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The role of glypican-4, thyroid stimulating hormone, and reproductive hormones in polycystic ovary syndrome progress among Iraqi women

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Abstract
Background: Polycystic ovarian syndrome affects 4% to 21% of women around the world, making it the most common endocrine disease in women who can have children. There are different types of this sickness, or phenotypes. It shows up in the body as oligo-ovulation or anovulation, changes in the ovary polycystic, and hyperandrogenism, which includes acne, hair loss, and hirsutism.
Aims of the study: Check the connection between glypican-4, TSH, and reproductive hormone levels and the development and identification of polycystic ovary syndrome.
Methodology: A case-control study was conducted at Al-Imamin Al-Kazemin Medical City, with the approval of the hospital's ethical committee. Patients' data, such as age and body mass index (BMI), were collected. Seventy women, 35 with PCOS and 35 healthy as controls, were recruited between February 2022 and September 2022. Women were between 18 and 45 years old in both groups. Patients with thyroid diseases, viral infections, or immune diseases were excluded, and 5 ml of blood was drawn and placed in a gel tube, then separated using centrifugation and hormones such as GLY-4, LH, FSH, prolectin, testosterone, and TSH were estimated using. ELISA technology.
Results: The results showed that there was no statistical significance in age and body mass index between the two groups. The results also showed a significant increase in the levels of GLY-4, LH, FSH, and Prolactin between the PCOS group and the control group. While the results showed no statistical significance in the levels of Testosterone and TSH between the two groups.
Conclusions: The findings showed that glypican-4 had a positive relationship with age, testosterone, prolactin, and follicle-stimulating hormone. On the other hand, it had a negative relationship with BMI and follicle-stimulating hormone. Women with polycystic ovary syndrome also showed higher levels of glypican-4 compared to women without the syndrome. This indicates the important role that the protein glypican 4 plays in influencing hormone levels and the relationship between them.

Keywords: Glypican-4, polycystic ovary syndrome, prolactin, *Follicle-stimulating hormone*, luteinizing hormone

Introduction
PCOS is a common metabolic and endocrine disorder that affects between 6% and 21% of women, based on their mean body mass index (BMI) and the diagnostic criteria that are used.in women who are overweight [1]. For PCOS to be identified, a woman must have hyperandrogenism, oligo/anovulation, and polycystic ovarian morphology. However, 30-75% of these women also have weight problems. Adipose tissue is an endocrine organ that affects a lot of chemicals. Hormonal problems may make the digestive problems that come with being overweight even worse [2]. Polycystic ovary syndrome is a common endocrine disease in women of childbearing age. Depending on the diagnostic criteria used, 4 to 21% of women with this disorder have it. A systematic review of studies found that the frequency was slightly higher in black and Middle Eastern populations than in Chinese and white populations9, but it's hard to make comparisons between ethnic groups because of differences in how people are diagnosed and how they are recruited. It looks like the number of diseases in the world is rising quickly. In 2019, statistics from 204 countries showed that the age-standardized point prevalence was 1677.8 per 100,000 people and the annual incidence was 59.8 per 100,000 people. These numbers show increases of 30.4% and 29.5% since 1990.11 Polycystic ovary syndrome needs to be seen as an international public health issue [3, 4] because it is becoming more common and is associated with higher rates of illness. An important example of a metabolic disease is polycystic ovary syndrome, which is linked to insulin resistance and can lead to heart and metabolic risk, which is made worse by being overweight.

Because of this, PCOS raises the chance of getting T2D, impaired glucose tolerance, dyslipidemia, non-alcoholic fatty liver disease, and obstructive sleep apnea (OSA). Between 6% and 10% of women of childbearing age have polycystic ovary syndrome [5]. PCOS can show up at any point in a woman's sexual life, but it most often starts in her teens. Polycystic ovary syndrome is marked by both reproductive and hyperandrogenic symptoms, such as irregular periods, trouble getting pregnant, hair growth, androgenic alopecia, and hirsutism. When someone has polycystic ovary syndrome, they often also have high levels of androgens [6, 7]. Obesity and PCOS have a reciprocal relationship in which they both increase one another in an ongoing cycle [8]. Androgen levels are higher in obese and overweight women, which causes hirsutism and excessive seborrhea [9]. Miscarriage rates are much higher, and fertility is severely decreased compared to women with normal body weight; the rate of spontaneous miscarriages is greater [10, 11]. Obesity may interfere with progesterone secretion, decrease follicle-stimulating hormone (FSH) and luteinizing hormone (LH) excretion, and disrupt ovulation [12, 13]. The PCOS has lasting repercussions, including an increased risk of diabetes and cardiovascular disease (CVD), which are significantly influenced by obesity. Deficiencies in minerals and obesity, as mentioned previously [14]. Obesity and insulin resistance (IR) are linked to PCOS. Yet, a growing number of lean, slim women are also diagnosed with PCOS. People have a gene called GPC4 that makes a protein called glycopican-4. Heparan sulfate proteoglycans on the surface of cells are made up of a protein core that is attached to the membrane and a number of heparan sulfate chains that can be different lengths. In the glypican-related integral membrane proteoglycan family (GRIPS), there is a core protein that is linked to the cytoplasmic membrane by a glycosyl phosphatidylinositol chain. These proteins might help control how cells divide and how much they grow. It is next to the 3' end of GPC3 and may also have something to do with Simpson-Golabi-Behmel syndrome [15, 16]. Protein Gpc4 helps control how cells grow, differentiate, and change form. It is a member of the heparan sulfate proteoglycan family. Gpc4 also played a big role in managing important proteins such as Sonic Hedgehog, Wnt, bone morphogenetic proteins, and fibroblast growth factor [17]. To control the differentiation of preadipocytes, the amount of Gpc4 in the cell may change how growth and differentiation factors connect to their unique high affinity signal-transducing receptors. Here you can see that the amounts of Gpc4 mRNA expression are very different in subcutaneous and abdominal fat. In human fat, there were strong links found between Gpc4 mRNA and both body mass index (BMI) and waist/hip ratio (WHR). This meant that Gpc4 might be very important in obesity and the spreading of body fat [17, 18]. We discovered that pregnant filial generation mice that were given low amounts of di-2-ethylhexylphthalate had more epididymal adipose tissue weight and Gpc4 mRNA expression. This makes me think that the Gpc4 gene might have something to do with storing fat. But no one knows for sure how Gpc4 decides where fat is kept [18, 19].

Methodology

A case-control study was conducted in Al-Imamain Alkadhimain Medical City. The Hospital ethical committee authorized this study's procedure. Age, body mass index (BMI), family history of hypertension, unfavorable pregnancy, multiple pregnancies, testosterone, androstenedione, and other personal and clinical treatment facts were obtained from patients. Seventy women included 35 women with PCOS and 35 healthy non-PCOS women as control were gathered between February 2022 and September 2022. Age was ranged between 18-45 years for the two groups. Patients with thyroid diseases, viral infections, or other immune diseases were excluded according to inclusion criteria. 5 ml of blood was drawn from each participant and placed in a gel tube and left at room temperature for 15 minutes until coagulation. Then it was separated using a centrifuge for 30 minutes at 3500 rpm, and the blood serum was isolated and kept at -20 degrees Celsius until use. Serum Glypican-4 (GLY-4), LH, FSH, Prolactin, Testosterone, and TSH hormones were measured using the ELISA technique.

Limitation

The sample size was determined based on a power calculation, ensuring that it was sufficient to detect a statistically significant effect. This approach supports the reliability of the findings despite the smaller sample size. Furthermore, while a larger sample size can enhance the generalizability of the results, the specificity of the population under study and the rigorous selection criteria have contributed to the robustness of the data, thus partially compensating for the smaller sample size. Lastly, resource and time constraints often limit the scope of research. In our case, these limitations were carefully balanced against the need for scientific rigor, resulting in the selection of a sample size that is both practical and methodologically sound.

Statistical analysis

Statistical analysis is often used to analyze quantitative data, and provides methods for data description, simple inference for continuous and categorical data. The procedure involves the collection of data leading to test of the relationship between two statistical data sets. In this study all data are presented as frequency and percentage. We used SPSS (Version 26) and the dependent t-test (Two-tailed) and independent t-test (Two-tailed) for variables that had a normally distributed distribution. For variables that did not have a normally distributed distribution, we used the Mann-Whitney U test, the Wilcoxon test, and the Chi-square test. $M < 0.05$ was seen as statistically significant.

Ethical approval

The study was approved by the human ethics committee of Al-Imamain Alkadhimain Medical City, Everyone who took part in the study was told about it and asked to sign a consent form. The patient was also guaranteed that his information would be kept private.

Results

Comparison of age and BMI between patients and controls: The table results show the mean and standard deviation for age and body mass index for both the patient group and the control group. Based on the data, the mean age of the patients was 28.50 years with a standard deviation of 7.37, while the mean age of the control group was 33.06 years with a standard deviation of 9.79; The calculated probability value was 0.06 and showed no statistical significance. As for the body mass index, its average value for patients was 27.40 kg/m2 with a standard deviation estimated at 3.95, while its average value for the control group was 26.70 kg/m^2 with a standard deviation estimated at 4.20. The calculated probability value was 0.5 and showed no statistical significance.

Table 1: The mean and standard deviation of age and BMI for the patients and control group

Variable	Mean±SD		P-Value
	Patients (N=35)	Controls (N=35)	
Age (Years)	28.50±7.37	33.06±9.79	0.06^{Ns}
BMI (kg/m^2)	27.40±3.95	26.70±4.20	0.5^{Ns}

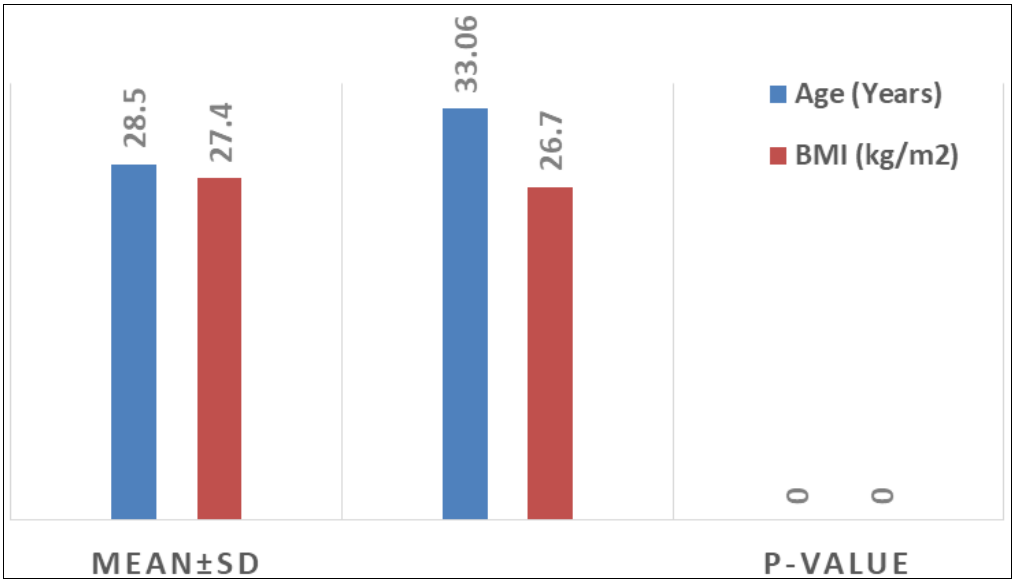

Fig 1: The difference of age and BMI for the patients and control group

Comparison of Hormone Levels between Patients and Controls

The results indicated that there were significant differences between the chemical variables of the patients and the control group. The value of glypican-4 (ng/ml) showed a mean of 18.40 with a standard deviation of 5.45 for patients, while the value for the control group was 9.20 with a standard deviation of 2.87, and the probability value was labeled as 0.00001. The values of the hormones LH (IU/L), FSH (IU/L), and TSH (mlU/L) also showed significant statistical differences between the two groups, while the probability value for the hormone prolactin (ng/ml) was not statistically significant (0.09). Finally, testosterone values (ng/dl) showed no statistically significant difference between patients and controls, with a p value of 0.51.

Table 2: The difference of biochemical variables for the patients and control group

Variables	Mean ± SD		P value
	Patients (N=35)	Controls (N=35)	
GLY-4 (ng/ml)	18.40±5.45	9.20±2.87	0.00001 S
LH (IU/L)	9.18±4.76	4.45±1.65	0.00001 S
FSH (IU/L)	5.32±1.44	4.36±1.38	0.01 S
Prolactin (ng/ml)	18.35±8.07	15.69±3.65	0.09 Ns
Testosterone (ng/dl)	0.27±0.13	0.29±0.14	0.51 Ns
TSH (mlU/L)	2.18±2.46	1.54±0.97	0.17 Ns

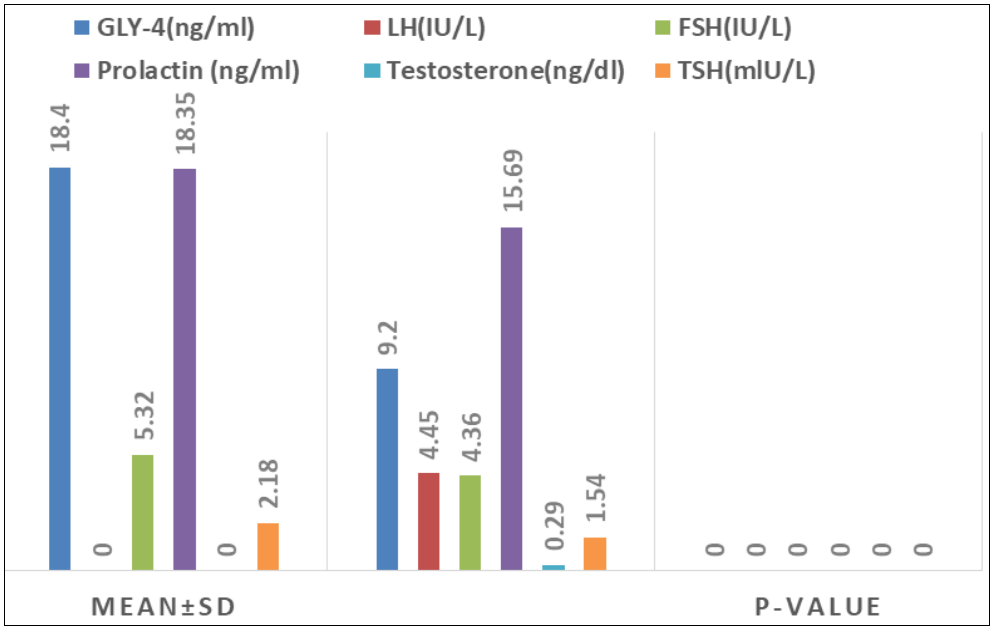

Fig 2: The difference of biochemical variables for the patients and control group

The correlations between anthropometric serum GLY-4 and other hormones in the study groups

Regarding age (years) the result show the value was 0.05 for patients and 0.37 for the control group, while the p value was 0.84 and 0.04, respectively. While the body mass index (kg/m2) the result show the value was 0.44 for patients and 0.15 for the control group, and the p-value was 0.44 and -0.14. Regarding FSH (IU/L) the value was 0.40 for patients and -0.17 for the control group, and the p value was 0.85 and -0.03. While LH (IU/L) the value was 0.96 for patients and 0.01 for the control group, and the probability value was 0.26 and -0.20. Regarding prolactin (ng/ml) the value was 0.73 for patients and 0.07 for the control group, and the probability values were 0.05 and 0.34 for the testosterone (ng/dl) the value was 0.15 for patients and -0.28 for the control group, and the p values were 0.71 and 0.07. While TSH (mlU/L) the value was 0.73 for patients and -0.07 for the control group, and the p values were 0.70 and 0.07.

Table 3: The correlations between anthropometric serum GLY-4 and other hormones in the study groups

Serum GLY-4 Variables	Patients (N=35)		Controls (N=35)	
	p	r	P	R
Age(Years)	0.05	0.37	0.84	0.04
BMI(kg/m^2)	0.44	0.15	0.44	-0.14
FSH(IU/L)	0.40	-0.17	0.85	-0.03
LH(IU/L)	0.96	0.01	0.26	-0.20
Prolactin (ng/ml)	0.73	0.07	0.05	0.34
Testosterone (ng/dl)	0.15	-0.28	0.71	0.07
TSH (mlU/L)	0.73	-0.07	0.70	0.07

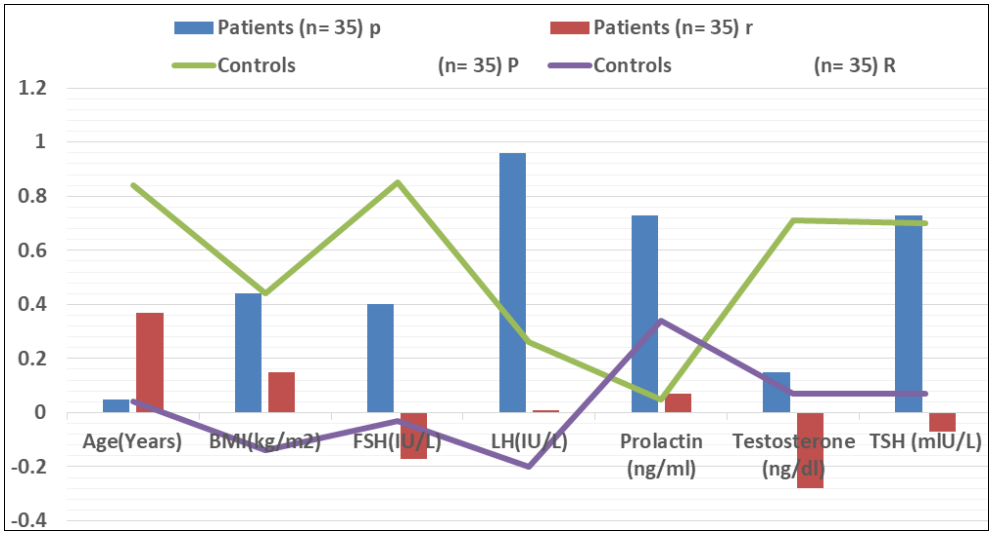

Fig 3: The correlations between anthropometric serum GLY-4 and other hormones in the study groups

The ROC curve analysis for the main variables in the study groups

The results of ROC curve analysis of the main variables in the study groups show that the glypican-4 level (ng/ml) had an AUC value of 0.957 with a sensitivity of 89.29 and a specificity of 91.18, and a P value of less than 0.0001. While the TSH level had an AUC value of 0.596 with a sensitivity of 50.00, a specificity of 73.53, and a probability value of 0.1923. Regarding testosterone level, it had an AUC value of 0.534 with a sensitivity of 7.14, a specificity of 100.00, and a probability value of 0.6471. As for the prolactin level, it had an AUC value of 0.646 with a sensitivity of 35.71, a specificity of 97.06, and a probability value of 0.0559.

Table 4: The ROC curve analysis for the main variables in the study groups

Variables	AUC	Sensitivity	Specificity	P-Value
GLY-4 (ng/ml)	0.957	89.29	91.18	< 0.0001
TSH	0.596	50.00	73.53	0.1923
Testosterone	0.534	7.14	100.00	0.6471
Prolactin	0.646	35.71	97.06	0.0559

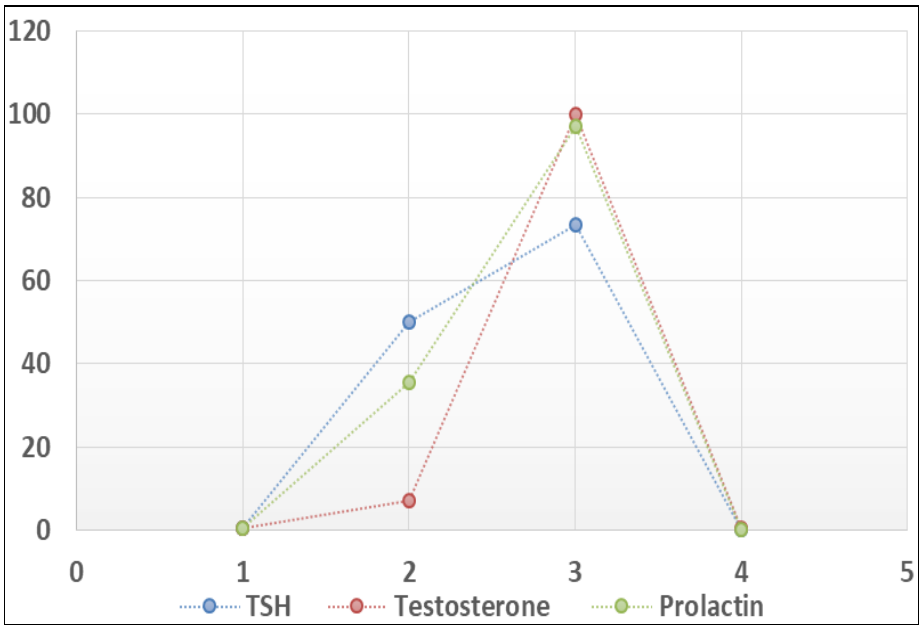

Fig 4: The ROC curve analysis for the main variables in the study groups

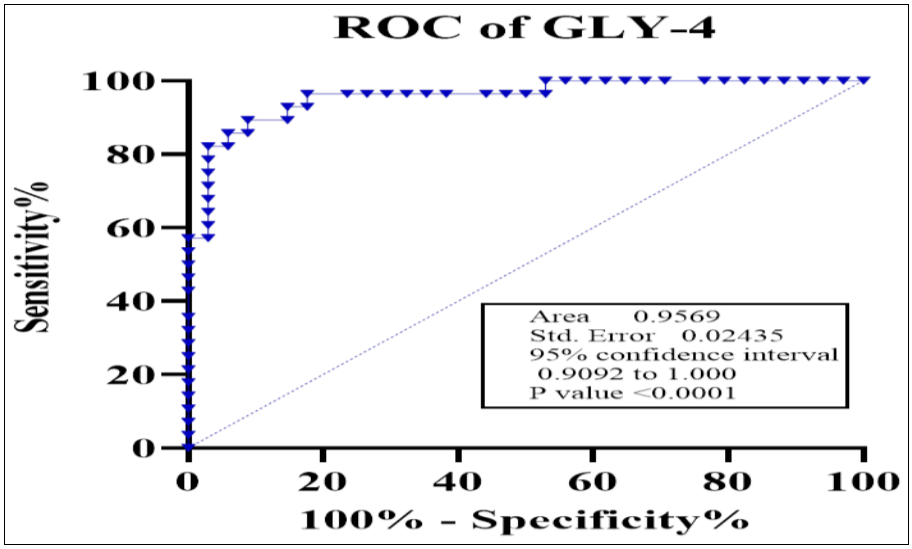

Fig 5: The ROC analysis of GLY-4

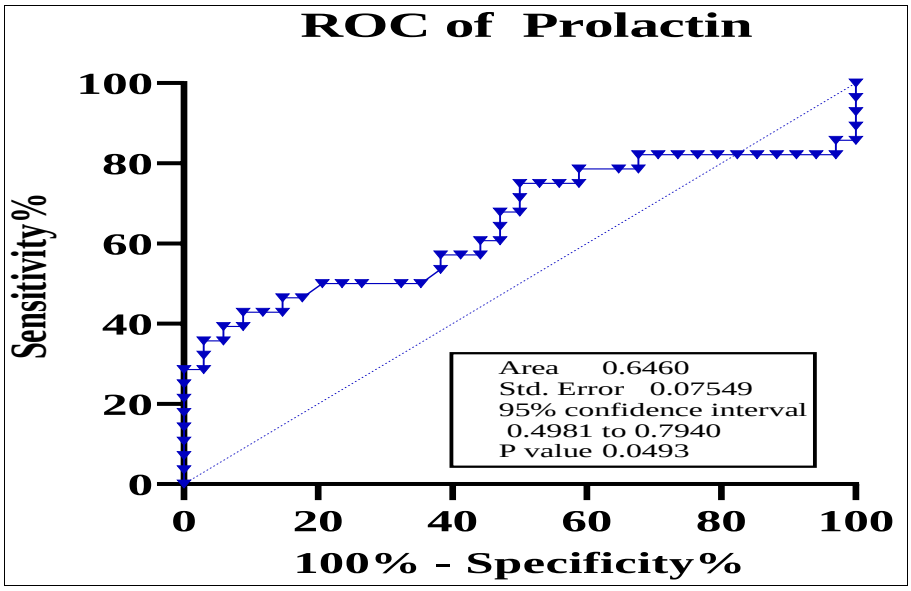

Fig 6: The ROC analyses of prolactin

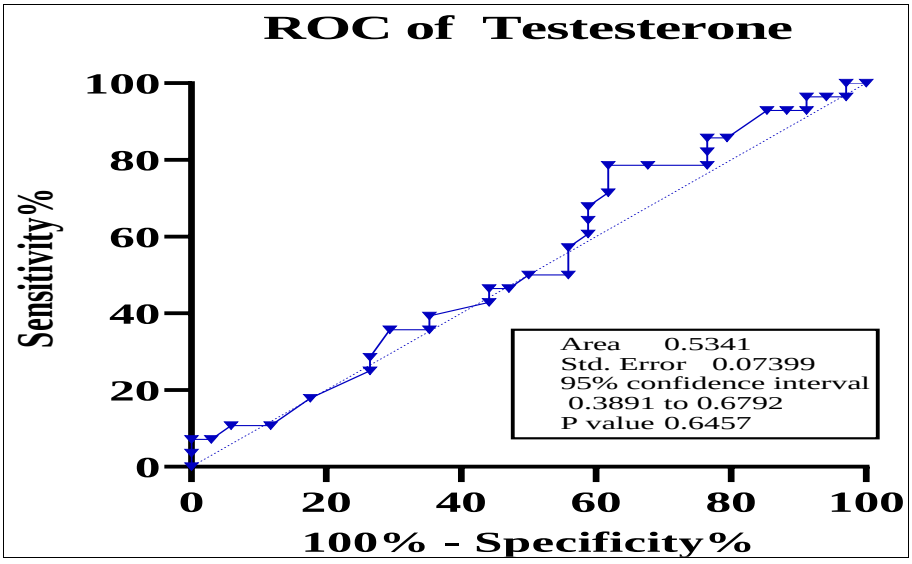

Fig 7: The ROC analyses of testosterone

Discussion

There was no statistically significant difference between the two groups in terms of age or body mass index. Also, there was a big difference between the polycystic ovary syndrome group and the control group in the amounts of GLY-4, LH, FSH, and prolactin. It was found that there was no statistically significant difference between the two groups in the levels of testosterone and TSH. In line with [20, 21], this finding is

correct. In 1995, Watanabe *et al*. discovered glycopican-4 for the first time in mice's kidneys and growing brain tissue. Gesta *et al*. showed that visceral and subcutaneous fat areas have different genetic expressions of glypican-4. The researchers discovered that having less glypican-4 in the subcutaneous and more in the visceral fat tissue is linked to having a higher waist-hip ratio (WHR) and body mass index (BMI), which means there are higher chances of metabolic and cardiovascular problems [22-24].

Glypican-4 is a new adipokine that was found not long ago. This is a cell surface proteoglycan that works directly with the insulin receptor to improve insulin receptor signaling and adipocyte development. This makes it different from other insulin sensitizers. Glypican-4 binds to the insulin receptor and makes it more sensitive to insulin. It then controls how insulin works and what signals are sent afterward. There is also more glypican-4 in the blood of people who have trouble handling glucose. These levels are connected to both body mass index (BMI) and waist-to-hip ratio (WHR), which is a part of the Homeostasis Model Assessment (HOMA) measure [25]. One small study was the only one that looked at glycopyran-4 levels in women with PCOS. They did a test study [26] to see if there was a link between glypican-4 and heart health in people with PCOS. They found that the amounts of glypican-4 were higher in women with PCOS compared to women who were not obese. Even though the women in the study had a low mean BMI of 22 kg/m^2 and no lipid disorders, these amounts were linked to heart disease risk factors, especially the spread of fat. As with this study, their results showed a positive link with androgenic markers and metabolic markers such as insulin and HOMA-IR. Multiple Linear Regression analysis in our study showed that BMI was a separate risk factor for glypican-4 values. In the single study [27], PCOS no longer had a big effect on the levels of glypican-4. We don't know a lot about how glypican-4 speaks. It's also not clear what role it plays in adipocytes or how it controls metabolism. We think that this subject should be studied in more genetic detail because we don't fully understand what takes place inside cells once the receptor binds. It is thought that if glypican-4 is working as a drug that makes insulin more sensitive, then it may cause glypican-4 levels to rise in people who are insulin resistant. Glypican-4 agonists can be used to treat things in this way [28]. So, our results show that women with PCOS have higher amounts of glypican-4 than women who did not have PCOS, and their serum galanin levels are lower. We need to do more study to find out if these adipokines can be used as extra signs for insulin sensitivity and cholesterol profile, and if they might have something to do with PCOS, which makes you more likely to get metabolic and cardiovascular diseases. Researchers looked at a lot of different factors and found that PCOS and BMI may be the best ways to tell if galanin levels will go up or down. Also, BMI was the best way to tell if glypican-4 levels would go up. More study needs to be done to find new ways to use medicine to treat insulin resistance. People with PCOS may not have enough galanin, which can make insulin resistance worse. Giving the body more galanin may make it more responsive to insulin [29]. They could also be used to treat insulin intolerance and too much androgen in future research. A small sample size could be bad for the plan that is being considered. There weren't enough patients in this study for the phenotypic features to be broken up into smaller groups. Even so, the fact that these adipokines are different in young, thin women with PCOS shows that we need to learn more about how these paths work [30]. GLY-4 is the name of adipokine, a proteoglycan that is present on the surface of cells and interacts directly with the insulin receptor. GLY-4 functions as an insulin sensitizer by binding to the insulin receptor, which in turn regulates the actions of insulin and the subsequent signals. Insulin receptor activity decreases when GLY-4 levels decrease, as demonstrated by *in vitro* studies. Circulating quantities are associated with BMI and insulin sensitivity (IS) [31]. The objective of the investigation is to determine the relationship between GLY-4 and PCOS. Women with PCOS had higher amounts of GLY-4 compared to women who did not have PCOS. These levels were also linked to CV risk factors, especially the way fat was distributed [32]. We don't know how Glypican-4 works in adipocytes or how it is connected to metabolic control or how it can send signals. It's not clear what happens inside cells once a receptor binds to it, but if GLY-4 is working as an agent to raise IS, then when IR is present in a person, GLY-4 levels may rise to raise IS [33]. In this manner, glycopican-4 agonists can be employed as medications. Therefore, our findings indicate that women with PCOS have elevated levels of GLY-4 in their bloodstream compared to those who do not have PCOS [33]. In order to determine whether these adipokines could serve as novel markers for lipid profile and IS, as well as whether they may contribute to the development of PCOS, which increases the likelihood of metabolic and cardiovascular diseases, additional research is required. GLY-4 levels were significantly influenced by BMI, which was the most significant predictor. In the future, glycopican-4 agonists may also be employed to treat IR and an excess of testosterone. The intended design may be rendered less adaptable due to the limited sample size [34]. The ROC curve analysis of serum GLY-4 demonstrated that this marker could differentiate between women with PCOS and other women (Table 4). In this table, the sensitivity was 89%, the specificity was 91%, and the area under the curve (AUC) was 0.957. The ROC test for GLY-4 was determined to be more specific and sensitive than the other tests conducted in this study by this study. Table 4 illustrates this. This is due to the direct interaction between the insulin sensor and GLY-4. When GLY-4 binds to the insulin receptor, it makes the body more sensitive to insulin. This changes the way insulin works and the cues that come after [34]. Studies done in a lab dish show that GLY-4 makes insulin sensors work less well. Levels in the blood change based on BMI and IS [35, 36]. GLY-4 drugs can be used to treat IS if GLY-4 makes it better. This means that women with PCOS have more GLY-4 than other women. It needs to be looked into further to see if these adipokines can be used to show IS and lipid profile or if they have anything to do with PCOS. The best way to guess GLY-4 levels was to look at BMI, and they went up over time [37]. Because the study's sample size was so small, the patients' physical traits could not be separated. There needs to be more research on these adipokines to fully understand how they work [36, 37], even though they are found in excessive amounts in young, thin women with PCOS. A hormone called thyroid hormone controls the hypothalamic-pituitary gonadal system in women. The connection between thyroid health and PCOS has been looked into a great deal because of this. One study looked at people who have Hashimoto's thyroiditis (HT) and found that they are more likely to have PCOS than people who don't have HT [38]. Natalia Zeber-Lubecka *et al*. did a study and found that people with PCOS and HT had more versions of mitochondria than people with PCOS who only had HT. This means that differences in mitochondrial DNA may play a big role in why PCOS and HT happen together [39]. This was found in another study with rats. Rats with hypothyroidism had fewer follicular cells than rats in the control group. Also, rats with hypothyroidism had a lower rate of ovulation, which is similar to the long-term lack of ovulation seen in PCOS [40]. Different people still have different ideas about how thyroid hormones change in people with PCOS. A big Danish study found that people with PCOS were twice as likely to have hyperthyroidism as people of the

same age who were not PCOS. Several studies, though, have shown that PCOS people are more likely to have hypothyroidism than hyperthyroidism [41]. But another study [42] found that people with and without PCOS had the same chance of getting thyroid cancer. So far, it's not clear if the link between thyroid function and PCOS is causal or not. This is because bias, confounding, and reverse causality can all affect observational studies.

Conclusion

There was more glypican-4 and less galanin in the PCOS group women than in the other groups of women. It needs more study to see if these adipokines can be used as extra signs of lipid balance and insulin sensitivity. It's also important to find out if these adipokines play a role in the biology of PCOS, which is linked to a higher chance of getting diabetes and heart disease. This study shows that we need to do more work to fully understand how these lipids affect PCOS and how they are linked to metabolic, cardiovascular, and cardiac risks.

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