Correlation of Immunological Variables of Interleukin-17 and Heat Shock Protein-70 In Ethnic Women with Polycystic Ovary Syndrome

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KEYWORDS

Immunological, Interleukin-17, Protein-70 In Ethnic Women

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a hormonal imbalance resulting from high levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in females, leading to immature eggs, ovarian cysts, irregular menstrual cycles, infertility, excessive facial hair growth, and acne. **Objective:** This study was conducted to follow up the therapeutic effect on some blood, hormonal, and immune variables of samples consisting of 35 patients with polycystic ovary syndrome (PCOS) from women suffering from infertility and infertility, and who visited Azadi Teaching Hospital and private laboratories in Kirkuk city, their ages ranged between (18 - 40 years). **Materials and Methods:** Sample collection and follow-up began by observing some clinical signs in addition to verifying the results of ultrasound examinations from early February 2023 to early November 2023. Each patient's examinations were followed up before treatment and three months after taking the treatment. The results we obtained after three months of treatment were compared with the results of the examinations of the patients in the pre-treatment stage and the control group. The treatment plan was based on modifying the patient's dietary lifestyle by following a healthy diet and giving treatment consisting of oral contraceptives, fertility drugs and the use of sugar-reducing drugs such as Glucophage or Metformin, in addition to nutritional supplements such as antioxidants. **Result:** The results of the therapeutic effect on the patients' diagnostic clinical signs showed a significant decrease in their percentages after three months of treatment compared to the pre-treatment stage such as (ovulation disorders - menstrual disorders - obesity - hirsutism - acne -). The results of the statistical analysis of the study showed that the therapeutic effect showed a significant difference in the body mass index (BMI) after three months of treatment compared to the pre-treatment stage and the control group. The therapeutic effect of the immune factors for each Hast Shock protein 70 and Interleukin-17 also showed a significant decrease at the level (0.000 > p) respectively after three months of taking the treatment compared to the pre-treatment stage and the control group. It was found that there is a significant positive correlation between the level of Heat Shock Protein 70 and the cellular kinetic IL-17.

1. Introduction

Polycystic ovary syndrome (PCOS) is a reproductive disorder of ovarian function in some women, associated with high levels of androgens, hyperinsulinemia, and chronic anovulation (1), but the cause is still unclear, as the endocrine system, reproductive system, and metabolism are affected by this syndrome and it is a major cause of infertility in women (2). One in every (5-6) women faces serious complications related to infertility and irregular menstrual cycles (3). This endocrine disorder affects women under the age of (18-44) years and affects (5-15%) of women worldwide (4). PCOS is characterized by many clinical symptoms such as diabetes, hyperinsulinemia, obesity, and coronary artery disease (5). The syndrome leads to disorders in fat distribution and cardiovascular diseases, which are one of the main causes of death worldwide (6). Heart diseases represent a group of disorders that affect the heart and blood vessels (7).

Polycystic ovary syndrome was first described by American pathologists Irvin Stein and Michael Leventhal in 1935, and was named Stein-Leventhal syndrome (8).

Heat shock proteins, including HSP70, have been studied in many cases due to their strong anti-apoptotic, anti-inflammatory, and antioxidant properties, and their regulatory role in gametogenesis and pregnancy (9), etc. Some studies have shown elevated HSP70 levels in the serum of non-obese PCOS patients (10).

The human interleukin-17A (IL-17A) gene is located on chromosome 6p12.2 and encodes IL-17A, a major pro-inflammatory cytokine. IL-17A is involved in regulating various immune functions, such as enhancing the production of other pro-inflammatory cytokines and cooperating with other cytokines. The cytokines IL-17A are produced by T-helper cells type 17. IL-17A is an important cytokines in

stimulating inflammation and autoimmune diseases. It affects the functions of neutrophils and macrophages and has the ability to enhance the effectiveness of T-helper cells type 1 to produce IL_2 and regulatory T cells to divide (11).

Aims of the study

Diagnosing the extent of the therapeutic effect on a group of clinical symptoms suffered by patients with polycystic ovary syndrome, such as menstrual disorders, body mass index (BMI), acne and hirsutism, after continuing treatment for three months compared to the pre-treatment stage and the control group, following up on the therapeutic effect followed for PCOS patients on some immune factors such as estimating the level of heat shock protein 70 and estimating the level of interleukin-17 after three months of taking treatment compared to the pre-treatment stage and the control group and studying the correlations between the variables studied above before treatment and three months after treatment for patients with polycystic ovary syndrome.

2. Methodology

Study site and sample collection:

The study samples were collected from the women's consultants and the infertility center at Azadi Teaching Hospital in Iraq, Kirkuk after diagnosing their condition with ultrasound and diagnosing the clinical signs of the syndrome in them, including menstrual disorders, infertility, hirsutism, weight gain and acne, according to a special questionnaire prepared for this purpose . The condition of each patient was followed up 3 months after treatment, and patients who had been treated previously were excluded. The ages of the patients ranged between (18_40) years. The sample collection and follow-up period took less than a year from (early February 2023 to November 2023). In addition to the patients' samples, blood samples were collected from healthy women who did not suffer from the above disorders, amounting to (20) blood samples, and each sample was subjected to a set of tests required to be followed up, which are blood, hormonal and immune variables.

Collection of blood samples:

Blood samples of 5 ml of venous blood were drawn after confirming that they had polycystic ovary syndrome during the follicular phase of the menstrual cycle in the morning. After that, 2 ml of blood were placed in a tube containing the anticoagulant EDTA for the purpose of measuring the total and differential count of white blood cells and measuring the percentage of hemoglobin in the blood, while 3 ml of blood were separated into a special tube of the Gel Tube type and left for a period of 10-15 minutes at room temperature, then centrifuged at a rate of 4000 rpm for 15 minutes to separate the blood serum, which represents the upper filtrate, using a micro pipette. After that, the serum was distributed into small Eppendorf tubes and several replicates were kept for each sample to prevent repeated dissolution. Then the samples were kept at a temperature of 20 Celsius for the purpose of conducting other serological and hormonal tests. Height and Weight

The height of the selected PCOS patients was measured using a centimeter scale (cm), and their weight was determined using a peraperson scale in kilograms (kg). Body mass index (BIM) The BIM was calculated by dividing the body weight in kilograms by the square of the height in meters, where patients are considered obese by knowing their BMI if it is greater than 30 kilograms per square meter.

Equation: BMI = weight (kg) / square of height (meter)

The concentration of immune variables in the blood serum of all women with polycystic ovary syndrome and healthy women was measured by following the steps accompanying the ready-made kit imported from the American company (USCN) for the ELISA.

3. Result and Discussion

After a 3-month treatment period, the results showed a significant improvement in the size of the eggs by 50% and the disappearance of a large number of cysts from the ovaries, indicating the effectiveness

of the treatment plan followed. The percentage of the study samples was 65.70% within the age range of (18-30) years, while it was 32.20% within the age range of (31-40) years, with 80% married and 20% unmarried, and 40% of them had type 2 diabetes. Figure (1) shows the percentages of women included in this study in terms of age, diabetes and marital status.

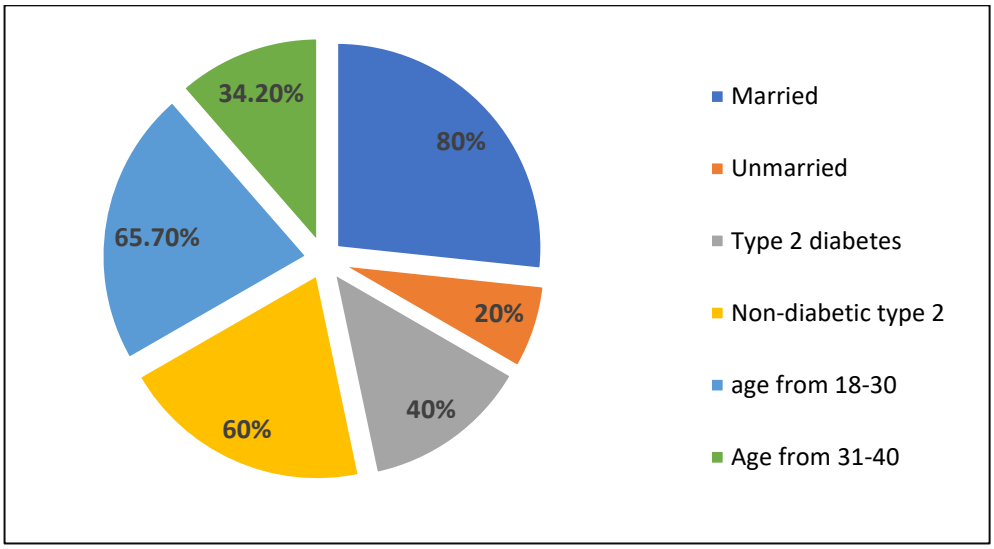


Figure (1): shows the percentage of each of age, diabetes and marital status.

Body mass index (BMI) measurement for women with polycystic ovary syndrome The results of the current study recorded a significant increase (p>0.000) in the BMI level in women with polycystic ovary syndrome before treatment (0.38±28.09) and after 3 months of treatment (0.30±26.40). The prevalence of obesity is increasing by about 40% among women worldwide. Women are more susceptible to weight gain due to excessive intake of carbohydrates, trans fats, sugars and lack of activity. Research evidence shows that 25% of overweight women may become obese, while excess weight and obesity are risk factors; Because it affects many systems including metabolism and hormones (12), weight gain and high body mass are among the most important apparent signs that indicate PCOS. Obesity plays a fundamental role in the reproductive functional changes that are linked to each other and are among the most important characteristics of PCOS. Rapid changes in body composition and fat amount should be monitored during the diet and exercise program. It is also noted that proteins have an effect on weight gain, as do carbohydrates, because they all add calories to the total number of calories in the body, which leads to an increase in body weight due to fat accumulation (13).

Table (1**):** Body mass index in women with polycystic ovary syndrome before and after treatment for 3 months.

Variables studied	**Number**	**Pre-treatment**	**After-treatment**	***P*_ Value**
Mean ± St. Error				
BMI	35	28.09±0.38	26.40±0.30	0.000

Therapeutic effect on diagnostic clinical signs of PCOS patients:

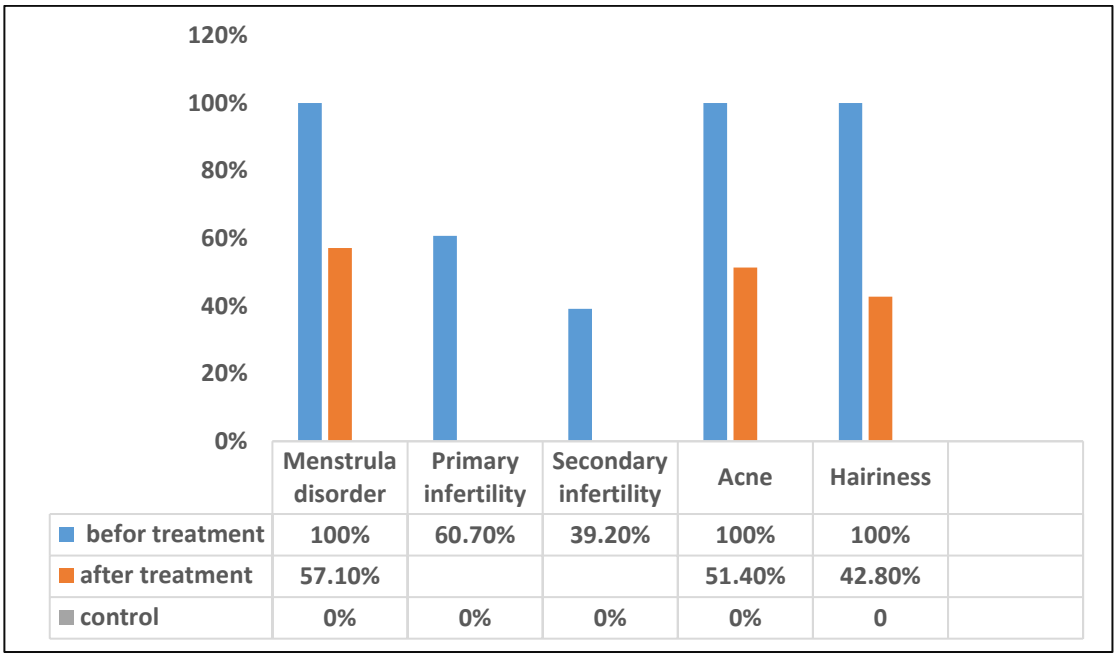
The percentages of therapeutic effect on diagnostic clinical signs of PCOS patients after three months of taking the treatment are shown compared to the pre-treatment stage and the control group, as shown in the figure

Hirsutism: In this study, we found that the percentage of hirsutism in the group of women with PCOS syndrome was very high, reaching (100%). Many studies agreed with research conducted by (14 and

15) that polycystic ovary syndrome is the primary cause of hirsutism, which is hair growth in areas that depend on androgen. Another study agreed with our results that polycystic ovary syndrome is the main cause of hirsutism by 72% to 82% (16). Another study agreed with our results (17) and (18).

Acne: In this study, we found that the percentage of acne in women with polycystic ovary syndrome was very high, reaching (100%) compared to the control group. This result was consistent with other studies (19), (20), and (21). Androgens affect the appearance of acne in women with this syndrome. Acne consists of black pimples, as a result of the accumulation of epithelial cell debris and the accumulation of fatty materials, which are colonized by Propionibacterium acnes bacteria. Inflammation of these pimples leads to the formation of nodules. The increase in androgens leads to the exacerbation of this process and the increase in the production of fatty materials by the sebaceous units. In addition, many women with polycystic ovary syndrome do not suffer from acne. These differences may be due to differences in androgen sensitivity (22).

Menstrual disorders: The study showed that the percentage of menstrual disorders in women with polycystic ovary syndrome was very high, reaching (100%). Disturbances in hormonal levels play the largest role in disorders. Menstrual cycle and increased menstrual disorders can affect fertility and this is due to the imbalance in the hypothalamic-pituitary axis which leads to an increase in the level of LH hormone, which causes an increase in the level of androgenic hormones (male hormone) above the normal limit, causing menstrual cycle disorders and irregular menstruation oligomenorrhea or absence of menstruation Amenorrhea, and thus leads to a disorder in the ovulation process and then infertility (23). Figure (2) showed a significant decrease in the percentage of hirsutism, which reached 42.80%, acne 51.40%, and menstrual cycle disorders 57.10% after taking the treatment for three months, meaning that anti-androgens and metformin played a major role in improving the patients' condition in terms of clinical symptoms represented by hirsutism, acne, and menstrual cycle disorders.



	Menstrula disorder	Primary infertility	Secondary infertility	Acne	Hairiness	
befor treatment	100%	60.70%	39.20%	100%	100%	
after treatment	57.10%			51.40%	42.80%	
control	0%	0%	0%	0%	0	

Figure (2): shows the percentages of the therapeutic effect on the diagnostic clinical signs of PCOS patients before and 3 months after treatment.

The studied variables of the control group and the patient group before and after treatment:

The effect of heat shock protein 70 in women with PCOS: The results of the current study recorded a significant increase ($p>0.000$) in the level of heat shock protein 70 in women with this syndrome before treatment (105.14±6.37 ng/ml), and after treatment (70.00±4.47ng/ml) compared to the control group (38.91±2.00ng/ml) and that the significant increase is due to insulin resistance in PCOS patients, as (15) indicated a positive correlation between the fasting insulin level and insulin resistance with the

Hsp70 level in PCOS women, while (24) indicated an increase in its production and activation in cells under a variety of stresses, high temperature, hypoxia and inflammation in the ovary, and may lead to lower fertility in PCOS women. The increase in its level in the serum of PCOS women is also associated with an increased risk of metabolic disorders and their complications in increasing infertility due to the reduction of autophagy regulation (25) as shown in Table (2) that the therapeutic effect led to a decrease in the level of Hsp70 after three months of the patients taking their treatment compared to the pre-treatment stage and the control group, while the results of our study did not agree with the results of study (26) which showed that the level of Hsp70 in the serum was low in the ovarian tissues of women with polycystic ovary syndrome.

Table (2): Statistical analysis of HSP70 protein in women with polycystic ovary syndrome before and three months after treatment.

Immune Factor	**Pre-treatment**	**After-treatment**	**Control**	***P*-Value**
Mean ± St. Error				
Hsp 70(ng/ml)	a 105.14±6.37	b 70.00±4.47	c 38.91±2.00	0.000

Effect of IL-7 in women with polycystic ovary syndrome: The results shown in Table (3) show a significant increase in the level of cytokines 17 (0.000>p) in women with PCOS in the group before treatment for 3 months (7.37± 109.14 pg/ml) and after treatment (5.41±77.75 pg/ml) compared to the control group (28.79±2.84 pg/ml). This result is consistent with many studies, including studies (27) and (28) of cytokines IL-17, which is one of the proinflammatory cytokines and contributes to regulating various immune functions, including stimulating the production of other proinflammatory cytokines. It was found that it contains a single polymorphism of the gene rs2275913, which contributes to inhibiting the nuclear factors of activated T cells, and thus the level of IL-17 increases, and its significant increase leads to major disturbances in the levels of Hormones and the appearance of pathological symptoms and this was confirmed by the researcher who pointed out that the cytokinesis interleukin 17 is considered a diagnostic marker for PCOS (29) and that the therapeutic effect led to a significant decrease in IL-17 after three months of the patients taking their treatment compared to the pre-treatment stage.

Table (3): Statistical analysis of cytokinesis IL-7 in women with polycystic ovary syndrome before and three months after treatment.

Immune Factor	**Pre-treatment**	**After-treatment**	**Control**	***P*-Value**
Mean ± St. Error				
IL-7 (pg/ml)	a 7.37± 109.14	b 5.41±77.75	c 28.79±2.84	0.000

4. Conclusion and future scope

The results of our current study and through the therapeutic follow-up of patients achieved the following conclusions: A clear improvement in the clinical diagnostic signs that patients with PCOS suffered from (ovulation disorders, menstrual disorders, obesity, hirsutism, acne, body mass index) after 3 months of taking continuous treatment, the therapeutic effect played a major role in reducing the level of immune factors Interleukin17 and heat shock proteins 70 and there is a high positive significant correlation between the level of heat shock protein 70 and the cytokines IL-17.

Recommendations

Study the syndrome in unmarried women, especially those aged between (16-25) years, and prepare educational and research plans in high schools and universities about this syndrome to reduce the risks of neglecting it. Study on other immune variables and their relationship to the syndrome. Study on large samples and follow-up treatment for periods of more than three months and their relationship to

immune indicators. Study on the specific genes responsible for polycystic ovary syndrome.

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