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Evaluation of the Role of some Myokines and Tumor Necrosis Factor-Alpha in Women with Polycystic Ovary Syndrome in Mosul City-Iraq

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ABSTRACT

Polycystic ovary syndrome (PCOS) is considered one of the endocrine disorders common in females of reproductive age. The study aims to evaluate the role of some myokines (hormones follistatin and irisin) among the causes of infection, tumor necrosis factor-alpha (TNF-α) is one of the inflammatory cytokines and is considered a primary indicator of inflammation in women infected with PCOS. The study included 75 blood samples, drawn from 50 married women (25 primary infertility, 25 secondary infertility) and 25 single women infected with PCOS, whose ages ranged between (15-45) years, collected from Al-Batoul Teaching Hospital in Nineveh Governorate, after they were diagnosed by obstetricians, gynecologists and infertility doctors. As well as 50 blood samples from women (25 married and 25 single) who were healthy and did not infect with PCOS and of the same ages were considered as a control group. Determination of follistatin, irisin and TNF-α in serum using kits based on the ELISA-sandwich principle.

The results showed a significant increase in follistatin, irisin and TNF-α levels in serum of both married and single women infected with PCOS, and in infected married women with primary and secondary infertility compared with the control. We conclude that follistatin and TNF-α are linked to the inflammatory state of women infected with PCOS, and irisin can be considered a biomarker to detect this syndrome.

Keywords: Polycystic ovary syndrom, myokines, follistatin, irisin, TNF-α.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is considered one of the endocrine disorders common in females of reproductive age with systemic metabolic manifestations and neuroendocrine-immune disorders. It is a disorder closely associated with chronic anovulation and is the leading cause of ovulatory infertility nowadays (Collée *et al*., 2021; McDonnell *et al*., 2022).

Muscles are among the largest soft tissues in the musculoskeletal system, which are basic tissues active in the metabolic process; it is the main site for metabolizing carbohydrates and fats to produce energy (Szentesi *et al.*, 2021). Skeletal muscle tissues have been associated with the production and secretion of active factors called myokines (de Oliveira dos Santos *et al*., 2021), which are cytokines or peptides that are synthesized and secreted by muscles in response to muscle contractions and exert autocrine, paracrine and endocrine effects, it represents a basic mechanism for regulating the body's balance, and it also participates in tissue regeneration and repair, maintaining healthy physical performance, immune modulation, cell signaling, expression, and symmetry (McDonnell *et al*., 2022).

One of the myokine hormones, Follistatin (FS), belongs to the transforming growth factors -β superfamily, it consists of one chain of glycosylated polypeptides, as it works as an important regulator of follicle development, a high level of FS inhibits the role of stimulating and secreting the hormone FSH, as FSH is important, it is very important for folliculogenesis, and its low level may stop follicle growth and increase androgen production in the ovary, both of which are characteristics of PCOS (Raeisi *et al*., 2021). It has recently been found that FS has anti-inflammatory activity, and in addition it has been linked to high-sensitivity C-reactive protein (hsCRP) as a marker of the inflammatory process in PCOS: It was found that women infected with PCOS have a higher level of hsCRP in the blood compared to healthy women, this indicates that females with this syndrome can have more severe of low grade inflammation, resulting in higher blood follistatin levels being associated with hsCRP level in females with PCOS (Elaalem *et al.*, 2023).

Irisin is also a muscle kinetics protein hormone, secreted mainly from skeletal muscles after exercise, to stimulate the transformation of white adipose tissue into brown adipose tissue, which leads to increased heat generation (thermogenic protein) and energy consumption (Cao and Hu, 2021). It is believed to play a role in reducing the amount of fat, irisin acts as a messenger from skeletal muscle to other parts of the body, and differentiation of bone cells, regeneration of neurons and B cells, many studies have indicated that the level of Irisin in the blood is associated with several of metabolic diseases characterized by insulin resistance (Bayraktar *et al.*, 2020).

Tumor necrosis factor-alpha (TNF-α) is an inflammatory cytokine, mainly produced by T lymphocytes, macrophages, natural killer cells, neurons, neutrophils and eosinophils, it has multiple effects on different types of cells, as it is a major regulator of inflammatory responses (Graza-Graza and Delgadillo-Guzman, 2020). It has an important functional role, as it stimulates the release of a series of different stimulating inflammatory molecules before other adipokines, in ovaries of females, tumor necrosis factor-alpha controls granulosa cell production, growth of follicle, ovulation, fertilization, implantation, steroid generation and prostaglandin production, It has been observed that TNF-α reduces FSH-induced estradiol production in immature follicles in the human ovary, TNF-α pathways can be related to problems in metabolic syndrome and steroidogenesis of ovarian. TNF-α has been bound to premature ovarian failure, a characteristic of PCOS (Azeez *et al.*, 2021).

This study aimed to evaluate the role of some muscle kinetics (follistatin and irisin) and TNF-α in the serum of females with polycystic ovary syndrome. It included married women (primary infertility and secondary infertility), and single women compared with Control (healthy women) in Mosul city to determine the extent of its influence and impact.

MATERIALS AND METHODS

Chemical materials used

This study used ready-made test kits from different international companies to estimate some myokines (follistatin, irisin) and TNF-α, which were supplied by the Chinese company BT-LAB.

Location and duration of study

The current study was conducted on women infected with PCOS visiting Al-Batoul Teaching Hospital for obstetrics and gynecology and some private laboratories in the city of Mosul for the period from January to August (2023). The study included 75 blood samples from women infected with PCOS (single and married) whose ages ranged between (15-45) years after they were diagnosed by female doctors specializing in obstetrics, gynecology, and infertility based on ultrasound diagnosis and clinical examination in addition to biochemical examinations according to a questionnaire form prepared. For this purpose, it was divided into 3 groups. The first group included 25 blood samples from married women infected with PCOS and those with primary infertility (who did not have children). The second group included 25 blood samples from married women infected with PCOS and those with secondary infertility (who had children). In contrast, the third group included 25 blood samples from unmarried (single) women infected with PCOS. The study also included 50 blood samples from women (25 married and 25 single) who were healthy, did not suffer from PCOS, did not suffer from any fertility problems or chronic diseases, had regular menstrual cycles, and were of the same ages, and were considered a control group.

Collect and preserve blood samples

Blood samples were collected by drawing 5 ml of venous blood, and placed in a jell tube free of any anticoagulant with clean and sterile. Leave it for 20 min. at room temperature until the blood coagulated. The blood is then separated using a centrifuge at 3000 rpm for 15 min. to obtain serum. The serum was placed in an Eppendorf tube and stored at (-18 to -20)°C in the freezer until the required tests were performed.

Statistical analysis

The results in this study were statistically analyzed by using a complete randomized design (C.R.D.), and the differences between two groups of sick and healthy women were determined using a (T-test) for the variables studied, while the differences between the groups of married women patients (with primary and secondary infertility), single women and control women were determined using duncun's multiple range test for the variables studied at the probability level ($p \leq 0.01$), which was considered a significant difference, using the ready-made SDS statistical program to find the mean ± standard error.

RESULTS AND DISCUSSION

The results in (Table 1) showed a significant increase at the probability level ($P \leq 0.01$) of hormone follistatin level in the serum of women infected with PCOS by 31% compared with the control.

Table 1: Concentration of some myokines and tumor necrosis factor – alpha (TNF- α) in the serum of infected woman with PCOS compared to control.

Studied groups / Variables	Control			Patients		
	#Mean±SE	%Conc.	%Increase	Mean±SE	%Conc.	%Increase
Follistatin (ng/ml)	319.18 ± 3.52	100	-	418.53 ± 4.53**	131	+31
Irisin (ng/ml)	6.62 ± 0.84	100	-	9.05 ± 1.75**	137	+37
TNF- α (ng/ml)	110.81 ± 6.88	100	-	138.55 ± 9.88**	125	+25

The numbers followed the sign (**) indicate a significant difference at ($p \leq 0.01$).

As well as the data in (Table 2) demonstrated that the level of follistatin in the serum of single women infected with PCOS, that a significant increase of 22%, while the percentage of increase in the serum of women infected with PCOS who were married was 34% compared with control, and the results in the (Table 3) showed a significant increase in the level of follistatin in serum of women infected with PCOS who were married and had primary infertility by 42%, while the rate of increase in serum of infected married women with secondary infertility was 26% compared with control.

Table 2: Concentration of some myokines and tumor necrosis factor – alpha (TNF–α) in the serum of infected single and married woman with PCOS compared to control.

Studied groups / Variables	Single control			Single patients			Married control			Married patients		
	#Mean ±SE	%Conc.	%Increase	#Mean±SE	%Conc.	%Increase	#Mean ± SE	%Conc.	%Increase	#Mean± standard error	%Conc.	%Increase
Follistatin (ng/ml)	319.52± 3.01b	100	-	388.74 ± 2.74a	122	+22	318.94 ± 4.11b	100	-	425.97 ± 4.63a	134	+34
Irisin (ng/ml)	6.89±0. 84b	100	-	8.69 ± 0.95a	126	+26	6.40 ± 0.84 b	100	-	9.14 ± 1.90a	143	+43
TNF- α (ng/ml)	112.30± 7.98b	100	-	136.09 ± 1.20a	121	+21	109.61 ± 6.55b	100	-	138.91 ± 9.75a	128	+28

The numbers followed by different letters horizontally indicate a significant difference at ($p \leq 0.01$) according to Duncan's Test.

Table 3: Concentration of some myokines and tumor necrosis factor – alpha (TNF – α) in the serum of primary and secondary infertility woman with PCOS compared to control.

Studied groups / Variables	Control			Primary infertility			Secondary infertility		
	# Mean±SE	%Conc.	%Increase	Mean±SE	%Conc.	%Increase	Mean±SE	%Conc.	%Increase
Follistatin (ng/ml)	318.94 ±4.11c	100	-	451.64±4.36a	142	+42	400.32±3.42b	126	+26
Irisin (ng/ml)	6.40 ± 0.84c	100	-	9.97±2.29a	156	+56	8.42±1.18b	132	+32
TNF- α (ng/ml)	109.62 ± 6.55b	100	-	142.44±9.27a	130	+30	135.65± 9.32a	124	+24

The numbers followed by different letters horizontally indicate a significant difference at ($p \leq 0.01$) according to Duncan's Test.

The results of the current study are consistent with the findings reached by (Raeisi *et al*., 2021; Elaalem *et al.*, 2023). Follistatin is a protein associated with activin and works to disrupt the function of activin *in vivo* and *in vitro* experiments, since follistatin prevents the action of activin. Changing the action of follistatin may be a possible means of influencing the growth of ovarian follicle, FSH release, insulin secretion and androgen synthesis. It has been shown that all of these events interfere with PCOS association with activin and affect to its action, that is stimulating the FSH hormone. thus, which may modify the function of follicle cells in an autocrine manner by linking and neutralizing the action of activin. Therefore, it is expected that overexpression of follistatin or an increase in its activity of functional will lead to a halt in follicle development, an increase in the production of ovarian androgen, and a reduction in circulating FSH levels, these are the distinguishing for PCOS syndrome (Raeisi *et al.,* 2021).

They noted (Elaalem *et al.,* 2023) that blood levels of follistatin are much higher among women infected with PCOS and those with fertility problems, regardless of age and body mass index (BMI). The sources of follistatin hormone in women are unknown, however this hormone is released from the cells. Granuloma in the ovary in response to FSH stimulation and increased

expression of follistatin is considered a possible mechanism through which follicle growth is inhibited. These results indicate that changes in the secretion of follistatin from the ovary or other organs may be related to pathophysiological disorders of PCOS. Higher levels of follistatin are associated with decreased pre-ovulatory follicle development in PCOS, therefore, modulating these hormones can be therapeutic targets for new drugs in women infected with PCOS (Raeisi *et al.,* 2021).

Recent studies have shown evidence indicating the participation of follistatin in anti-inflammatory processes. Therefore, high follistatin levels in the blood of women infected with PCOS may be a compensatory response to chronic low-grade inflammatory state independent of insulin resistance and obesity (de Oliveira dos Santos *et al*., 2021).

The results in (Table 1) showed a significant increase in the level of hormone irisin, as the percentage of increase reached 37% in the serum of women infected with PCOS compared with control. The results in (Table 2) showed also a significant increase in level of irisin in serum of single women infected with PCOS increased by 26%, while the percentage of increase in serum of women infected with PCOS who were married was 43% compared with control, while the results in (Table 3) also showed a significant increase in level of irisin in serum of women infected with PCOS who were married and who were they had a rate of primary infertility of 56%, while the rate of increase in the serum of married infected women who had secondary infertility was 32% compared with control.

The results of the current study are consistent with the findings of (Sharan *et al*., 2021; Behboudi-Gandevani *et al.,* 2021) that there is a significant increase in level of irisin in the serum of women infected with PCOS compared with control. Irisin, a muscle-derived brown adipose differentiation factor, is identified as a cleavage and secreted product of the FNDC5 protein. Irisin can stimulate the transport of type 4 glucose (4-Glut) to plasma membrane and stimulate glucose uptake in differentiated muscle (Castillo-Quan, 2021). The higher level of irisin in the blood serum of women infected with PCOS is associated with increased energy production and improved diet-induced insulin resistance (Behboudi-Gandevani *et al.,* 2021).

There is no clear explanation for the increased levels of irisin in serum in women infected with PCOS, and this may be due to its association with the metabolic syndrome itself, which is linked to this syndrome, there is another opinion that attributes the reason for the high level of irisin in serum to protection in the pre-diabetic state of PCOS, before it develops into diabetes (Sharan *et al*., 2021). Elevated irisin levels in women infected with PCOS may be interpreted as a state of irisin resistance similar to insulin resistance, in which high circulating hormone levels fail to affect irisin level, or by suggesting that it acts as a protective factor, given the fact that irisin typically increases energy production in brown and beige adipose tissue in cases of PCOS as a defense system against excess energy flow (Mubarak and Ali, 2020). Abnormal changes in irisin may contribute to the development of risk factors that lead to increased incidence of diseases, such as insulin resistance, lipid profile disorder, and obesity in women infected with PCOS, it is expected that one of the main mechanisms behind the high irisin level of the hormone irisin in the serum of women infected with PCOS may be the secretion of irisin as a compensatory means to confront metabolic disorders such as insulin resistance in people who do not have diabetes (Masaeli *et al*., 2019).

The results in (Table 1) showed a significant increase in level of tumor necrosis factor-alpha (TNF-α), as the percentage of increase reached 25% in serum of patients with PCOS compared with control. The results in (Table 2) also showed a significant increase in level TNF-α in serum of single women infected with PCOS increased by 21%, while the percentage of increase in serum of married women infected with PCOS was 28% compared with control, while the results in (Table 3) showed a significant increase in the level of TNF-α in serum of women infected with PCOS. PCOS in married women with primary infertility increased by 30%, while the rate of increase in serum of infected women who were married with secondary infertility was 24% compared with control. The

results of the current study are consistent with the findings of (Azeez *et al.*, 2021; Atakul *et al.*, 2020; Vasyukova *et al.*, 2023).

TNF-α is a pro-inflammatory cytokine that plays a key role in mediating insulin resistance. In PCOS (polycystic ovary syndrome), a pro-inflammatory environment is created, which is further exacerbated by obesity. Both conditions interfere with insulin signalling pathways, leading to increased insulin resistance and contributing to metabolic syndrome (Vasyukova, *et al.,* 2023). Azeez *et al.,* (2021) reported elevated TNF-α levels in women with this syndrome, but it has not been confirmed what causes this increase, inflammation in this syndrome leads to the emergence of insulin resistance, and the level of TNF-α increases with increasing BMI in women infected with PCOS.

Evidence suggests that TNF-α is a contributing factor in biochemical and clinical disorders such as obesity and hyperandrogenism, chronic low-grade inflammation has been studied in women infected with PCOS, through the existing links between PCOS and changes in immune cells, in adipose tissue and ovaries. Macrophages are the most abundant immune cells, as they work to balance destructive and protective cell-mediated immunity in inflammation. Obesity is associated with increased levels of blood fats, including non-esterified fatty acids (NEEA). The inflammatory response may be due to lipotoxicity, which occurs when these acids accumulate in the blood, research indicates that obesity and a low-grade inflammatory state are two major factors that contribute to the development of PCOS (Vasyukova *et al.,* 2023).

The one mechanism of persistent low-grade inflammation in this syndrome is the hypertrophy of adipocytes associated with PCOS. It is known that adipocytes are prone to enlargement when exposed to excessive androgen stimulation, as is the case in women with this syndrome. Androgen excess and hypertrophy of adipocytes lead to compression of the stromal blood vessels which leads to decreased blood flow in adipose tissue (Atakul *et al.*, 2020). As a result of hypoxia, it stimulates the release of various inflammatory cytokines, including interleukins (IL-18, 6, 10), TNF-α, Interferon-gamma, and transforming growth factors beta. Hypoxia in adipose tissue leads to the recruitment of macrophages into adipose tissue, which maintains the inflammatory state and leads to a defect in the function of adipose cells and leads to cell necrosis, there is evidence that an inflammatory state can itself promote androgen production and hyperandrogenism in the blood of women infected with PCOS (Sabbir *et al*., 2021).

More than one study is mentioned indicates that the cause of low-grade chronic inflammation in thin women infected with PCOS is hyperandrogenic in the blood, as studies conducted on healthy, thin women who do not suffer from PCOS and who were treated with dehydroepiandrosterone-sulfate (DHEA-S) increase in parameters of inflammatory after increasing androgen levels (Hu *et al*., 2020). Elevated inflammatory markers such as TNF-α in women infected with PCOS are signals of altered immune function (Aboeldalyl *et al*., 2021).

CONCLUSIONS

We conclude from the current study that the level of the hormone Folli statin in the blood serum of women infected with PCOS is high, as it participates in the pathophysiological changes of this syndrome, and that it may be associated with a low-grade chronic inflammatory condition. Also, the high level of the hormone irisin in serum of women infected with PCOS can be considered a vital indicator to detect infection with this syndrome in the future, as well as it can be used as an indicator to follow the syndrome under different conditions. We also conclude from this current study that TNF-α increases in women infected with PCOS and that these markers of inflammation can be used to diagnose patients with PCOS. The low-grade inflammation that appears in women infected with PCOS is essential for monitoring this syndrome.

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تقييم دور بعض الحركيات العضلية وعامل النخر الورمي-الفا في النساء المصابات بمتلازمة تكيس المبايض في مدينة الموصل-العراق

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الملخص

تعد متلازمة المبيض المتعدد الاكياس (PCOS) احد اضطرابات الغدد الصم الشائعة في الاناث عند سن الانجاب، اذ هدفت الدراسة الحالية الى تقييم دور بعض الحركيات العضلية (هرموني الفولسيتايتن والايرسين) بشكل واضح ضمن اسباب الاصابة وعامل النخر الورمي–الفا (TNF-α) احد السايتوكينات الالتهابية وكمؤشر اساسي على الاصابة بالالتهابات لدى النساء المصابات PCOS، اذ شملت هذه الدراسة 75 عينة دم من نساء مصابات كان 50 منهن متزوجات (25 ذوات عقم اولي، 25 ذوات عقم ثانوي) و25 عازبات، و تراوحت اعمارهن بين (15–45) سنة، جمعت من مستشفى البتول التعليمي لأمراض النسائية والتوليد في محافظة نينوى، بعد تشخيصهن من قبل اطباء النسائية والتوليد والعقم، فضلا عن 50 عينة دم لنساء (25 متزوجات و25 عازبات) سليمات وغير مصابات بـ PCOS ولايعانين من اي مشاكل في الخصوبة والامراض المزمنة وبنفس الاعمار وعدت كمجموعة سيطرة.

أظهرت النتائج زيادة ملحوظة في تركيز الفوليستاتين والإيريسين وTNF-α في مصل كل من النساء المتزوجات والعازبات المصابات بمتلازمة المبيض المتعدد الاكياس، وفي النساء المتزوجات المصابات ولديهن العقم الأولي والثانوي مقارنة بمجموعة السيطرة. نستنتج أن الفوليستاتين وTNF-α مرتبطان بالحالة الالتهابية للنساء المصابات بمتلازمة المبيض المتعدد الاكياس، ويمكن اعتبار الإيريسين بمثابة علامة حيوية للكشف عن هذه المتلازمة.

الكلمات الدالة: متلازمة المبيض المتعدد الكيسات، ميوكينات، فوليستاتين، ايريسين، عامل نخر الورم ألفا.