the adrenal gland and gonads (Menegatti et al., 2022).

A deficiency of 3β-HSD classically presents with some degree of salt wasting, mild virilization, or symptoms suggestive of polycystic ovary syndrome (PCOS)(Edwards et al., 2021).

Estradiol E2 is a potent naturally produced Estrogen by the placenta and ovary, additionally a smaller amount is secreted by the adrenal cortex. Estradiol secrete into blood stream via binding to sex hormone binding globulin (SHBG). Whereas, Estradiol receptor complex greatly affects estrogenic activity (Syndrome et al., 2024).

Dehydroepindrosterone (DHEA), a 19-carbon endoganous steroid hormone , is naturally produced in the body via the cholesterol to pregnenolone route. DHEA is primarily synthesized in the zona reticularis of the adrenal gland and is thereafter transformed into its sulfated form, dehydroepiandrosterone sulfate (DHEA-S), by the enzyme sulfotransferase. DHEA and DHEA-S are the predominant steroid hormones in humans, exhibiting a gradual reduction with advancing age (Lin & Tsui, 2021).

DHEA-S is easily converted back to DHEA via hydrolysis by a sulfatase in peripheral target tissues and acts as a precursor to androgens and estrogens. DHEA-S can infiltrate the ovarian follicle and serves as a crucial source of ovarian testosterone, which significantly contributes to follicular growth (Astapova et al., 2019).

Materials and Methods:

Study Design

Women diagnosed with PCOS according to the Rotterdam 2003 criteria were enlisted for a case-control study. present study included 60 individuals divided into two groups: 30 patients with obesity and polycystic ovary syndrome (PCOS) , referred as (G1) and 30 patients with PCOS and normal body mass index (BMI) referred as (G2). All participants, aged 18 to 40, satisfied the Rotterdam consensus criteria (2003) for diagnosing PCOS.,The research started in September 2024 and was undertaken for patients at Babylon Teaching Hospital Maternity and Children in Babylon province. The practical investigation was carried out at the biochemistry laboratory of "AL-Furat Al-Awsat Technical University" and Babylon Teaching Hospital. Participants who were pregnant or nursing, smoked, had diabetes, were unsuitable for transvaginal ultrasonography (such as virgins or non-compliant patients), or had previously utilized hormonal prescriptions,

medications affecting ovarian function, or drugs influencing metabolism within a month prior to enrollment in the study were disqualified. Moreover, other prominent disorders characterized by elevated testosterone levels were excluded.

Samples Collection

Blood samples were collected from all females in periods between the 2nd and 5th day of the cycle.

At room temperature, venous blood samples were drawn from all subjects by using a disposable syringe (5 mL) in the sitting position, and slowly pushed into tubes for study .,blood was drained into plain tube gel for serum preparation, which was used in hormonal test after separation centrifuged at 450Xg for approximately 5-10 minutes, the blood test tubes used were manufactured of high-purity polypropylene created without plasticizers or biocides to provide the best quality of the study, tubes have a safe-lock seal to prevent inadvertent tube opening and sample loss (Eppendorf, Germany), then the serum was divided into four parts and stored at -20˚C until analysis..

Laboratory Determination

The ELISA technique was utilized to measure the activities of Aromatase enzyme and the 3β-Hydroxysteroid Dehydrogenase and the level of Estrogen hormone in patient samples. And using miniVIDIS was used to assess the level of DHEA-s hormone in patients serum Subsequently , the results were then statistically evaluated.

Statistical Analysis

Each data value was explained as mean ± standard deviation (SD). A one-way ANOVA was employed to evaluate the data. The Statistical Package for Social Sciences (SPSS) software, version 20.0, was utilized for statistical analyses, with significance referred by P-values < 0.05.

Results &Discussion

Table (1) Biometric variables in the Studied Groups.

Biometric variables	Groups	Means ±SD	95% Confidence Interval		P-value
			Lower	Upper	
Age (Years)	(G1)	26.00±6.27	23.65	28.34	0.855
	(G2)	26.95±6.41	24.56	29.35	
	(G3)	26.13±6.16	23.83	28.43	
BMI (Kg/m^2)	(G1)	32.06±1.76	31.40	32.72	<0.001
	(G2)	23.67± 0.94	23.32	24.02	
	(G3)	23.70± 0.85	23.38	24.01	
Waist/ Hip ratio (W/HR)	(G1)	1.06±0.12	1.01	1.10	<0.001
	(G2)	0.86±0.04	0.85	0.88	
	(G3)	0.76±0.02	0.75	0.77	

(G1) are obese polycystic ovarian syndrome patients,(G2) are polycystic ovarian syndrome patients with normal weight,(G3) control women with normal weight (BMI) body mass index,P-value <0.05 was significant.

The effect of Age on PCOS

No significant age differences Was reported between the patient groups (obese and normal weight), (P-value Is 0.855) (Table 1).

These results have revealed that the age has no impact on the obesity in PCOS patients.

The Effect of Body Mass Index on PCOS

The results Have reported the significant elevation for BMI value in the patients with obese PCOS patients (G2)(32.06±1.76), Compared with normal weight patient (G3)(23.67± 0.94) respectively as shown (Table 1).

A higher BMI is directly correlated with elevated levels of androgens (male hormones), which can lead to excessive hair growth and acne. A higher BMI in women with PCOS is associated with an increased risk of acquiring diseases such as type 2 diabetes and metabolic syndrome (Neubronner et al., 2021).

Elevated BMI is directly correlated with worse metabolic condition , with insulin resistance and abnormal lipid profiles are more prevalent among PCOS patients with higher BMI (Neubronner et al., 2021).

Women with PCOS , are particularly those Who exhibit central obesity and have an increased propensity for developing dyslipidemi, anovulation, and hyperandrogenemia, which are the metabolic and reproductive manifestations of PCOS (Yadav & Tarware, 2019). Obesity often triggers the clinical manifestations of PCOS. Indeed, hirsutism and irregular menstrual cycle are more prevalent in obese persons compared to those without obesity-related PCOS. Due to the above listed problems, the quality of life for a PCOS patient may deteriorate with time (Wayadande & Honklas, 2023).

Obesity is associated with reduced fertility, And consequently women with PCOS may already encounter difficulties in this regard. Weight reduction in obese patients with PCOS might beneficially promote the reproduction quality (Mohapatra & Samantaray, 2024).

The Effect of Waist to Hip(W⁄H) Ratio on PCOS

According to table 1 , the results have shown that mean ± SD of the W⁄H ratio was significantly increased in obese women with PCOS (1.06±0.12), compared with the women with PCOS (0.86±0.04)with a P-value of 0.001 indicating greater significance.

In the females uterus , androgen excess may contribute to an adverse metabolic condition resulting in dyslipidemia and an abnormal fat distribution (android patterns), which subsequently leads to PCOS (Yadav & Tarware, 2019).

A previous study have. observed that waist circumference is more closely associated with obesity-related risk factors than BMI (Janssen et al., 2004) Remarkably , all WHR > 0.8 are substantially linked with PCOS, demonstrating its impact on patients' clinical characteristics, metabolic abnormalities, and endocrine profiles (Kayali et al., 2022).

Table (2): The enzymological aspect in the Studied Groups.

Enzymes	Groups	Means ±SD	95%Confidence Interval		P-value
			Lower	Upper	
Aromatase	G1	3.56±2.40	2.66	4.46	0.139
	G2	5.25±4.88	3.43	7.07	
	G3	16.54 ±4.58	14.83	18.26	
(3β-HSD)	G1	3.39±1.09	2.98	3.80	0.010
	G2	4.66±1.43	4.13	5.19	
	G3	4.66±1.43	4.13	5.19	

The results of table 2 have reported that aromatase activity was non significantly decreased in G1 (3.56±2.40) compared with G2(5.25±4.88) revealing that the obesity has a minimal impact on aromatase activity.

In this regard , a recent study has demonstrated a reduction of aromatase in women with PCOS and indicated that Hyperandrogenism as a hormonal imbalance is caused by reduced aromatase enzyme activity (Panghiyangani et al., 2020).

Aromatase deficiency inhibits the conversion of testosterone to estradiol, potentially resulting in hyperandrogenism. Hyperandrogenism affects normal folliculogenesis, hence enhancing the onset of insulin resistance and the formation of polycystic ovaries (Ikhtiyarova et al., 2024).

The results of table 2 have reported that 3β-HSD activity was significantly decreased in G1 ((3.39±1.09) compared with G2(4.66±1.43) , p=0.010

Interestingly , a lower 3β -HSD activity has been documented in 12–33% of women exhibiting clinical manifestations of hyperandrogenism. The reduction in this enzyme's activity leads to insulin resistance and the buildup of DHEA and DHEAS. Both normoandrogenic and hyperandrogenic PCOS may have analoguous 3 β -HSD activity (Panghiyangani et al., 2020).

Table (3): The hormonal aspect in the Studied Groups.

Enzymes	Groups	Means ±SD	95%Confidence Interval		P-value
			Lower	Upper	
Estrogen	G1	82.20±25.10	72.83	91.58	<0.001
	G2	169.24±38.25	154.95	183.52	
	G3	348.54±90.43	314.77	382.31	
	G1	347.13±90.56	313.31	380.95	

DHEA-s	**G2**	243.06±83.71	211.80	274.32	<0.001
	G3	123.21±29.63	112.14	134.27	

The significant difference in Estrogen (P<0.05) among studied groups (obese patients and normal weight), mean ± SD of PCOS groups for (obese and normal weight) were (82.20±25.10), (169.24±38.25) respectively with highly significant difference (P< 0.05), as in Table (3).

The abnormality of estrogen secretion, which elevates the prevalence of PCOS, is the primary cause of female infertility. Polycystic Ovary Syndrome (PCOS) primarily presents as a condition of reproductive dysfunction and endocrine abnormalities, predominantly characterized by elevated testosterone levels and anovulation. The hyperandrogenic condition of the ovaries in individuals with PCOS is the primary risk factor for the reduction of aromatase synthesis in luteinized granulosa cells (Liu et al., 2021).

The reasons for increasing obesity in menopausal women are not clear. Some researcher arguments that the absence of estrogens may be an important obesity-triggering factor. Estrogens deficiency enhances metabolic dysfunction predisposing to DM type 2, the metabolic syndrome, and cardiovascular diseases . As a result of increases of life expectancy in developed countries, many women will spend the second half of their lives in a state of estrogen deficiency. Thus, the contribution of estrogen deficiency in the pathobiology of multiple chronic diseases in women., A growing body of evidence now demonstrates that estrogenic signaling can have an important role in obesity development in menopausal women. Menopausal women are three times more likely to develop obesity and metabolic Syndrome abnormalities than premenopausal women (Lizcano & Guzmán, 2014).

As reported by table 3 , DHEA-s level was significantly increased in G1 (347.13±90.56) compared with G2 (243.06±83.71) , p< 0.001.

Adrenall hyperandroganism is prevalent in people with PCOS and may be evaluated using many approaches, including the measurement of circulating DHEAS levels. In clinical practice, serum DHEAS serves as the primary measure for adrenal hyperandrogenism. Nonetheless, it has been unable to establish any

distinction between women with PCOS exhibiting elevated DHEAS levels and those with normal DHEAS levels, leaving the potential involvement of adrenal androgens in the pathophysiology and clinical manifestation of PCOS ambiguous (Carmina & Longo, 2022).

Conclusion

The present study reported that the activities of aromatase and 3β-hydroxy steroid dehydrogenase and estrogen level were decreased in Iraqi obese women PCOS compared with other Iraqi PCOS women with normal weight. In contrast , DHEA-s level was increased in the same patients . These results submit a novel finding to the field of research regarding the diagnosis of PCOS.

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