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Estimation of Galanin hormone levels and additional biochemical markers in polycystic ovary syndrome patients

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Abstract
Polycystic ovarian syndrome (PCOS), which is typified by irregular menstrual cycles, hyperandrogens, and polycystic ovaries, is the most prevalent endocrine and metabolic disorder affecting premenopausal women. Women with PCOS are more likely to develop metabolic syndrome because they are at a higher risk of acquiring hypertension and dyslipidemia.
Twenty health screen examinees who were admitted to the Tikrit Teaching Hospital's primary accommodations between January and March 2024 were referred to our organization. Forty patients were selected on the basis of their history of Polycystic Ovarian Syndrome, blood test results, abdominal ultrasound results, and whether or not they provided written informed consent. For those who have Polycystic Ovary Syndrome, blood galanin levels were significantly elevated, independent of age or the results of laboratory tests (144.9±9.2vs. 30.18±6.6, p<0.01) and serum Irsin levels (6.1±0.6 vs. 0.9±0.3, p<0.01). Polycystic Ovary Syndrome patients exhibited significantly higher mean serum 8 Oxoguanine DNA glycosylase levels than the control group (13.6± o.4 versus 2.3± 0.3 (p>0.01). Polycystic Ovary Syndrome patients had significantly higher serum hormones (TSH, Prolactin, LH and FSH) compared to controls (0.05±0.02 versus 2.29± 0.8), (35.5±3.5 versus11.2±3.8), (17.7±1.5 versus 9.07±2.7) and (28.01±3.8 versus 9.6±1.5) respectively with p values <0.01. As well as Patients with Polycystic Ovary Syndrome had significantly higher Lipid profiles (Cholestrol, Triglyceride) and D3 concentrations compared to controls (294.2±22.2versus 92.1±4.7, 212.4±6.4 versus 73.6±9.4) and (8.1±2.0versus 51.8±9.2) respectively with p-values < 0.01.
Aim of the study: This study aimed to evaluate the lipid profile (Cholestrol, triglycerides), blood levels of galanin and irisin, hormone levels (TSH, prolactin, LH, and FSH), and D3 concentrations in individuals with Polycystic Ovary Syndrome condition and compare them to control groups.

Keywords: Polycystic ovary syndrome, Galanin, Irisin, BMI, TSH, prolactin, LH, FSH, cholesterol, triglyceride, D3 concentrations

Introduction
The most prevalent endocrinopathy, polycystic ovarian syndrome (PCOS), affects seven to ten percent of women who are of reproductive age [1]. Hyperandrogenism, irregular menstrual periods, and polycystic ovaries are symptoms of this complex endocrine condition. Three criteria are used to diagnose PCOS: high androgen, ovulatory failure, or polycystic ovaries [2]. PCOS can be caused by a variety of hereditary and environmental factors. Family history, inactivity, diabetes mellitus, and obesity are risk factors for the development of PCOS. PCOS increases the risk of osteoporosis, cancer, high blood pressure, diabetes, lipid problems, mental health problems, and insulin resistance. PCOS is known to have a significant influence on female infertility, affecting between 0.6 and 3.4% of infertile couples. Recent advancements in lab equipment, sonography, and conventional laparoscopic testing for infertility have led to a large increase in the prevalence of PCOS. Women with PCOS need to be properly diagnosed and treated because of their elevated risk of diabetes, hypertension, cardiovascular disease, and cancers linked to hyperestrogen [3]. Both sexes' reproductive systems and the central and peripheral nervous systems have high concentrations of galanin, a neuropeptide composed of 29 amino acids [4]. Numerous physiological functions and illnesses, including feeding, hunger, inflammation, Alzheimer's

disease (AD), epilepsy, neuroregeneration, convulsions, and pain, are linked to it. It belongs to the three members of the G-protein-coupled receptor (GPCR) superfamily (GAL1-3), each of which has unique signaling pathways and distribution patterns. Galanin, galanin-like peptide (GALP), galanin, alarin, and galanin-message associated peptide (GMAP) make up the so-called galaninergic system, which uses galanin as its primary signaling component [5]. In the hypothalamus, some of the neurons that produce GnRH also produce galanin. Estrogen significantly increases the synthesis of the galanin gene in these GnRH neurons. GAL2 has been shown to be present in the ovary. Galanin is believed to have a major role in the development of the gonadal secretory and preovulatory LH spike in animals. There may be more, as yet unidentified functions for galanin. It is thought that neurological influences over GnRH secretory neurons, especially galanin, are important in the development of polycystic ovarian syndrome since neuroendocrine abnormalities are frequently associated with unregulated gonadotropin production in patients with this illness. PCOS is one of the most prevalent conditions affecting women in their reproductive years. It is defined as metabolic and endocrine issues linked to modifications in the ovarian and pituitary-gonadal axis's function [6]. A myokine that muscles make called irisin was recently discovered to act as a messenger between skeletal muscle and other body parts. The Greek goddess Iris, a god-to-god mediator, is the source of the name Irisin [7]. When fibronectin type III domain containing 5 (FNDC5) is broken down by the circulating factor Irisin, white adipose tissue (WAT) experiences thermogenic responses, often known as browning. Increased oxygen demand and mitochondrial density are linked to weight loss and heat production caused by WAT browning. The pathogenesis of insulin-resistant conditions like type 2 diabetes and metabolic syndromes may involve irisin. Muscle mass, adipose tissue mass, and body mass index (BMI) have all been linked to higher irisin levels. Irisin plasma levels and body fat are correlated in both PCOS patients and healthy individuals [8].

This study sought to determine whether irisin and gelanin, two hormones, were associated with PCOs. To do this, we evaluated the relationship between the hormone levels of irisin and gelanin and another biological trait.

Materials and Methods

The patients included in this study were recruited during the period from January 2024 and March 2024.

Patients Group: Patients diagnosed with PCOs by upper abdomen ultra-sonogram were included in the study. This study investigated 40 patients, they ranged in age from 20 to 40. Submitted to the primary accommodations from Tikrit Teaching Hospital.

Controls Group: For standardization and comparison, 20 seemingly healthy persons, neither smokers nor PCOs matched for age, and weight with the patient groups, were chosen as controls. The ages varied from 20 to 40 years. The control group was assessed to be free of sickness, not taking any drugs, and consisted primarily of medical staff and friends.

Data Collection: The primary source of data was gathered directly from patients and control individuals through interviews and the use of a specifically designed questionnaire that recorded detailed information about each patient and control subject. It includes the following information: name, age (Years), mobile number, weight (kg), height (m), BMI, smoking history, family history of PCOs, length of sickness, kind of treatment, duration of treatment. Data received indirectly from patient records were also analyzed and documented.

Sample Collection

A sterile disposable syringe was used to extract approximately 8 milliliters of venous blood from each subject, which was then placed into gel tubes. After the blood in a gel tube was centrifuged for 10 minutes at 4000 rpm and allowed to clot at room temperature for a while, the serum was extracted and divided into Eppendorf tubes for every sample. After that, the samples were stored at -20 °C in a deep freezer until analysis time. Samples from control subjects were collected and processed using the same protocols.

Exclusion criteria

Patients with the following criteria were excluded;

- Anemia patients (Iron deficiency anemia).
- Viral Hepatitis.
- Alcoholic.
- Malignancies.
- Pregnant women.
- Gender male

Inclusion criteria

Patients with the following criteria were included;

- Patient with Polycystic Ovary Syndrome.
- Age group 20-40 years.
- Female

No.	Chemical tests	Company	Origin
1	(FSH, LH, prolactin, TSH,) kits	Linear	Spain
2	(Cholesterol, Triglyceride, D3) kits	Linear	Spain
3	(Irisin, galanin, 8 Oxoguanine DNA glycosylase (OGG1)) kits	Linear	Spain

Statistical analysis

A datasheet was created using all of the test findings and questionnaire information. Real Statistics Resource Pack for Excel 2016 and IBM SPSS version 28.0 were used for data analysis.

Results

The distribution of control groups and individuals with polycystic ovary syndrome considering demographic characteristics

There are 40 polycystic ovarian syndrome patients and 20 control groups in the study. The study's findings indicate that the two groups' demographics, as displayed in Table 1, explain the variation in the mean ± standard deviation of age (Years) between the control group and patients with polycystic ovary syndrome, which was 28.35±5.21 and 32.7±5.3, respectively. ($p>0.001$) The change was not statistically significant.

Patients with m PCOS had a mean BMI (kg/m2) that was 36.1±2.7 against 29.47±0.99 for the control group. The difference was significantly larger as a result ($p>0.01$).

Table 1: Polycystic Ovary Syndrome patient and control group distribution Based on demographic traits

Characteristic	PCOS No.40	Control No.20	P-Value
	Mean ± SD	Mean ± SD	
Age (Years)	32.7±5.3	28.35±5.21^{NS}	0.06
BMI (kg/m²)	36.1±2.7	29.47±0.99	0.01
Total	40	20	

* $p<0.05$, NS: non significance

Polycystic Ovary Syndrome Patients' Galanin Serum Level in Comparison to the Control Group. Among patients with polycystic ovarian syndrome, the average serum level of galanin was 144.9±9.2 contrary to 30.18±6.6 in the control group, as indicated in table (2). The outcome was noteworthy (p>0.01).

Table 2: Comparison of the control group's and Polycystic Ovary Syndrome patients' mean ± SD of galanin

Study groups	n	Galanin Mean ± SD	P. value
Patient with Polycystic Ovary Syndrome	40	144.9±9.2	0.01 Significant difference
Control group	20	30.18±6.6	

3. Irsin Serum Level in Patients with Polycystic Ovary Syndrome in Comparison to the Control Group: According to table (3), the mean ± standard deviation of the serum level of Irsin in patients with Polycystic Ovary Syndrome was 6.1 ±0.6 as opposed to 0.9±0.3. The result was significant (p>0.01).

Table 3: Comparison between patient with Polycystic Ovary Syndrome and Control group regarding the mean ± SD of Irsin

Study groups	n	Irsin Mean ± SD	P. value
Patient with Polycystic Ovary Syndrome	40	6.1±0.6	0.01 Significant difference
Control group	20	0.9±0.3	

4. Compared to the control group, the serum level of 8 Oxoguanine DNA glycosylase (OGG1) was higher in patients with polycystic ovary syndrome.

In individuals with Polycystic Ovary Syndrome, the mean serum level of 8 Oxoguanine DNA glycosylase (OGG1) was 13.6±o.4, compared to 2.3±0.3 in the control group (table 4). The outcome was significant (p>0.01).

Table 4: Comparison between patient with Polycystic Ovary Syndrome and Control group regarding the mean ± SD of 8 Oxoguanine DNA glycosylase (OGG1)

Study groups	n	8 Oxoguanine DNA glycosylase (OGG1) Mean ± SD	P. value
Patient with Polycystic Ovary Syndrome	40	13.6±o.4	0.01 Significant difference
Control group	20	2.3±0.3	

5. Polycystic Ovary Syndrome patients' serum levels of TSH, prolactin, LH, and FSH were compared to those of the control group

The mean blood TSH level in patients with Polycystic Ovary Syndrome was 0.05±0.02 versus 2.29±0.8, respectively, compared to the control group, as indicated in table (5). The outcome was noteworthy (p>0.01).

As well as Prolactin in patient with Polycystic Ovary Syndrome comparing with the control group (35.5±3.5 versus11.2±3.8) respectively. The result was significant (p> 0.01).

While LH patient with Polycystic Ovary Syndrome comparing with the control group (17.7±1.5 versus 9.07±2.7) respectively. The result was non-significant (p> 0.01).

Additional FSH in patient with Polycystic Ovary Syndrome comparing with the control group (28.01±3.8 versus 9.6±1.5) respectively. The result was significant (p> 0.01).

Table 5: Serum Level of TSH, prolactin, LH and FSH in patient with Polycystic Ovary Syndrome compared with the control group

Study groups	n	Mean ± SD			
		TSH	Prolactin	LH	FSH
Patient with Polycystic Ovary Syndrome	40	0.05±0.02	35.5±3.5	17.7±1.5	28.01±3.8
Control group	20	2.29±0.8	11.2±3.8	9.07±2.7	9.6±1.5
P. value		0.01	0.01	0.01	0.01

6. Serum Level of D3, Cholestrol and TG in patient with Polycystic Ovary Syndrome compared with control group

Polycystic Ovary Syndrome patients had an average serum D3 level of 8.1±2.0, compared to 51.8±9.2 in the control group (Table 6). The result was significant ($p<0.001$).

Furthermore, the control group's cholesterol levels were 92.1±4.7, while those of patients with Polycystic Ovary Syndrome were 294.2±22.2. The outcome was noteworthy ($p<0.01$).

The Polycystic Ovary Syndrome group's average blood triglyceride level was 212.4±6.4, while the control group's was 73.6±9.4. The outcome was noteworthy ($p<0.01$).

Table 6: Serum Level of D3, Cholestrol and TG in patient with Polycystic Ovary Syndrome compared with control group

Study groups	n	Mean ± SD		
		D3	Cholesterol	TG
Patient with Polycystic Ovary Syndrome	40	8.1±2.0	294.2±22.2	212.4±6.4
Control group	20	51.8±9.2	92.1±4.7	73.6±9.4
P. value		0.001	0.01	0.01

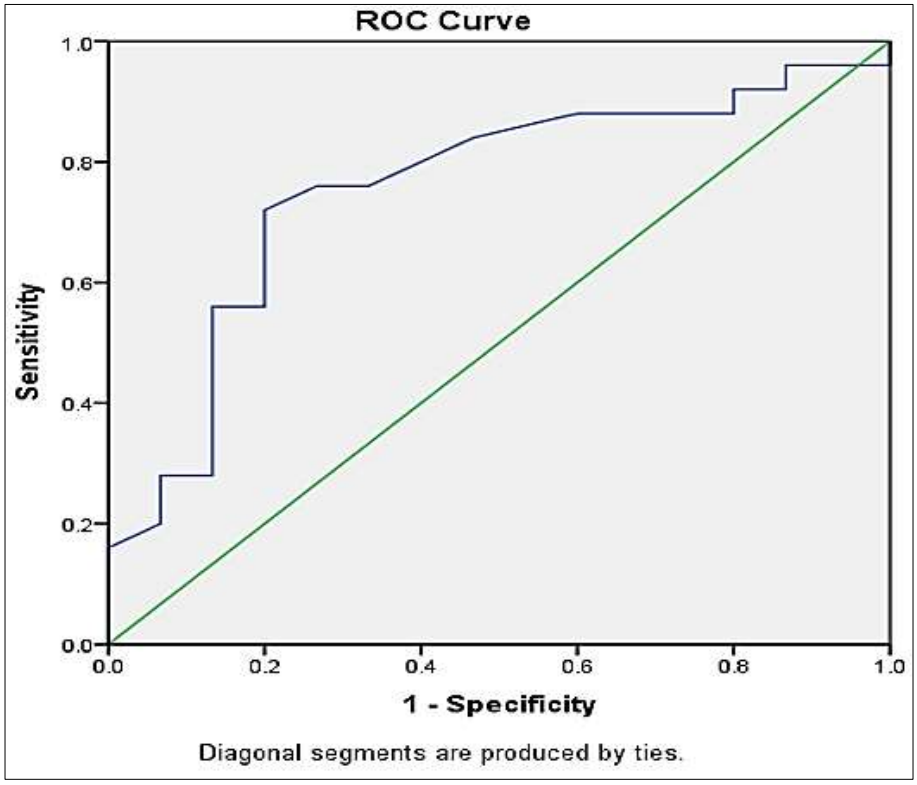

Fig 1: ROC Curve Comparison between BMI and Galanin

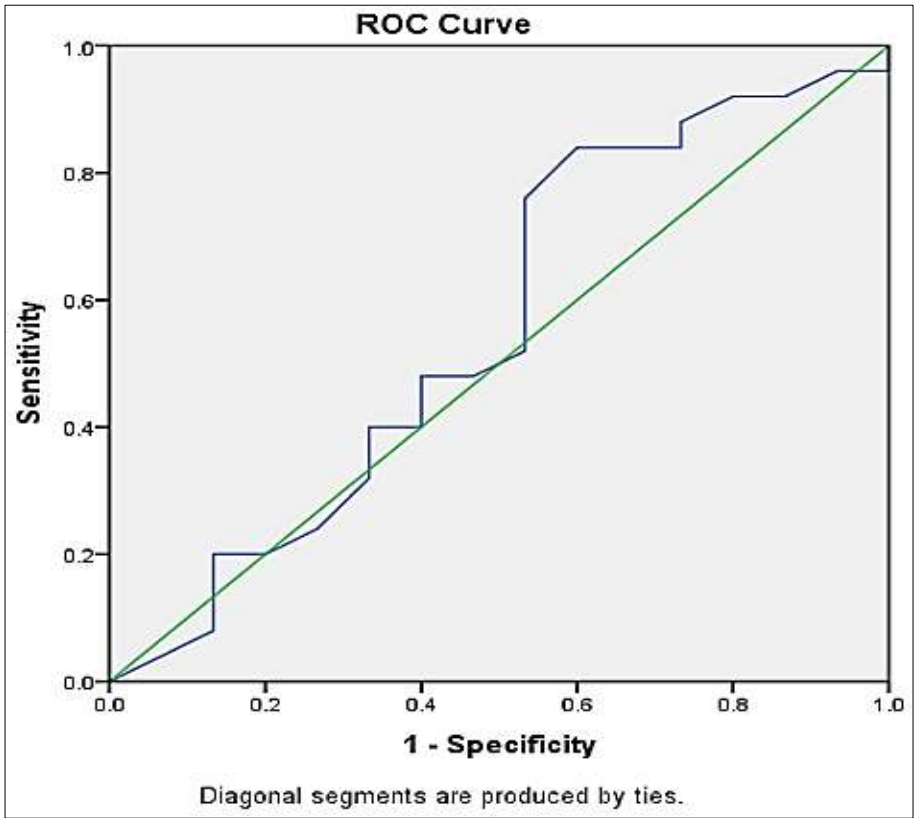

Fig 2: ROC Curve Comparison between BMI and Irsin

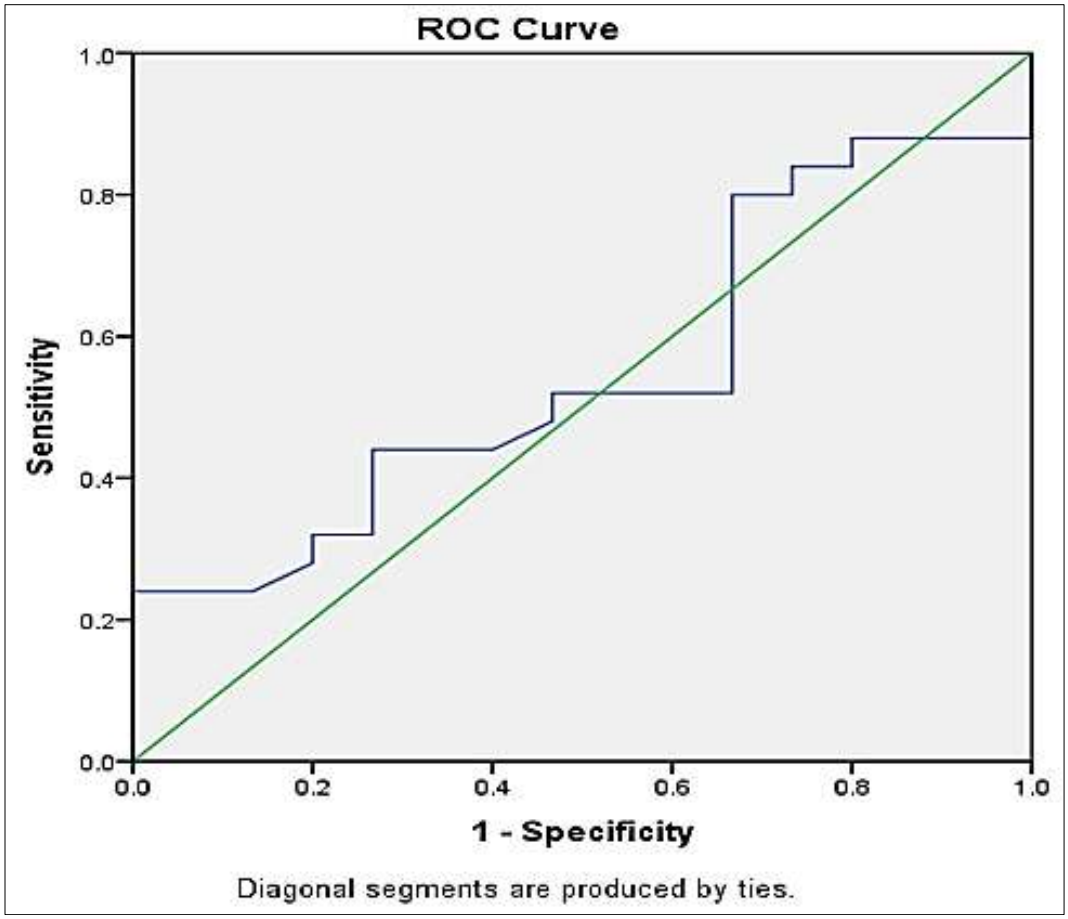

Fig 3: ROC Curve Comparison between BMI and OGG1

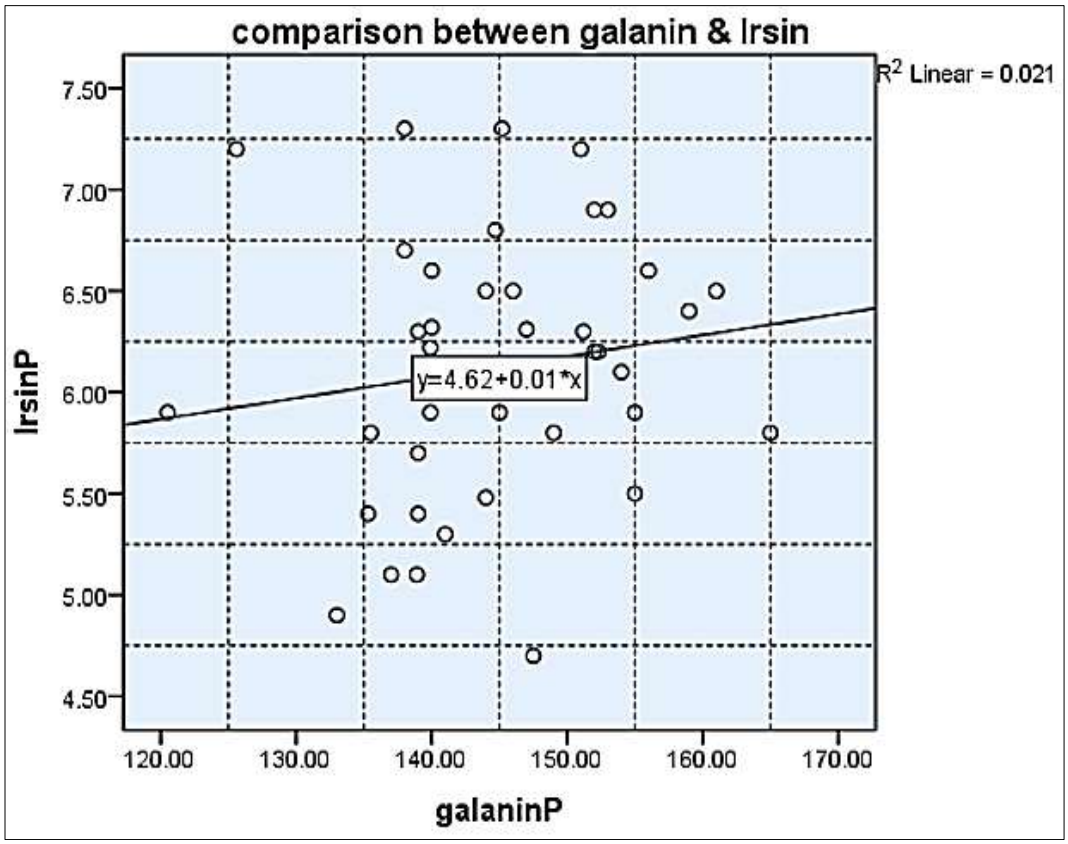

Fig 4: Relationship between Polycystic Ovary Syndrome patients' galanin and irisin levels

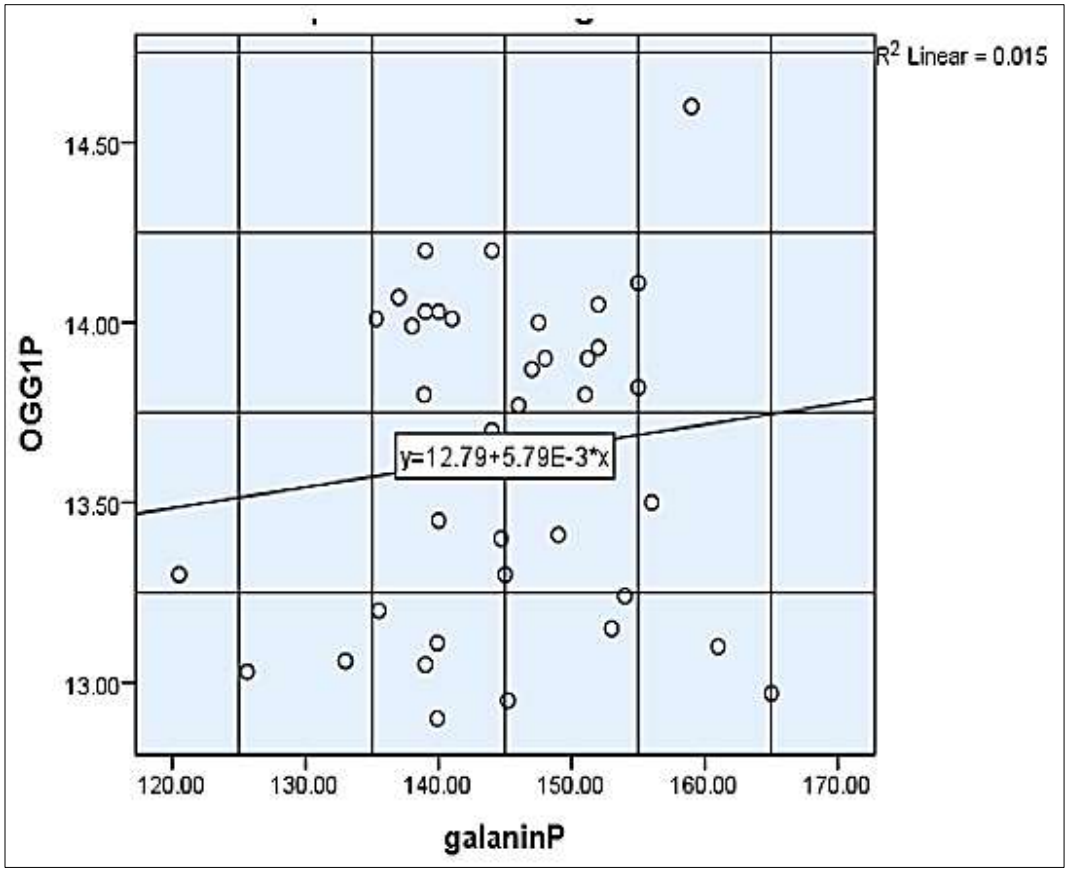

Fig 5: OGG1P and galanin levels in patients with polycystic ovary syndrome are correlated

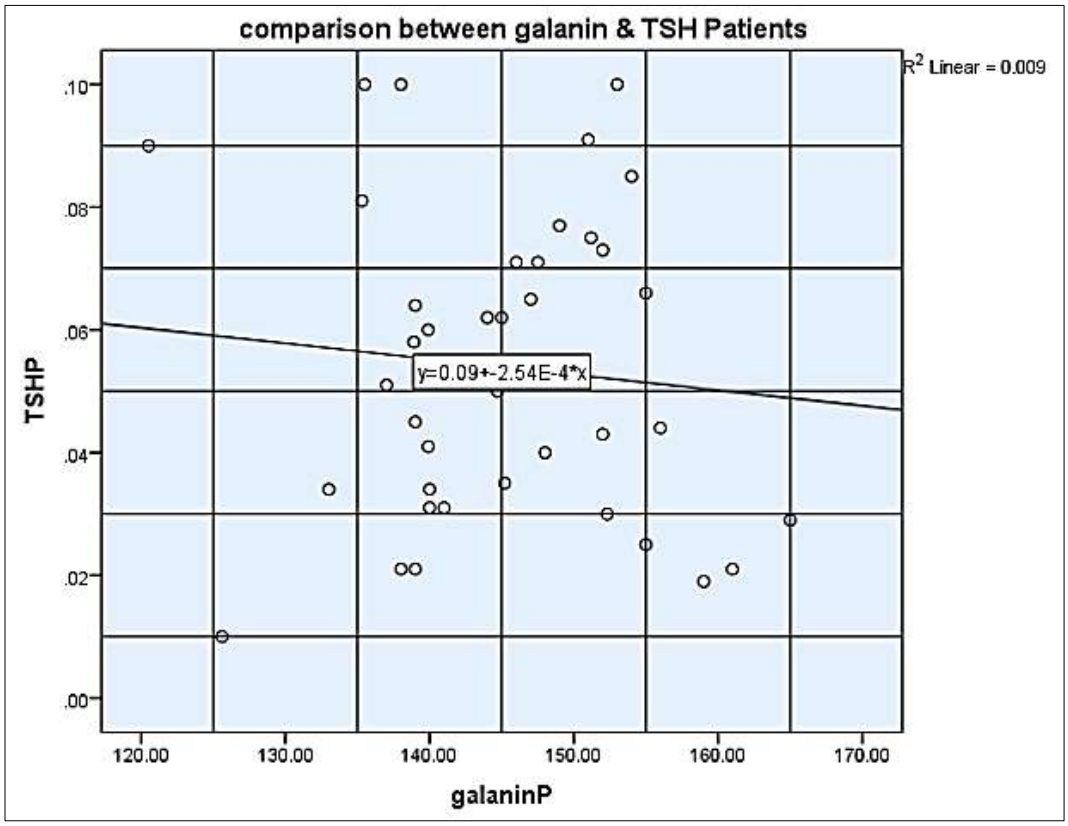

Fig 6: TSH and galanin levels in patients with polycystic ovary syndrome are correlated

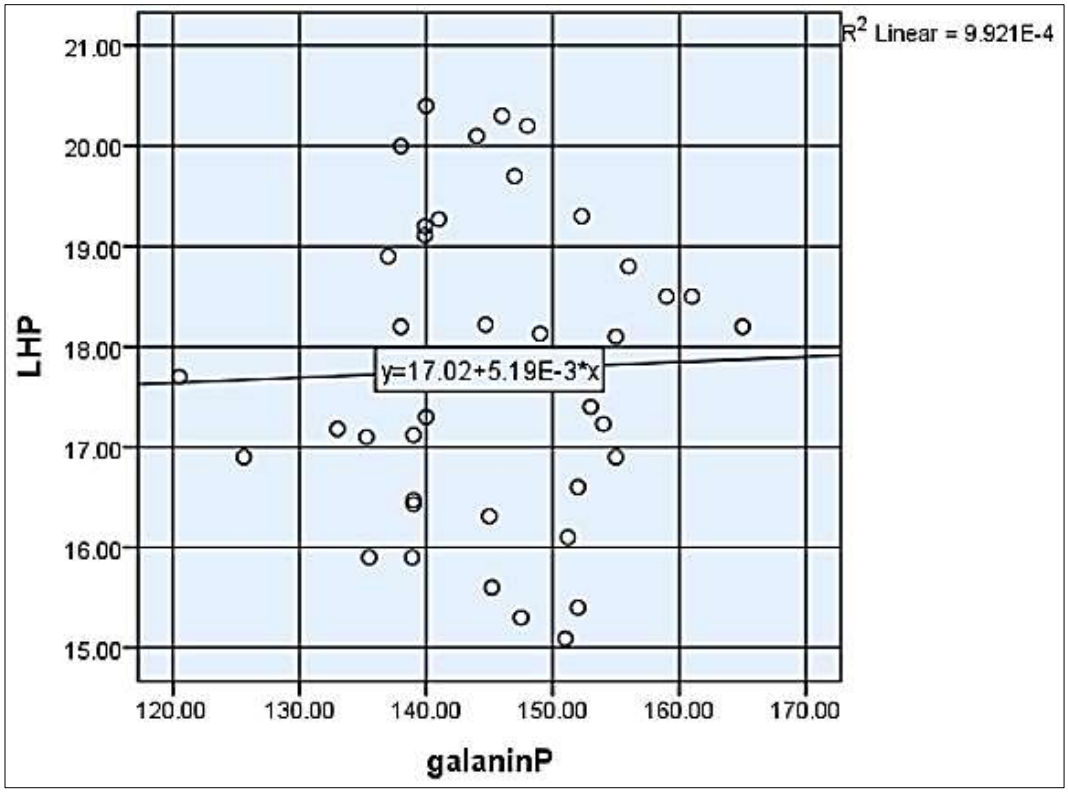

Fig 7: LH and galanin levels in patients with polycystic ovary syndrome are correlated

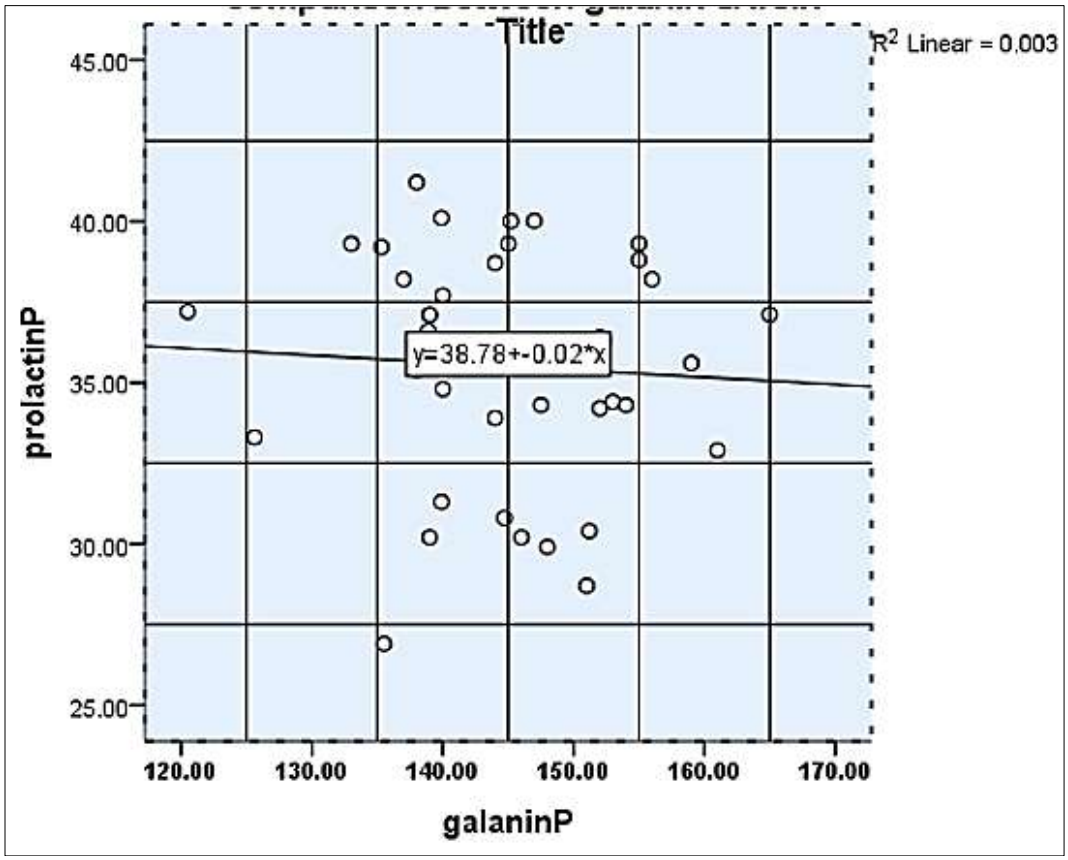

Fig 8: Prolactin and galanin levels in patients with polycystic ovary syndrome are correlated

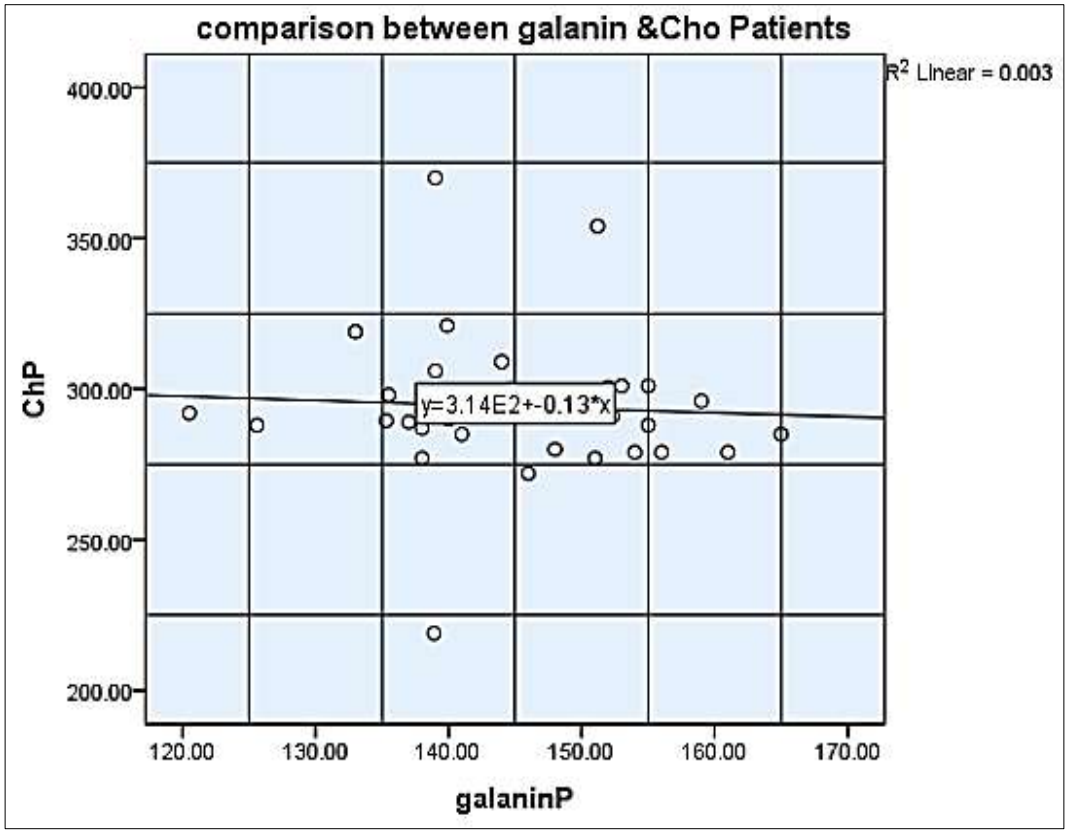

Fig 9: Relationship between Cho and Galanin Level in Polycystic Ovary Syndrome Patients

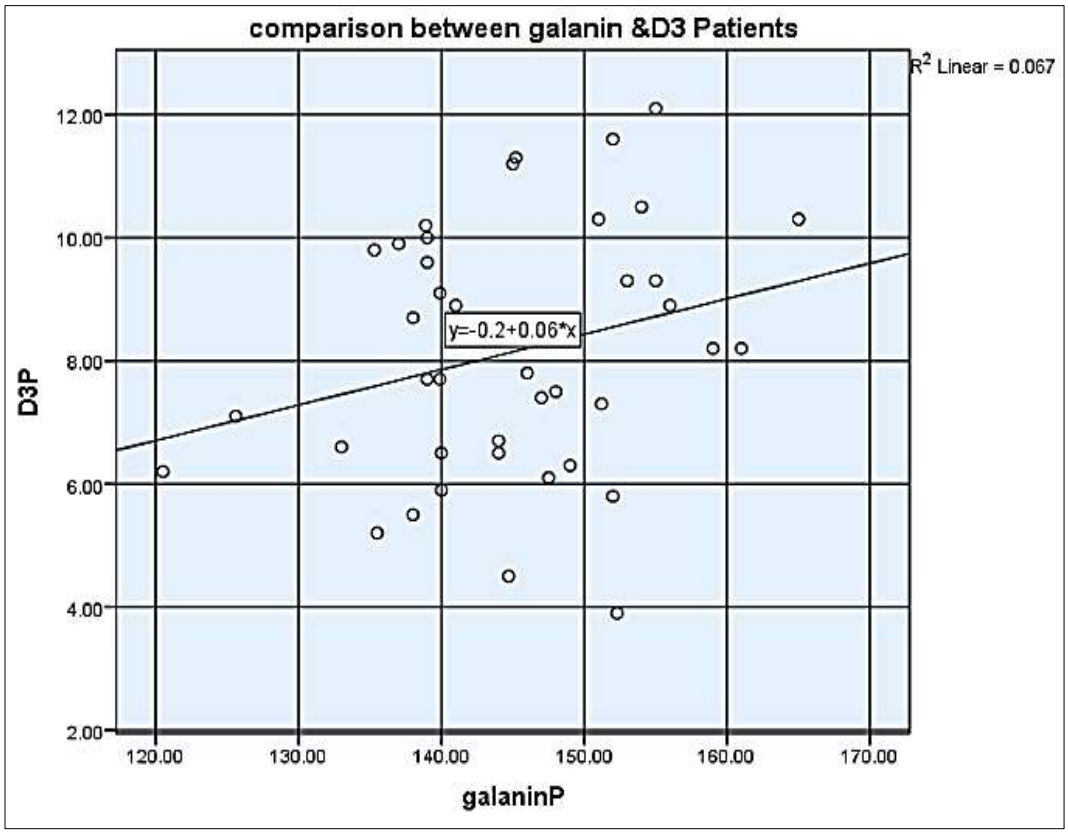

Fig 10: Relationship between Polycystic Ovary Syndrome patients' galanin and D3 levels

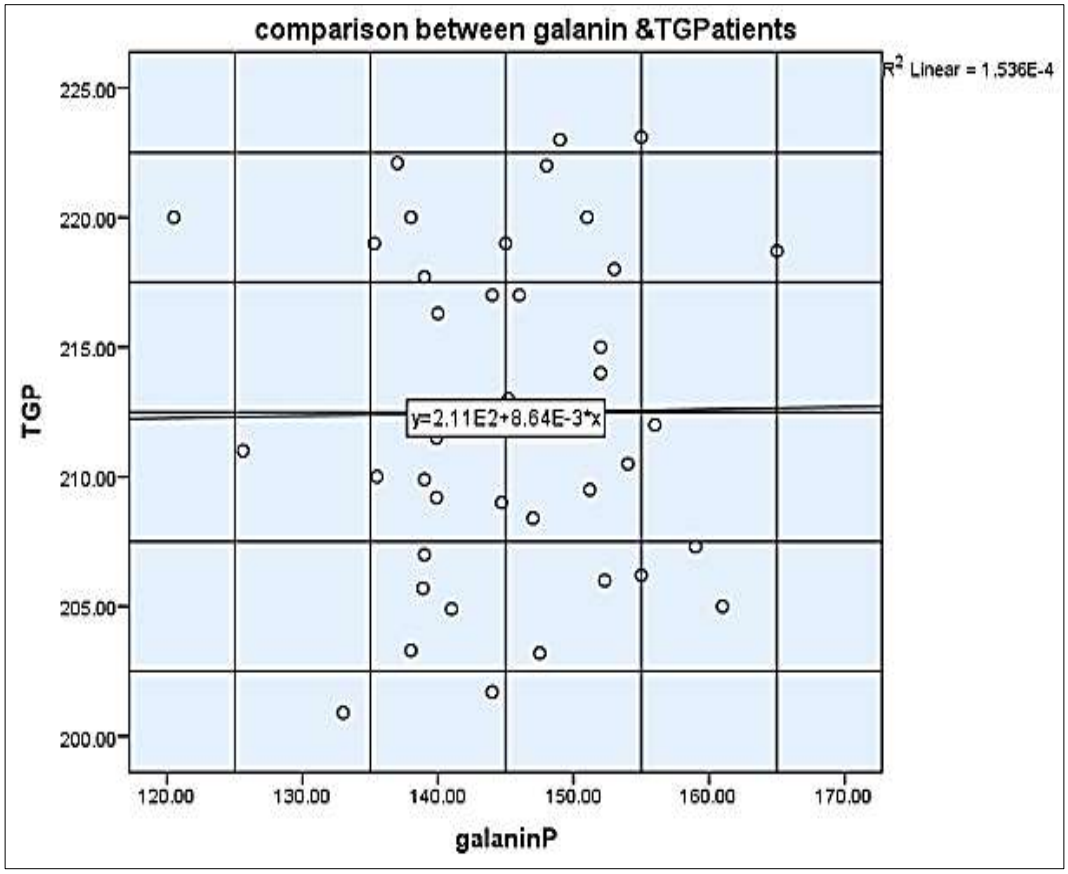

Fig 11: Galanin and TG levels in patients with polycystic ovary syndrome are correlated

Discussion

Insulin resistance and metabolic syndrome are frequently associated with PCOS, based on the ASRM/ESHRE criteria. Even after adjusting for BMI, female PCOS patients had a 4.5-fold higher risk of Metabolic Syndrome (MBS), which rose by 3.8 times for each quartile increase in bioavailable testosterone, per a study by COVIELLO [9].

The study's PCOS patients had irisin levels that were abnormally high in comparison to what is typically seen in PCOS patients. Although its exact cause is uncertain, PCOS is associated with metabolic problems, as was previously indicated. Serum irisin levels were greater in patients with metabolic syndrome, per a study by Park *et al*. [10]. Serum fasting irisin levels may be higher in PCOS patients than in control subjects because of the metabolic syndrome, which is linked to PCOS. Another theory suggests that a high serum irisin level may provide protection during the prediabetic stage of PCOS. Furthermore, it has been suggested that irisin resistance may be linked to PCOS, as it is comparable to leptin resistance in people who are obese and/or have metabolic syndrome [11]. Gelanin levels were higher in PCOS patients than in controls, according to our findings. Slim PCOS patients exhibited considerably greater galanin levels than the BMI-matched controls, according to group analysis that further separated the subjects depending on their BMI; however, this difference was not present in the overweight/obese group [12].

Insulin resistance, diabetes, appetite, obesity, dyslipidemia, hypertension, metabolic syndrome, and reproduction are all impacted by galanin. Numerous investigations have discovered a link between galanin and PCOs [13].

This result is consistent with other research regarding the negative association between PCOs and TSH in the healthy control group. One study found that a drop in TSH levels was correlated with a decrease in the number of antral follicles generated [14]. AMH is released by both antral and pre-antral follicles, hence it seems logical that the two chemicals would be inversely correlated in healthy people [15]. According to a number of studies, women with PCOS had higher blood levels of the hormone LH. Ambiger *et al*. found that compared to healthy women, women with PCOS had higher levels of LH [16].

Elevated blood levels of LH are consistent with our investigation's findings. But keep in mind that a lot of obese women without PCOS also have elevated LH levels. This suggests that LH secretion control is altered by fat, which may cause PCOS-like symptoms in obese women. On the other hand, the blood LH levels of obese women with PCOS did not differ statistically significantly from those of women without the illness. Therefore, PCOS cannot be diagnosed by LH levels alone [17].

The primary glycosylase that catalyzes the removal of DNA oxidation products is 8-oxoguanine DNA glycosylase (OGG1). In this work, we looked into possible routes and OGG1's function in PCOS development. When we initially looked at OGG1 levels in PCOS patients' blood and follicular fluid (FF), we found that these patients had much higher OGG1 levels. In line with a study conducted in 2022 by Xia, Jing, *et al*. [18], we likewise found a notable increase in OGG1 expression levels in the ovarian tissue of PCOS brought on by dehydroepiandrosterone (DHEA). 8-oxoguanine DNA glycosylase (OGG1) is the main glycosylase that catalyzes the elimination of DNA oxidation products. In this study, we investigated potential pathways and the role of OGG1 in the development of PCOS. When we first examined OGG1 levels in the blood and follicular

fluid (FF) of PCOS patients, we discovered that these patients had significantly higher OGG1 levels. We also discovered a significant rise in OGG1 expression levels in the ovarian tissue of PCOS caused by dehydroepiandrosterone (DHEA), which is consistent with a study done in 2022 by Xia, Jing, *et al*. [18]. This study found that vitamin D treatment did not stabilize the women's serum profiles or mitigate the effects of the condition. Drug administration may still be an important area of research, but it should not be overlooked in the fight against PCOS [20]. Rashidi *et al*. (2016) examined the relationship between serum 25-OH-Vit D3 levels and metabolic variables in women with polycystic ovarian syndrome who were not obese. A normal weight group and an overweight group (based on BMI) were established among non-fat women with polycystic ovarian syndrome. Serum levels of 25 (OH) D, FBS, CRP, TC, TG, LDL, HDL, INS, and IR were measured. 84.1% of women were found to be vitamin D deficient; yet, there was no discernible difference between the two groups. Their main finding was that there was a significant relationship between HDL serum levels, age, and 25 (OH) D. However, Rashidi *et al*. discovered that a decrease in 25 (OH) D levels was not statistically significantly associated with an increase in IR risk in non-obese women with polycystic ovarian syndrome [21].

Conclusion

The information gathered suggests that irisin could be used as a PCO diagnostic biomarker. PCOS patients had significantly higher serum levels of galanin and irisin than the control groups, and obese PCOS patients had higher levels than participants of normal weight. PCO patients had higher levels of blood irisin, galanin, 8 oxoguanine DNA glycosylase, lipid profiles (TG and cholesterol), and D3.Elevated irisin levels in PCOS patients can lead to risk factors like insulin resistance and dyslipidemia. The polycystic ovarian syndrome can be identified by changes in blood levels of the irisin biomarker.

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Ethical Approval

On December 24, 2023, the Tikrit University College of Medicine's Ethical Committee approved this study under file number 68/240. Consent from participants or their guardians was obtained prior to study participation and blood collection.

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