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Original Article

Estimation of Dehydroepiandrosterone (DHEA) and Tumor Necrosis Factor–Alpha (TNF-α) Levels Women Diagnosed with Polycystic Syndrome

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Abstract - The most common endocrine condition that affects fertile women worldwide is known as polycystic ovarian syndrome, or PCOS. Depending on the diagnostic criteria applied, the prevalence might range from 5% to 15%. The presence of two of the three criteria—polycystic ovaries, clinical or biological hyperandrogenism, and chronic anovulation—must be present for PCOS to be diagnosed under the recommendations of specialized societies. DHEA and DHEA-S levels of androgens are frequently high in women with PCOS. Acne, irregular menstruation periods, and hirsutism excessive hair growth can all be indications of this hormonal imbalance. Two groups were involved in the study: one hundred women in the patient group, which is the initial group. Between October 1, 2023, and March 1, 2024, 50 patients and 50 healthy individuals served as controls, as determined by a team of experienced gynecologists at the Fertility Center in AL-Sadder Medical City in Najaf Province, Iraq. 5 ml of blood were drawn from a brachial vein using sterile medical syringes on female subjects who were in the luteal phase for this investigation. The blood was then put in a gel. To determine LH, FSH, Prolactin, DHEA, TNF-α, and IL-6 using an ELISA kit (Elabscience, USA) and lipid profile using an automated biochemistry analyzer (Beckman Coulter, USA), the serum was extracted, put in Eppendorf tubes, and deep frozen at -20 °C. According to the current study's findings, POCS women's levels of DHEA are much higher than those of healthy women. Additionally, the research demonstrated that, when compared to healthy controls, women with polycystic syndrome had significantly higher blood levels of IL6 and TNF-α. The results clearly show a correlation between PCOS and increased DHEA, IL-6, and TNF-α levels.

Keywords - Polycystic ovary syndrome, DHEA, TNF-α.

1. Introduction

A complex endocrine condition known as polycystic syndrome (PCOS) affects fertile women. It is typified by polycystic ovarian morphology, ovulatory problems, and hyperandrogenism [1]. Based on the diagnostic standards and populations under study, its estimated prevalence can range from 6% to 20%, making it one of the most common endocrine diseases globally [2]. Even though PCOS has a major negative influence on women's health and quality of life, its prevalence and clinical symptoms are still little understood in Iraq, especially in the region of Al-

Najaf. Dehydroepiandrosterone (DHEA) changes and disruption of interleukin 6 (IL-6) are two of the many hormonal abnormalities linked to PCOS that have received a lot of interest. DHEA is a key player in controlling androgen production and metabolism, an androgen precursor mostly generated by the adrenal glands [3]. Increased DHEA levels have been linked to the hyperandrogenic phenotype seen in PCOS patients, possibly as a part of the disease's pathophysiology [4].

Tumor Necrosis Factor-alpha (TNF-alpha) is a cytokine that promotes inflammation and has a role in systemic inflammation. It is produced by the immune system. Research has revealed that women with Polycystic Ovary Syndrome (PCOS) had elevated levels of TNF-alpha compared to those without PCOS. It is plausible that the aforementioned increase in TNF-alpha is a contributing factor to the pathogenesis of PCOS, given its involvement in insulin resistance, inflammation, and androgen production [5]. Moreover, it is now known that Interleukin 6 (IL-6), a proinflammatory cytokine, may have a role in developing PCOS. Elevated IL-6 levels have been identified in PCOS-afflicted women, and these levels are associated with obesity, hyperandrogenism, and insulin resistance, the three primary hallmarks of the condition [6].

In order to evaluate the levels of DHEA, TNF-alpha, and IL-6 in women with PCOS in the Iraqi province of Al-Najaf, this study was conducted. Through the process of quantification, we aim to clarify any possible correlations between these biomarkers and the severity and clinical presentation of PCOS in this particular group. Understanding PCOS's inflammatory and hormonal profiles may also illuminate the condition's pathophysiology, etiology, and possible therapeutic targets for treatment and intervention. Women of reproductive age who suffer from PCOS, a complicated endocrine illness, often experience polycystic ovaries, hyperandrogenism, and irregular menstrual periods. This study aims to improve the knowledge of the pathophysiological processes behind PCOS by estimating levels of DHEA and TNF-α.

2. Methods and Methods

2.1. Subject of Study

The study involved the creation of two separate groups: The first group is the patients' group, which consists of 50 patients who were chosen by specialized gynecologists of the "Fertility Center in AL-Sadder Medical City in Najaf Province/Iraq during the period from the 1st October 2023 to the 1st March 2024." These patients meet the requirements of the Rotterdam criteria (2003), which dictate the presence of at least two of the following characteristics: irregular menstruation, hyperandrogenism, and polycystic ovaries on ultrasound exams. Patients in this age group varied in age from 19 to 45. Gynaecologists decided that the qualifying standards for the second group, referred to as the control group, were met by 50 women who had regular menstrual cycles, normal ovulation, and normal ovarian function.

2.2. Blood Sample

Using sterile medical syringes to extract 5 milliliters of blood from the brachial vein, the research participants were females in the luteal phase. The blood was then put in a gel tube. The samples were centrifuged (3000 rpm/min) for 5 minutes to extract the serum from the remaining components of the blood after the gel tube had been left at room temperature for 30 minutes to coagulate the blood. To determine LH, FSH, Prolactin, DHEA, TNF-α, and IL-6 using an ELISA kit (Elabscience, USA) and lipid profile using an automated biochemistry analyzer (Beckman Coulter, USA), the serum was removed using a micropipette and then put in two repeaters of Eppendorf tubes and deep frozen at -20 °C.

2.3. Statistical Tests

The Statistical Package for Social Sciences (SPSS) version 25 for Windows was developed by IBM in the US in 2017. It was used to enter, arrange, and interpret data from research participants, polycystic women patients, and controls. Every variable was checked for mistakes or discrepancies before the study was conducted. It was

established if age, BMI, cholesterol, TG, HDL, LDL, VLDL, FSH, LH, PRL, testosterone, DHEA, TNF-α, and IL6 were continuous variables using histograms and normal distribution curves. The statistical importance of variations in the frequency of categorical parameters between patients with polycystic women and controls was assessed using the chi-square test. Mean comparison among the research parameters subjected to a t-test independent analysis to determine group variances. Substantial levels of significance are defined as P values of 0.05 or less.

3. Results

The sociodemographic features of the study sample are shown in Table 1. These included age, BMI, education level, marital status, and place of residence. All of these variables indicated the presence of variances between the study groups, with the level of probability for age, BMI, and place of residence being significant, while other factors showed no significant differences between the patient and control groups.

Table 2 indicates that there was a significant difference (P <0.001) in the mean levels of cholesterol, Triglycerides (TG), LDL, and VLDL among the PCOS patients group. When comparing POCS to control, the results similarly showed a significantly significant drop in HDL levels. According to Table 3, the FSH levels of POCS women were significantly lower than those of the control group at (P.<0.001), while their levels of LH, prolactin, and testosterone were greater. Additionally, the results of the current study indicate that POCS women have much greater levels of DHEA than normal women, based on the information in Table 4 and Figure 1. Furthermore, as indicated by Table 5 and Figures 2 and 3, respectively, the research findings revealed a highly significant rise in the blood levels of serum IL6 and TNF-α among women with polycystic syndrome as compared to healthy controls.

Table 1. Features of the studied groups

Age (Years) Mean ± SD*	28.5 ± 5.4	30.3 ± 2.9	< 0.05 †
BMI (kg/m²) Mean ± SD	30.4 ± 5.4	25.12 ± 1.2	< 0.001 †
Education			
Primary: N (%)	19 (38.0)	12 (24.0)	0.294 ¥
Secondary: N (%)	21(42.0)	24 (48.0)	
University: N (%)	10 (20.0)	14 (28.0)	
Marital Status			
Married: N (%)	33 (66.0)	35 (70.0)	0.668 ¥
Unmarried: N (%)	17 (34.0)	15 (30.0)	
Residence			
Urban: N (%)	34 (68.0)	37 (74.0)	< 0.05 ¥
Rural: N (%)	16 (32.0)	13 (26.0)	
*: Standard Deviation; †: T. Test; ¥: Chi-Square Test; P<0.05 Considered Significant .			

Table 2. Serum lipid profile among patients and control

Parameters	Study Groups		T. Value	P.Value
	POCS	Control		
Cholesterol (Mg/Dl)	140.9 ± 30.4	127.8 ± 23.5	2.3	<0.001
Triglyceride (Mg/Dl)	148.6 ± 65.3	122.8 ± 55.7	2.6	<0.001
HDL (Mg/Dl)	39.8 ± 12.7	45.7 ± 22.3	1.9	<0.001
LDL (Mg/Dl)	75.8 ± 12.4	42.9 ± 6.76	6.7	<0.001
VLDL (Mg/Dl)	30.02 ± 11.5	24.9 ± 8.7	1.8	<0.001

Table 3. Serum hormone among POCS women in comparison with control

Hormone	Pocs	Control	T. Value	P.Value
FSH	3.6 ± 0.98	6.4 ± 1.5	8.9	<0.001**
LH (MIU/ML)	17.6 ± 2.4	13.3± 4.09	6.3	<0.001
Prolactin (NG/ML)	29.6 ± 6.3	17.8± 4.2	8.4	<0.001
Testosterone (NG/ML)	1.9 ± 0.87	0.87± 0.29	7.6	<0.001

Table 4. Serum level of DHEA in POCS women in comparison with control

Parameter	Groupd	Mean	P. Value
Dhea	Pocs No. (50)	188.7 ± 30.06	<0.001
	Control No. (50)	65.4 ± 20.2	

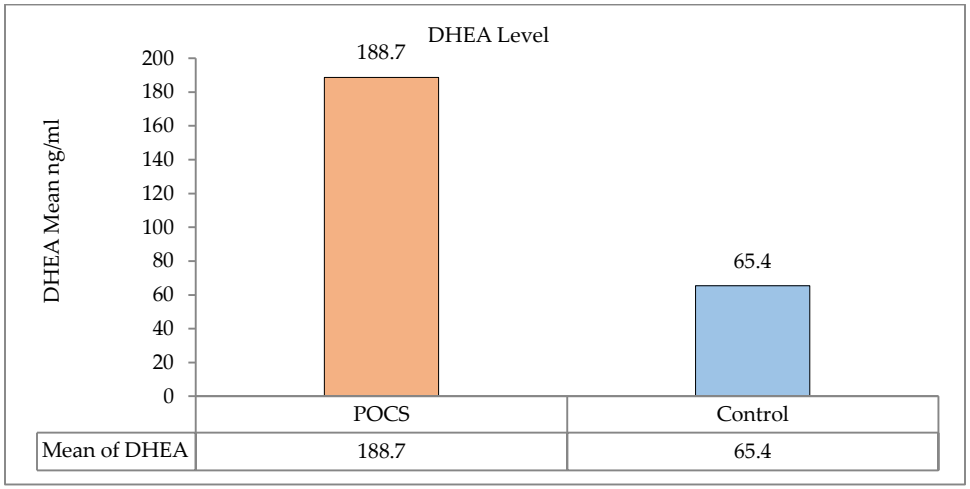

Fig. 1 Mean of DHEA level in POCS women and healthy control

Table 5. Serum level of inflammatory markers (IL6 and TNF- α) among POCS women in comparison with control

Inflammatory Markers	Pocs Patients	Control	Statistical Evaluation	
			T. Test	P. Value
IL6 (PG/ML)	88.9± 23.4	6.76± 2.3	18.12	<0.001**
TNF-A (NG/ML)	13.42± 2.3	4.52± 1.2	6.43	<0.001**

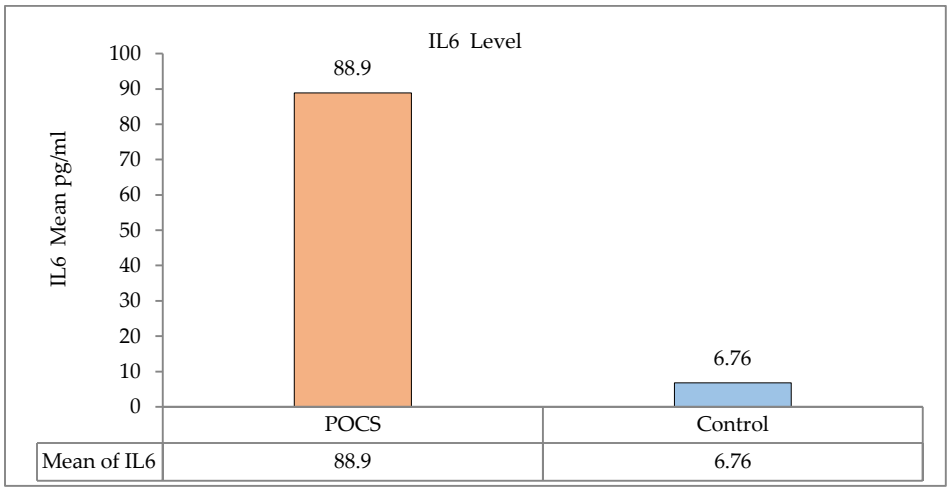

Fig. 2 Mean level of IL6 among POCS women and healthy women

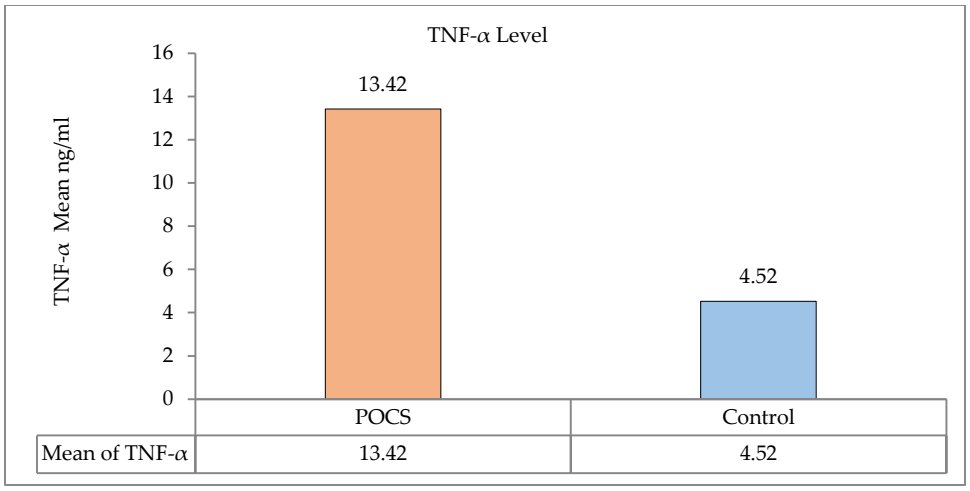

Fig. 3 Mean level of TNF-α among POCS women and healthy women

4. Discussion

Among the endocrine diseases that affect people the most often is PCOS. As a matter of fact, the importance of it is underscored by the fact that even among young, thin women with PCOS, low-level inflammation is possibly partially responsible for the increased incidence of heart-related illnesses and related mortality, which is a feature shared by the majority of these women and is associated with excess visceral/ectopic fat [7]. Fifty PCOS-afflicted women made up the study's participant sample. The mean age was 28.5 ± 5.4 years, which is in line with Mehde & Resan's (2014) findings [8] but lower than ELMekkawi et al.'s (2010) findings [9] and higher than Ateia et al.'s (2013) data [10] and Agacayak et al., [11] investigations. The fact that PCOS manifested at menarche and the females developed symptoms later; however, given that most women with polycystic ovarian syndrome are diagnosed between the ages of 20 and 30, it becomes sense that there would be similarities between these studies concerning the administration of the same age range. [12].

Women diagnosed with PCOS are often obese; data demonstrating changes in these variables during loss of weight suggests a connection between obesity and the hyperandrogenic condition and irregular menstruation associated with PCOS [13]. Increases in androgen hormones, adipose mass, and dyslipidemia are triggered in obese PCOS women [14]. BMI is lowered by low adiponectin levels, but testosterone, IR, and BMI can be raised by high leptin levels brought on by fat mass. Comparing PCOS women with obesity or overweight to control participants, they revealed higher levels of leptin and lower levels of adiponectin [15]. Variations in BMI can also be brought on by eating habits, lifestyle choices, and genetics [16]. In line with Kim et al. (2014) [17], the majority of women who suffer from PCOS do not engage in regular physical activity, despite the fact that food consumption and exercising are important components of PCOS therapy

There were notable variations in TC, TG, LDL cholesterol, HDL cholesterol, and VLDL cholesterol between PCOS patients and control subjects. Conversely, PCOS patients who showed higher TG, TC, and LDL cholesterol also had lower apoA-I and HDL cholesterol than the control group [18]. The hyperandrogenemia and hyperinsulinemia associated with PCOS may be the cause of dyslipidemia. This enabled the release of free fatty acids into the bloodstream and enhanced lipolysis of adipocytes brought on by catecholamines. Hypertriglyceridemia is brought on by increased VLDL production as a result of elevated free liver fatty acid content [19]. Reduced HDL cholesterol and elevation of LDL cholesterol are caused by hypertriglyceridemia via the reverse cholesterol transfer route. Adipocytes are predisposed to dyslipidemia linked to PCOS by additional androgenic priming during early life [20].

The disturbance of the normal gonadotrophin axis in PCOS women leads to a reversal of the LH/FSH ratio, with LH levels rising and FSH levels dropping [21]. Furthermore, the pituitary and brain exhibit reduced susceptibility to the inhibitory effects of exogenous progesterone on LH synthesis, and there is a scarcity of cyclic progesterone production by a corpus luteum in PCOS [22]. Modified synthesis of sex steroids, obesity, or metabolic disorders may all contribute to changes in LH secretion patterns. A significant increase in the numerical census of total and progressive pain is the cause of the growth in the duration of interleukin [23]. In the beginning, research on patients with polycystic ovaries revealed higher serum levels of prolactin. On the other hand, new research that used serial serum samples to rule out temporary prolactin spikes has revealed a less common correlation between both conditions [24].

The research of Lerchbaum et al. (2014) [25], the two main androgens that are raised in PCOS are testosterone and androstenedione. They concluded that whereas women with PCOS who have high androstenedione/free testosterone ratios have beneficial metabolic profiles, women with PCOS who have high levels of free testosterone have poor metabolic profiles. Severe stress triggers the blood to have high amounts of DHEA, which can affect ovarian, endocrine, and metabolic processes. Small quantities of circulating DHEA are also created and released by the testis and ovaries in a physiological manner [26]. Conversely, in hirsute PCOS women, the main source of androgen production is the ovaries rather than the adrenal glands [2]. Theca cell dysfunction in polycystic ovaries can explain this, which leads to overexpression of steroidogenic enzymes, especially CYP17A1, which increases the synthesis of DHEA [27]. A clinical manifestation of anxiety and depression is elevated DHEA, which is a reflection of psychosocial stress. Furthermore, an excess of androgens can induce a range of symptoms, from moderate ones like acne to severe ones like infertility, by interfering with the manufacture of feminine hormones [27].

The recent study also revealed elevated inflammatory markers such as TNF-α and IL6; however, the precise cause of inflammation in PCOS patients remains unclear. Excessive fat, a well-known proinflammatory condition, is highly common in women with PCOS and is linked to ongoing low-grade inflammation because of immune cells found in adipose tissue and hypertrophied adipocytes [28].

There doesn't seem to be any evidence supporting a hereditary basis for the chronic low-grade inflammation observed in PCOS patients. There has been limited research on the connection between proinflammatory genotypes, such as those that encode TNF-α, type 2 TNF receptor, and IL-6, and PCOS [29]. It has also been proposed that hyperandrogenism linked to PCOS may be the underlying cause of inflammation in adipose tissue. According to several research studies, adipocyte hypertrophy brought on by androgen compresses stromal arteries, resulting in tissue hypoxia, a condition known to set off inflammation [30].

Estimated serum TNF-α concentrations in six investigations revealed that PCOS women had higher serum TNF-α levels than healthy controls. But rather, when circulating IL-6 was examined in five different papers, the results showed that PCOS women had much greater levels than healthy controls [31]. A significant inflammatory environment associated with PCOS is highlighted by the presence of elevated TNF-α levels in these women, which may exacerbate insulin resistance and metabolic syndromes, increase androgen production, and exacerbate PCOS symptoms such as irregular menstruation and infertility [32]. Moreover, raised TNF levels in PCOS give rise to worries regarding the potential for enhanced cardiovascular risks in those with the condition. Given that PCOS is associated with chronic inflammation, which is a known risk factor for cardiovascular illnesses, treating the inflammatory aspect of the condition with a comprehensive strategy is imperative [33].

5. Conclusion and Future Studying

In order to determine the differences between women with the Polycystic Ovarian Syndrome (PCOS) and a control group of women without PCOS, the levels of Tumor Necrosis Factor-alpha (TNF-α), Interleukin-6 (IL-6), and Dehydroepiandrosterone (DHEA) were compared. The findings indicate that DHEA, IL-6, and TNF-α were

much greater in the PCOS group. The study reveals that elevated levels of DHEA, IL-6, and TNF-α are directly associated with PCOS. More studies on sizable patient samples are necessary to explore the underlying processes and identify suitable solutions for treating these biochemical abnormalities and improving the lives of women suffering from PCOS [34].

Authors' Contributions

Nibras Abbas AL-Mansouri and her team work conceived the idea and wrote the original draft of the manuscript, and the author reviewed and edited the final version.

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