Comparative study of the levels of some biochemical and immunological variables between patients with polycystic ovarian syndrome with and without insulin resistance

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Abstract

This study aimed to determine the changes occurring in some immune variables in women suffering from polycystic ovary syndrome in Tikrit City to study the effect of this elevation on the emergence and development of the disease. These variables included (Adeponectin, resistin, Beta klotho, and Endophilin A2). The study included non-infected women as the control group, women having polycystic ovarian syndrome who do not have insulin resistance, as well as those who have the disease but are insulin resistant. All the methodology of the immunological variables was done by ELIZA technic according to the instructor of the company leaflet. According to the findings, there were no significant changes ($p<0.05$), in adiponectin levels between the control group and the polycystic ovarian group with and without insulin resistance. the results indicated a significant increase in the level of Resistin in the blood serum of the patient's group (group 2 and grpup3) compared with the control group, there were no significant differences in the level of Beta Klotho and Endophilin A2 mean in blood serum between the control group and the other groups of patients ($p<0.05$). It concluded from this study the most variable that can be affected by polycystic ovarian syndrome disease is resistin which increases in the patient groups Resistin plays an important role in polycystic ovary syndrome (PCOS) due to its association with insulin resistance in women with polycystic ovary syndrome, resistin is associated with androgens, especially testosterone, Therefore, polycystic ovarian syndrome increases both.
Keywords: Adiponectin, Endophilin A2, Polycystic ovary syndrome, resistin.

Introduction

One of the complicated conditions affecting the ovaries is a polycystic ovarian syndrome, which is brought on by a combination of environmental and genetic factors (Fatima et al. 2019). In it, the natural ovulation process is disturbed due to a hormonal imbalance in the body, and it is accompanied by several symptoms that appear together in the patient, so it is called polycystic ovary syndrome. These symptoms are most common in adolescence, during the onset of the menstrual cycle (Ismayilova and Yaya 2022).

One prevalent cause of anovulation and infertility is this syndrome. Ovarian follicles contain eggs, but in women with PCOS, the ovary does not produce enough hormones to mature the eggs, so follicles or follicles remain and accumulate in the ovary, and thus ovulation is prevented. In addition, the ovaries tend to produce high levels of androgens. Therefore, it negatively affects ovulation and fertility (Branfield 2019), It has been found that 35-50% of women with this syndrome are overweight. Polycystic ovary syndrome can be detected and diagnosed through an ultrasound examination. Ovarian enlargement can be observed, meaning it is larger than the normal size, and several eggs of up to 14 or more small-sized eggs can be seen. This condition does not apply to women who take birth control pills. Because the main function of these pills is to reduce the size of the egg (Ehrmann 2005; Ortiz-Flores, Luque-Ramírez, and Escobar-Morreale 2019).

Adiponectin is a lipoprotein (lipocytokine) or protein hormone consisting of 224 amino acids that is produced exclusively by adipose tissue in the body. It is symbolized by APN and is also known as Acrp 30 or Adipo Q (Thornton, Von Wald, and Hansen 2015).

It has various biological functions and effects, including regulating the level of glucose and fatty acids in the blood, as it has an effective role in suppressing the production of glucose in the liver and stimulating its absorption and catabolism in the liver and muscles. Adiponectin (APN) stimulates the rapid oxidation of fatty acids in skeletal muscle and thus inhibits their synthesis. Adiponectin binds to three receptors: T-cadherin, AdipoR1, and AdipoR2 (Shorakae et al. 2018).

Resistin is a polypeptide protein consisting of 94 amino acids and rich in cysteine. It is expressed by the RETN gene in humans. In mice, it is derived from white adipose tissue, while in pigs it is secreted from immune and epithelial cells. Resistin levels increase in obese people and decrease with anti-diabetic treatments. This proves that resistance has a strong relationship with insulin resistance (IR) and type 2 diabetes (T2DM) (Steppan et al. 2001). Resistin is produced in humans by mononuclear cells found in the peripheral circulation. Resistin interacts with lipopolysaccharide when binding with the Toll-like receptor 4, thus contributing to anti-inflammatory activities.

Resistin is found in humans largely in Macrophages and stromal cells (connective tissue cells in any organ) within fat cells (Raeisi et al. 2021).

Beta klotho is a protein or hormone that is encoded by the klotho gene. It was discovered by a scientist named Makotokoro in 1997. This hormone is known as an anti-aging hormone, in addition to other activities, which are anti-oxidative and anti-inflammatory (Bednarska, Fryczak, and Siejka 2020).

This protein can be found in two different forms: soluble secreted form and membrane-bound form. When the klotho gene is fully transcribed and translated into a protein, the membrane-bound form is created; however, when the gene is clipped to eliminate the membrane region, the soluble secreted form is created. According to (Hua et al. 2021) this protein can be classified into three subfamilies: alpha, beta, and gamma, based on how the gene is cut. The most common form of this protein is the soluble alpha klotho.

Living cells need proteins and large molecules of food to perform their vital functions, which cannot be entered through the bilayer plasma membrane or special transport channels located through the membrane. Therefore, these molecules are entered through the process of endocytosi (Lin et al. 2022).

Recently, the awareness and understanding of the process of endocytosis has been expanded by identifying two pathways that endophilin A2 has an important role in regulating (Malinova et al. 2020). They are the clathrin-independent endocytic route, which is called clathrin-dependent fast endophilin-mediated endocytosis, and the other is clathrin-dependent endocytosis. -dependent endocytic, Endophilin, a crucial member of the BAR domain protein superfamily of membrane trafficking proteins, is required for the FEME pathway to function (Kjaerulff, Brodin, and Jung 2011).

Martial and methods

The study design

This study was carried out on several women with polycystic ovary syndrome, and women who suffer from both polycystic ovary syndrome and insulin resistance who visited Salah al-Din General Hospital and some outpatient women's clinics in the city of Tikrit during the period from "April 2022 to September 2022", Diseases were diagnosed through laboratory tests, monitoring of some clinical symptoms, and ultrasound examination by physician, in addition to collecting information for each group according to the questionnaire, thus selecting the appropriate cases for

the subject of the study. 90 blood samples were collected from women aged between 20-50 years. The studied samples were distributed into two main groups, as follows:

1. Control group

It included 14 blood samples from healthy women who did not suffer from any diseases

2. Patients Group

It has been distributed into two groups:

A- The first group of patients: It included 26 blood samples from women suffering from polycystic ovary syndrome (PCOS).

B_The second group of patients: It included 24 blood samples from women who suffering from polycystic ovarian syndrome and insulin resistance together.

The blood was collected using a plastic syringe, 5 ml of blood was collected, and placed in gelatin tubes with a cap. It is airtight and free of EDTA. The blood is left for (15 minutes) at room temperature until it coagulates. After that, it is centrifuged for 10 minutes at a speed of 3000 rpm. Then the blood serum is withdrawn using a micropipette and placed in a test tube, then stored (-20°C) until the required tests are carried out.

Determine of immunological and biochemical variables

Results

The results in Table (1) showed that there were no significant differences($p<0.05$), in adiponectin level between the control group, and the two groups of patients: the second group (women with polycystic Ovarian syndrome ,and for the third group (those with polycystic ovary syndrome and insulin resistance together) these results agree with (Beyazit et al. 2021; Cardoso et al. 2020), while the results of the current study did not agree with (Echiburú et al. 2018; Shirazi, Khodamoradi, and Jeddi 2021) who indicate a decrease in adiponectin level in the three groups of patients.

The variables / The groups	Adeponectin ng/ml	Resistin ng/ml	Beta klotho ng/m	Endophilin A2 ng/m
	Mean ±SD			
Control (first group)	a 7,606±1,534	b 4,134±1,066	a 13,65±2,810	a 43,84±6,49
Women with polycystic ovary syndrome (Group 2)	a 7,752±1,150	a 5,310±1,191	a 16,54±1,168	a 45,10±7,90
Women having polycystic ovary syndrome in addition to insulin resistance (Group 3)	a 7,924±1,202	a	a 16,58±2,030	a 45,89±6,84

		5,008±0,92 2		

The findings, as presented in Table (1), demonstrated that there were notable variations in the blood serum concentrations of resistin between the patient and control groups :the second group (women having PCOS) and the third group (women having PCOS and insulin resistance together), as these groups showed an increase Significantly compared to healthy women, while there are no significant differences between the two groups of patients at a significant level (p<0.05). The results agree with the findings of earlier research (Raeisi et al. 2021) (Moustafa et al, 2020), which indicate an increase in the level of resistin within patient groups.

The results, as shown in Table (1), showed that there were no significant differences in the concentrations of Beta KLotho in blood serum between the control group and the other groups of patients (p<0.05).

These results differ with the researcher (Bednarsk et al, 2020), who indicates that Beta KLotho concentrations show an increase in groups of patients suffering from type 2 diabetes and polycystic ovarian syndrome, as measuring Beta KLotho levels can be useful in diagnosing polycystic ovarian syndrome, as opposed to its decrease. It allows for normal pregnancy and normal ovarian function, thus reducing the incidence of ovarian cysts (Zeng et al. 2023).

The current study's results showed that there were no significant differences in the concentrations of Endophilin A2 in blood serum between the control group and the other two patients groups at a significant level (p≤0.05).

Discussion

Hyperandrogenism associated with polycystic ovarian syndrome, which has an inhibitory effect on adiponectin. The hormone testosterone inhibits the production of adiponectin, in addition to the low gene expression of the adiponectin receptors Adipo R1 and R2 (Dos Santos et al. 2012). Adipocytes produce adiponectin, which is involved in several clinical and psychological processes such as inflammation, atherosclerosis, metabolic syndrome, and fat metabolism (Behboudi-Gandevani et al. 2017).

One adipocytokine linked to the pathophysiology of polycystic ovarian syndrome (PCOS) is adiponectin. According to (Talat et al. 2021), adiponectin possesses anti-inflammatory, anti-diabetic, anti-atherogenic, and insulin-sensitizing effects. It is also negatively correlated with

obesity in healthy individuals. The effects of adiponectin regulation on the synthesis and formation of steroidogenesis is associated with the PCOS pathogenesis (Lagaly et al. 2008).

Since adiponectin stops glucose production in the liver and enhances the oxidation of fatty acids in skeletal muscle, it has a preventive role in protecting against diabetes, especially type 2. It acts to inhibit cell death, inhibit inflammation, and enhance cell survival (Adya, Tan, and Randeva 2015).

It was found that obese women who suffer from polycystic ovary syndrome show a decrease in adiponectin levels less than its levels in non-obese women who suffer from the same disease. Adiponectin has anti-inflammatory activity, anti-atherosclerosis properties, and anti-coronary artery disease, and it protects against insulin resistance (Pekcan et al. 2019).

An increase in the level of resistin within patient groups, and it was interpreted that the mRNA levels of resistin show an increase Production by thiazolidinediones medicine antidiabetic drugs, which may linked with the peroxisome proliferator-activated receptor -g (PRAR-g) in fat cells.

The expression of the resistin gene in adipocytes is pathologically higher in obese people than in thin people (Raeisi et al. 2021; Zhang et al. 2011). Because resistin is associated with insulin resistance in women with polycystic ovarian syndrome (PCOS), it plays a significant role in the condition. Resistin is associated with androgens, especially testosterone, so both are increased in polycystic ovary syndrome, where it overlaps with PCOS and its obvious clinical symptoms (Yilmaz et al. 2009).

(Escobar-Morreale 2018) indicated that the levels of resistin in the serum of obese women are higher than those of non-obese women, and this may explain its relationship to polycystic ovarian syndrome women which already have obesity. (Munir et al. 2005) discovered that there is an increase in resistin levels in women with polycystic ovary syndrome, in addition to a positive relationship between resistin, body mass, and testosterone (Raeisi et al. 2021).

Beta KLotho is an essential component of the (α-β)-Klotho-FGFRs Complex, which is a major key to activating the factors FGF19 and FGF21 after its association with them to stimulate the signaling chain in order to maintain metabolic processes, especially glucose metabolism and energy release, in addition to its antidiabetic functions and Anti-adepogenic, FGF21 suppresses appetite and improves insulin sensitivity (Bednarska, Fryczak, and Siejka 2020).

In performing endophilin's functions and effectiveness, depends on two functional domains: the BAR domain (Bin -amphiphysin -Rvs) N terminal, which has a curved, concave shape resembling a banana shaped that mediates the curvature of the membrane and the double protein, and the

second site is the SH3 C terminal (Src homology3) Which binds to proline-rich regions (PRDs) in the target protein (Kato, Takayama, and Sunagawa 2021; Liu et al. 2020).

Conclusion

It was concluded from this study the most variable can be affected by polycystic ovarian syndrome dieses is the resistin which increase in the patients groups Resistin plays an important role in polycystic ovary syndrome (PCOS) due to its association with insulin resistance in women with polycystic ovary syndrome, resistin is associated with androgens, especially testosterone, so both are increased in polycystic ovary syndrome.

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