



## Estimation of Spexin level and some biochemical indicators in obese and non-obese woman's with polycystic ovary syndrome

Zainab Eskander Mahmood<sup>1\*</sup>, Sayran Sattar Saleh<sup>2</sup>

<sup>1,2</sup>Department of Chemistry –College of Science- Kirkuk University –Iraq

\*Corresponding author: Zainab Eskander Mahmood, Department of Chemistry –College of Science- Kirkuk University –Iraq, Email: Zainabeskander78@gmail.com

Submitted: 17 February 2023; Accepted: 10 March 2023; Published: 06 April 2023

### ABSTRACT

The research aimed to measure the level of the hormone spexin and a number of biochemical variables, including (glucose, cholesterol (ch), triglycerides (T.G), very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL) in obese, non-obese, non (PCOS) women.

The research was conducted on (90) blood samples, 60 samples from patients with (PCOS) and 30 of them were healthy females. Blood samples were collected from women attending outpatient clinics in Kirkuk Governorate, after their diagnosis. By obstetrics and gynecology doctors for the period from 11/20/2021 to 25/2/2022, as their ages ranged between (17-42) years, and they according to the body mass index, into three groups was divided, The first group (30) samples for patients with (PCOS) who are obese with BMI of more than 30 kg / m<sup>2</sup>, and the second group (30) samples for ladies without obesity who have polycystic ovarian syndrome with BMI less than 25 kg / m<sup>2</sup>, and the third group (30) samples for non-infected women Those with BMI more than 30 kg/m<sup>2</sup> as the control group.

The findings of this research show following:

\*A notable reduction ( $P \leq 0.05$ ) in the level of the hormone spexin when comparing the obese group of PCOS women with the healthy female, However a significant decrease shown P- value ( $P \leq 0.01$ ) when comparing the obese women with PCOS with the non-obese.

\* There are non-significant differences in the value of body mass index when comparing between female suffers (PCOS) and healthy individuals, while the results reported rose in (BMI) when comparing two groups, the probability level ( $P < 0.01$ ) women with PCOS who are obese and non-obese.

\* P- Value rise ( $P < 0.01$ ) in glucose concentration when comparing obese PCOS patients with control, while the reported showed raised ( $P \leq 0.05$ ) when comparing obese PCOS patients with non-obese PCOS patients.

P- Value rise ( $P \leq 0.01$ ) in the booth's concentration triglycerides and very low-density proteins when comparing obese PCOS patients with control, and also when comparing between PCOS individuals with and without obesity.

\* Increase that is statistically significant ( $P < 0.05$ ) in the concentration both cholesterol and low-density lipoproteins when comparing obese women with PCOS with control, and no significant differences when comparing between PCOS-afflicted obese and non-obese women.

\* A non-significant decrease when concentrating of LDL in obese PCOS patients to compared with healthy individuals. It reported lowered when comparing obese PCOS female with non-obese ones.

**Keywords:** *Periodontitis, Estimation, Control*

## INTRODUCTION

A complex condition, polycystic ovary syndrome, clinically heterogeneous, hormonal condition that impacts about (15-25)% childbearing age (1). There is differentiating a small cysts containing ovarian fluids, which are endocrine and metabolic disorders. It is more common in the premenopausal period, and symptoms of this syndrome appear during the early adult years (2) , additionally, PCOS is frequently linked to insulin resistance and abdominal obesity, cardiac risk factors, and metabolic disorders. PCOS was first discovered by researchers Stein& Leventhal in 1935 AD and known by their names (3). This disease is diagnosed clinically, depending on the symptoms that appear on the patient, and these symptoms include high levels of androgens, amenorrhea or lack of ovulation, the appearance of acne on the face, hirsutism and weight gain (4) , and polycystic ovarian syndrome is also diagnosed by ultrasound examination Ultrasound, and although a long time has passed since the discovery of the syndrome, the pathogenesis of the syndrome remains unclear (5) .

### *Hormone spexin*

It is a new peptide hormone called a neuropeptide (NPQ), which was first identified by Mirabeau and his group in 2007 in to analyze the human genome, and then confirmed by a biochemical method. , It was discovered for the first time in the esophagus and stomach of rats, and the human spexin was encoded by a propeptide by the C12 or F39 gene, and it contains 116 amino acid residue units, and ( spexin gene) was more disruptive in person fats and obesity (6) , moreover, spexin plays crucial roles in metabolism. In many different human organs, including the ((pancreas, liver, visceral fat, thyroid, and adrenal glands)), the spexin gene is highly expressed. Also, obese participants had reduced blood Spexin levels. Spexin prevented mice's adipocytes from absorbing long-chain fatty acids from obese people of normal weight (7).

Through studies and research, it has been established that Spexin levels fall in conditions like (obesity, diabetes, and insulin resistance). In addition, it has been established that Spexin is the root cause of conditions like {diabetes, obesity, polycystic ovarian syndrome, cardiovascular disease, and kidney disease}.

### *Objectives of the resorts*

The study aims:

- 1- Investigating the level of the hormone spexin in polycystic ovary syndrome patients who are obese, not obese, and not infected.
- 2- Study of biochemical indicators including glucose and blood lipids (cholesterol, (HDL), (LDL) and (VLDL).

## MATERIALS AND METHODS

### *The Sample of the study*

Blood specimen (90) accumulate, 60 blood samples taken from polycystic ovary syndrome sufferers and 30 blood samples from healthy women between the ages of (17-42) years from outpatient clinics for the period from 11/20/2021 to 25/2/2022. These samples were obtained by taking five milliliters (ml) of venous blood from the (2nd-3rd) day of their menstrual cycle after confirming that they have Polycystic Ovary Syndrome and putting the blood directly into Gel tubes and leaving it for (20) minutes at room temperature for the purpose of blood clotting.

After that it was spun at 3000 revolutions per minute for ten minutes in a centrifuge and the blood serum free of red blood cells was separated, and the serum was frozen after its fractionation in several test tubes (Appendroff tubes 1.5 ml) to perform the required biochemical tests.

### *Body mass index calculation*

The Body mass index (BMI), which determines the level of obesity in the body, is calculated by as shown:

$$\text{BMI} = \frac{\text{weight}(kg)}{\text{length}(m^2)}$$

### ***Estimation level of the hormone spexin in the blood serum***

The level of spexin in blood serum is estimated using a ready-made analysis kit using the enzyme-linked immunosorbent assay (ELISA) technique, which is an immunoassay quantitative method based on the sandwich principle, consisting of 96 holes in one plate where the conjugated antibody is used Biotin as detection antibodies, while it was added to standard solutions, samples, and conjugated antibodies for biotin detection, HRP-Streptavidin was added after being washed with Wash buffer, and the unbound conjugates were removed with the washing solution.

After adding the acidic stop solution, TMB was catalyzed by HRP to create a blue-yellow complex.

### ***Estimation of blood serum glucose concentration***

The enzyme glucose oxidase (GOD), which catalyzes the oxidation of glucose to gluconate, was used in the test kit created by the Canadian company Taytec to measure the blood's glucose levels. Hydrogen peroxide is detected by the enzyme peroxidase (POD), which contains 4-Aminophenazone (4-AP), which is present in the enzyme glucose oxidase (GOD) (9).

### ***Estimation of cholesterol concentration in blood serum***

With the diagnostic kit created by the French company Biolabo, the amount of cholesterol in the blood serum is estimated using an enzymatic approach. The enzyme Cholesterol esterase in the analysis kit activates the decomposition of cholesterol ester, which is present in the blood serum to cholesterol and free fatty acids, then cholesterol is oxidized by enzyme cholesterol oxidase and in the presence of oxygen to produce hydrogen peroxide. This interacts with phenol

and PAP in the presence of peroxidase enzyme to produce a pink quinone amine, which is absorbed at the wavelength (500nm).

### ***Estimation of triglycerides concentration in blood serum***

Triglyceride levels in blood serum were calculated using an enzyme-based approach with a diagnostic kit made by the French company Biolabo (10).

### ***Estimation of (VLDL) concentration in blood serum***

According to the methodology used by Ash Wood and his team (11), the value of (VLDL-C) was computed as equation:

$$\text{VLDL}(mg/dl) = \text{Triglycerides} / 5$$

### ***Estimation of (high-density lipoprotein cholesterol) concentration in blood serum***

HDL-C in serum was determined, the other lipoproteins, VLDL and LDL-C, should be precipitated using magnesium chloride and phosphotungstic acid, and HDL-C is obtained from the saliva after being separated by a centrifuge (12).

### ***Estimation of LDL-cholesterol concentration in blood serum***

The value of (LDL - C) was computed using the equation below in accordance with the technique developed by Andréoli and his team (13)

$$\text{LDL -C} (mg / dl) = \text{Total cholesterol} - (\text{HDL} + \text{VLDL})$$

## **RESULTS AND DISCUSSION**

### ***Body mass index, spexin level and serum glucose concentration***

The blood serums of female have PCOS and healthy group, ( BMI), the hormone spexin, the concentration of glucose were measured shown in Table. (1).

**TABLE 1:** Average body mass index, the average level of the hormone spexin, and the glucose concentration in the blood serums of the two groups of infected and healthy women.

Groups Parameter	Patients Obesity and Control Mean±SD			Patients Obesity and Non obesity Mean±SD		
	Control n=(30)	Patients Obesity n=(30)	p-value	Patients Obesity n=(30)	Patients Non obesity n=(30)	p-value
BMI(kg/m <sup>2</sup> )	30.26±3.2	33.49±6.3	Ns	33.49±6.3	23.11±6.29	0.01
Spexin(pg/ml)	98.56±11.4	87.82±8.82	0.05	87.82±8.82	114.58±22.1	0.01
Glucose(mg/dl)	90.37±22.6	107.5±32.2	0.01	107.5±32.2	96.93±20.65	0.05

***Body mass index for women with (polycystic ovary syndrome)***

According to Table (1), showed no statistically differences in the value in BMI between two groups, but there was a significant difference in the BMI value (P0.01), obese women with PCOS were compared to non-obese patients. This information was used to determine the prevalence of obesity among patients (PCOS) concur with those of Al-Mahdawi and his team's study (14)

Those who indicated no differences in body mass index in obese PCOS women compared with the control group, while the results of this study contradicted the results of the study of Esmailzadeh and his group (15), as they found that women with polycystic ovary syndrome have a body mass index significantly higher than in the control group, higher mean weight gain was indicated to be associated with less exercise sports among women and wrong eating habits with high calories approved. (16). Excess adipose tissue in the abdomen leads to metabolic and endocrine disorders that impair insulin action. This combines with the development of hyperandrogenism, leading to decreased glucose absorption, which again leads to increased visceral fat deposition, regardless of BMI (17).

***The level of the hormone spexin in the blood serum***

When comparing obese women with PCOS with healthy group, the findings in Table (1) revealed drop in the hormone spexin p-value (P0.05). The results also showed a significant decrease at the

level of probability (P≤0.01) in the level of spexin when comparing The group of obese and non-obese women with PCOS, glucose homeostasis and fatty acid uptake And that its activities are mediated by three subtypes of Galanin (GAL) receptors, as Galanin Receptor (GALR2) and Galanin Receptor3 (GALR3) are active with high strength and high affinity (18) (19), the results of the current investigation coincided with the results of the study of Asli (20) and his colleagues.

***The concentration of glucose in the blood serum***

When obese female PCOS were compared to controls, the reported revealed a rise in glucose concentration at (P- 0.01), while the same table's results revealed a significant rise in probability (P0.05) when obese PCOS patients were contrasted with non-obese PCOS. The results of the study concur with those of Daghestani and his team's study (39), The study also concurred with those of Zhu's study (21), which found that PCOS patients frequently have postprandial blood sugar dysregulation and an elevated risk of DMT2, particularly in obese individual. (22). Moreover, studies have revealed that women with PCOS are at an increased risk of developing diabetes. There were closely related to weight gain.

***Blood lipid concentrations in the groups under study***

The concentrations of total cholesterol, triglycerides, and lipoproteins (HDL-LDL-VLDL) were measured in serum of patients with

PCOS compared to the healthy group. The results were as shown in Table (2).

**TABLE 2:** shows the typical blood lipid levels for the two groups of infected women and the control group.

Group parameter	Patients Obesity and Control Mean± SD			Patients Obesity and Non obesity Mean± SD		
	Control N=(30)	Patients obesity N=(30)	P- valu	Patients obesity N=(30)	Patients Non obesity	P-valu
Cholestrol (mg/dl)	103.96±21.7	116.8±21.5	0.05	116.8±21.5	113.07±27.4	Ns
Triglyceride (mg/dl)	76.30±11.2	119.6±23.0	0.01	119.6±23.0	83.97±15.4	0.01
HDL (mg/dl)	35.41±8.5	28.87±5.72	Ns	28.87±5.7	33.50±6.56	Ns
LDL (mg/dl)	51.26±6.6	67.39±7.1	0.05	67.39±7.1	61.46±5.46	Ns
VLDL (mg/dl)	15.25±2.2	23.93±4.6	0.01	23.93±4.6	16.79±5.1	0.01

Results in Table (2) showed a statistically significant rise in LDL and total cholesterol levels at a probability level (P<0.05) in obese (PCOS) women compared to controls, and also showed a non-significant increase in total cholesterol, LDL when comparing sera obese women with PCOS with control. Non-obese patients, as well showed in Table (2) a raised in triglycerides and VLDL P- value (P<0.01) in sera obese women (PCOS) when compared with healthy groups, as well as a significant increase at the level of probability (P<0.01) at Comparing obese PCOS patients with non-obese patients, Also, results in Table (2) appeared a non-significant lowering in HDL-C concentration in obese PCOS women when compared with control. It also showed a non-significant decrease when comparing obese PCOS women with non-obese women. The occurrence of an imbalance in the level of lipids in the blood, including an increase in the level of cholesterol, triglycerides, LDL, and lowered of HDL-c. These results vary depending on the body weight and diet of the woman (23)(24) , and the results of this study agreed with what Rashidi and his group reached (25) They indicated that the concentration of triglycerides and cholesterol in obese women is higher than in Based on this review of the results

of the study, it is clear to us that there are disturbances in blood lipid concentrations in women with this syndrome, which were more evident in obese women than in thin women. The high blood lipid level is one of the characteristics of this syndrome, as it is attributed The reason for the increase in triglycerides in obese women is the increase in body mass index (BMI), and the increase in BMI leads to an excess in the concentration of LDL-c in obese women compared to the lean women, which proves that the increase in BMI leads to an increase in lipoproteins. Kim and his group (26) , have indicated that. Lowered in HDL-C levels and the higher in triglycerides the reason for the increase may be related to an increase in androgens (27) , several studies have conducted In studies of non-obese women with PCOS, complete lipid profiles and lipid routines were obtained.

#### REFERENCE

1. Liu, H., Xie, J., Fan, L., Xia, Y., Peng, X., Zhou, J. and Ni, X. (2022). Cryptotanshinone protects against PCOS-induced damage of ovarian tissue via regulating oxidative stress, mitochondrial membrane potential, inflammation, and apoptosis via regulating ferroptosis. *Oxidative Medicine and Cellular Longevity*.

2. Witchel, S.F.; Oberfield, S.E. and Peña, A.S.(2019). Polycystic ovary syndrome: pathophysiology, presentation, and treatment with emphasis on adolescent girls. *Journal of the Endocrine Society*; 3(8); 1545-1573.
3. Shaaban, Z., Khoradmehr, A., Amiri-Yekta, A., Nowzari, F., Jafarzadeh Shirazi, M. R. and Tamadon, A. (2021). Pathophysiologic Mechanisms of Insulin Secretion and Signaling-Related Genes in Etiology of Polycystic Ovary Syndrome. *Genetics Research*, 2021. 19-
4. Halder, A. and Kumar, H. (2020). Polycystic Ovary Syndrome (PCOS): The Pros and Cons of Various Diagnostic Criteria. *EC Gynaecology*, 9, 39-
5. Liao, B., Qiao, J. and Pang, Y. (2021). Central regulation of PCOS: Abnormal neuronal-reproductive-metabolic circuits in PCOS pathophysiology. *Frontiers in Endocrinology*, 12, 667422. 41.
6. Bacopoulou F, Apostolaki D, Mantzou A, et al(2019).Serum Spexin is Correlated with Lipoprotein (a) and Androgens in Female Adolescents *Journal of Clinical Medicine*;8(12):2103.
7. Walewski JL, Ge F, Lobdell IV H, et al.( 2014) . Spexin is a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet-induced obesity. *Obesity*;22(7):1643-52.
8. Champe, P. C., Harvey, R. A. and Ferrier, D. R.(2008). "Metabolism of Biochemistry" 4th ed USA:Lippic Williams and wilkins, Pp. 173.
9. Kaplan, L.A.Amadeo, J.Pesce and steren, C.K.(2003)." Methods in clinical chemistry " .4th ed. Mosby . U.S.A:P:113.
10. Young D.S., (1995)Effect of Drugs on Clinical laboratory Tests, 4th Ed..p3-573 to 3-589.
11. Tietz,N. (1999). Textbook of clinical chemistry . 3reded.C.A.Burtis, E.R. Ashwood , W.B.Saunders.Pp:819-861,1250..
12. Finley PR, Tietz , NW. (1996) . editors. Clinical guide to laboratory tests .WB Saunders company.
13. Andreoli TE, Carpenter J , Griggs RC.(2001) . Cecil essentials of medicine:disorder of lipid metabolism. Herbert PN Philadelphia WB Saunders company. London, Toronto;16:526-32 .
14. Al-Mahdawi, M. A.; Khadhem, H. K. and Al-Jebori, S. R. (2018). Effect of physical activity on sex hormones in polycystic ovary syndrome Iraqi women. *Iraqi J of Biotechnology*; 17(1): 98-107.
15. Esmaeilzadeh, S.; Andarieh, M.G.; Ghadimi ,R. and Delavar, M.A. .( 2015) Body mass index and gonadotropin hormones (LH & FSH) associate with clinical symptoms among women with polycystic ovary syndrome. *Global J of health science*; 7(2): 101.
16. Daghestani, M.H.; Daghestani, M.; Daghistani, M.et al( 2018). A study of ghrelin and leptin levels and their relationship to metabolic profiles in obese and lean Saudi women with polycystic ovary syndrome (PCOS). *Lipid in Health and Disease*; 17(1): 1-9.
17. Sprung, V. S.; Jones, H.; Pugh, C. J.et al.(2014). Endothelial dysfunction in hyperandrogenic polycystic ovary syndrome is not explained by either obesity or ectopic fat deposition. *Clinical Science*; 126(1): 67-74.
18. Liu Y, Sun L, Zheng L, et al(2020) . Spexin protects cardiomyocytes from hypoxia-induced metabolic and mitochondrial dysfunction. *NaunynSchmiedeberg's Archives of Pharmacology*. 1;393(1):25-33.
19. Reyes-Alcaraz A, Lee YN, Son GH, et al (2016). Development of spexinbased human galanin receptor type II-specific agonists with increased stability in serum and anxiolytic effect in mice. *Scientific reports*. 24;6(1):1-0.
20. Asli, G. and Ismail, D. et al.(2021). Decreased levels of spexin are associated with hormonal and metabolic disturbance in subjects with polycystic ovary syndrome;41(3):408-413.
21. ZHU, J.P.; TENG, Y.C.; Zhou. J.; Lu, W.; TAO, M.F. and JIA, W.P. (2013). Increased mean glucose levels in patients with polycystic ovary syndrome and hyperandrogenemia as determined by continuous glucose monitoring. *Acta obstetricia et gynecologica Scandinavica*92(2):165-171.
22. Rosenfield, R. L. and Ehrmann, D. A. (2016). The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocrine reviews*: 37(5): 467-520.
23. Guo, F., Gong, Z., Fernando, T., Zhang, L., Zhu, X. and Shi, Y. (2022). The Lipid Profiles in Different Characteristics of Women with PCOS and the Interaction Between Dyslipidemia and Metabolic Disorder States: Chinese in Study Retrospective A in Population .*Frontiers Endocrinology*,13.
24. Liu, Q., Xie, Y. J., Qu, L. H., Zhang, M. X. and Mo, Z. C. (2019). Dyslipidemia involvement in the development of polycystic ovary syndrome. *Taiwanese Journal of Obstetrics and Gynecology*, 58(4), 447-453.
25. Rashidi, H., Tafazoli, M., Jalali, M. T. and Mofrad, A. M. E. (2018). Serum lipid profile and insulin resistance in women with polycystic ovary syndrome.

- syndrome (PCOS). *Journal Diabetes Metabolic Disorder Control*, 5(3):148-52.
26. Kim, J. J. and Choi, Y. M. (2013). Dyslipidemia in women with polycystic ovary syndrome. *Obstetrics and gynecology science*, 56(3), 137-142.
27. Osibogun, O., Ogunmoroti, O., & Michos, E. D. (2020). Polycystic ovary syndrome and cardiometabolic risk: opportunities for cardiovascular disease prevention. *Trends in cardiovascular medicine*, 30(7), 399-404.