Spexin as a marker of ovarian dysfunction in PCOS related infertility

La espexina como marcador de disfunción ovárica en la infertilidad relacionada con el síndrome de ovario poliquístico

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Abstract

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in young women with complex underlying pathophysiology including obesity and insulin resistance as a key feature. Spexin is a protein hormone involved in diverse body metabolic events and disorders of central and peripheral gonadal function that is the core of this study which aims to evaluate its role in PCOS-related infertility.

Material and methods: Sixty women with PCOS are divided into two groups, [group I=30 fertile and group II=30 non-fertile] and 30 healthy fertile women as a control group. Spexin, AMH, HOMA-IR, and levels were measured by ELISA and Cobas E411.

Results: Spexin levels were significantly lower in PCOS subjects compared with the control group (p< 0.001), while the statistical data showed no significant differences between the fertile and non-fertile groups with a p-value (0.79). Spexin showed a negative correlation with insulin resistance, BMI, LH, and Testosterone in PCOS women.

Conclusion: The significant association of Spexin with LH, IR, and androgens suggest its possible role in PCOS pathophysiology, additionally Spexin showed a highly significant screening value and reliable diagnostic methods to differentiate PCOS cases versus control, as well as good diagnostic behavior to diagnose infertile cases but with less screening value.

Keywords: Spexin, PCOS, HOMA-IR, BMI, Hyperandrogenism

Resumen

Antecedentes: el síndrome de ovario poliquístico (SOP) es un trastorno endocrino común en mujeres jóvenes con una fisiopatología subyacente compleja que incluye la obesidad y la resistencia a la insulina como una característica clave. Spexin es una hormona proteica involucrada en diversos eventos metabólicos del cuerpo y trastornos de la función gonadal central y periférica que es el núcleo de este estudio que tiene como objetivo evaluar su papel en la infertilidad relacionada con el SOP.

Material y métodos: Sesenta mujeres con SOP se dividen en dos grupos, [grupo I=30 fértiles y grupo II=30 no fértiles] y 30 mujeres fértiles sanas como grupo control. Spexin, AMH, HOMA-IR y los niveles se midieron por ELISA y Cobas E411.

Resultados: Los niveles de espexina fueron significativamente más bajos en las pacientes con SOP en comparación con el grupo control (p< 0,001), mientras que los datos estadísticos no mostraron diferencias significativas entre los grupos fértiles y no fértiles con un valor de p (0,79). Spexin mostró una correlación negativa con la resistencia a la insulina, el IMC, la LH y la testosterona en mujeres con SOP.

Conclusión: La asociación significativa de Spexin con LH, IR y andrógenos sugiere su posible papel en la fisiopatología del SOPQ, además, Spexin mostró un valor de detección altamente significativo y métodos de diagnóstico confiables para diferenciar los casos de SOPQ del control, así como un buen comportamiento diagnóstico para diagnosticar la infertilidad. casos pero con menos valor de cribado.

Palabras clave: Spexin, PCOS, HOMA-IR, IMC, Hiperandrogenismo

Introduction

Polycystic ovarian syndrome (PCOS) is a global health problem with ovulatory dysfunction is one of the diagnostic criteria that includes additionally androgen excess and polycystic ovarian morphology[1]. It is a common condition with a worldwide prevalence of around 10%[2]. Menstrual irregularities, infertility, and obesity are among the presenting features, whereas diabetes, impaired lipid metabolism, and metabolic syndrome are among the long-term health consequences[3]. The underlying pathophysiology is not fully understood with a wide diversity involving many pathways and biomarkers. Different phenotypes had been described according to clinical presentation whether hyperandrogenism, ovarian dysfunction, or both as the dominant presenting symptoms[4].

Spexin is a hormone involved in different body organs' metabolism and energy expenditure, it was involved in the regulation of hypothalamic gonadal axis[5] studies found a lower level of spexin in PCOS compared to control with a significant negative correlation with Luteinizing hormone[6,7], that is the cornerstone behind this work which investigates the implication of this biomarker in PCOS related infertility.

Methods

The protocol of this research was approved by the ethical committee of Al- Mustansiriyah university (MOG-146 on 22nd December 2020) where a total of 90 women were involved in this case-control study that had been conducted over one year from January till December 2021. Sixty of these participants were case group who were diagnosed as PCOS according to Rotterdam's criteria: two out of three criteria which are oligo-ovulation, hyperandrogenism, and polycystic ovarian morphology[1]. Case group patients were further subdivided into 30 fertile women who constitute those with at least prior one pregnancy and 30 infertile women who failed to achieve a prior pregnancy despite attempts to achieve pregnancy for more than one year. The cases attended the gynecology department of Al Yarmouk hospital in Baghdad. The control group constitutes 30 healthy fertile women with no medical diseases or gynecological problems and were age-matched with cases.

After clarification of the study with the written agreement of participants to be enrolled in the study, detailed history was taken and a proper thorough physical examination was performed including general examination for body weight, height, waist circumference, and hip circumference measurements in addition to blood pressure estimation. All cases were sent for pelvic ultrasound looking for polycystic ovarian morphology according to Rotterdam criteria ≥12 ovarian follicles, 2–9 mm size or an ovarian volume ≥ 10 ml[1] and to exclude pelvic pathology.

The blood sample was taken from all participants and biochemical study including the following: LH, FSH, prolactin, testosterone, AMH, FBS, Insulin, HOMO-IR, and Spexin.

Results

The findings for the waist and hip were not statistically significant when are to the control group as shown in **Table (1)**. Using the ANOVA test, the waist of the control group averaged (37.20±5.42), whereas the waists of the fertile and non-fertile groups averaged (37.53±6.21) and (37.83±5.46) respectively. In contrast, the results of the ANOVA were indicating significant differences in BMI among the levels of the group. The mean of BMI for Control 23.02 ± 2.46 was significantly smaller than for Fertile (28.13±5.64, $p < .001$) and non - fertile (28.06± 5.03, $p < .001$) groups.

In comparison to the control group, the mean ± SD of luteinizing hormone (LH), follicular stimulating hormone (FSH), prolactin (PRL), anti-Mullerian hormone (AMH), and testosterone levels in the serum of fertile and non-fertile women suffering from the polycystic ovarian syndrome are shown in **Table (2).**

To investigate the differences between the variables, paired t-tests were calculated between each pair of measurements. The mean value of **LH** in non-fertile patients (14.03±3.25), was extremely significantly elevated with P values (< 0.001) when compared to the mean value of fertile patients (10.47±7.01) and control group (7.13±2.88). In contrast, the concentrations of **FSH** did not differ significantly among the studied groups. Serum levels of FSH have an average of (7.60 ± 1.77) for the control group while the fertile and non-fertile groups have an average of (7.06 ± 2.07), (7.31 ± 2.60) respectively Figure (3-3). Similarly, there were no significant differences were found for serum levels of **PRL** ($p = 0.09$) between the control group and patients' group. The means and standard deviations are presented in **Table (2)**. The data in **Table (2)** was demonstrated the mean value of **AMH** in non-fertile patients (8.92±1.82). It was highly significantly increased with p- values (< 0.001) compared with the mean value of fertile patients and control group (8.15 ±1.77), (5.95± 1.32) respectively. Consistent with this, T**estosterone** serum levels showed significant variations with p-values equal to ($p<0.001$) among the

groups. This was indicating that the level of testosterone in non-fertile women recorded highly significant differences compared with the control group. Furthermore, the mean of testosterone for fertile women (0.60± 0.18) was significantly lower than for non-fertile (0.89± 0.14, p < 0.001).

Table 1. Description of clinical parameters of study groups

Parameters	Groups			P- ANOVA
	Control	Fertile	Non - Fertile	
	Mean± SD	Mean± SD	Mean± SD	
Age (years)	27.37± 5.39	28.43± 5.82	26.13± 5.49	.282
Height(cm)	162.20± 4.99	160.67± 4.80	161.10± 5.96	.512
Weight(kg)	62.50± 9.04	72.30± 15.92	72.33± 14.43	.007
Waist circum. (inches)	37.20± 5.42	37.53± 6.21	37.83± 5.46	.912
Hip circum. (inches)	38.33± 6.43	38.23± 5.12	38.80± 6.27	.926
BMI (kg/m^2)	23.02± 2.46	28.13± 5.64	28.06± 5.03	< .001

Table 2. Comparison of baseline biochemical parameters of study groups

Parameters	Groups			P-value
	Control	Fertile	Non - Fertile	
	Mean± SD	Mean± SD	Mean± SD	
LH (mIU/mL)	7.13± 2.88	10.47± 7.01	14.03± 3.25	< .001
FSH (mIU/mL)	7.60± 1.77	7.06± 2.07	7.31± 2.60	.623
PRL (ng/mL)	17.78± 6.46	17.31± 7.22	21.57± 10.27	.091
AMH (ng/mL)	5.95± 1.32	8.15± 1.77	8.92± 1.82	< .001
Testosterone (ng/mL)	0.40± 0.26	0.60± 0.18	0.89± 0.14	< .001

Table 3. Comparison of study core biochemical markers among study groups

Parameters	Groups			P value
	Control n= 30	Fertile n= 30	Non – Fertile n= 30	
	Mean± SD	Mean± SD	Mean± SD	
FBS mg/dL	101.24± 12.94	113.02± 12.01	114.30± 6.08	< .001
INSULIN uU/ml	9.45± 8.66	22.43± 19.18	33.42± 42.49	.005
HOMA_IR	1.99± 0.80	5.44± 3.37	9.89± 12.14	< .001
SPEXIN pg/ml	7382.19± 1638.54	3670.64± 1466.42	3441.45± 3190.63	< .001

Figure 1. Spexin pg/ml scatter gram demonstrating significant differences between research groups' means and medians.

Table 4. Correlations of serum Spexin levels with other core study biomarkers in Fertile and Non-fertile groups.

Correlation matrix (spearman) / Fertile Group		
	Spexin (SPX)	
Variables	*r*	*P*
INSULIN uU/ml	-0.22	0.25
HOMA-IR	-0.12	0.54
AMH ng/ml	0.13	0.51
Correlation matrix (spearman) / Non - Fertile Group		
	Spexin (SPX)	
Variables	*r*	*p*
INSULIN uU/ml	-0.10	0.59
HOMA IR	-0.09	0.63
AMH ng/ml	-0.44	0.02

The correlations of the serum levels of the core biomarker, **SPX**, with other core study biomarkers in both PCOS groups were further displayed in Table (4). Weak positive correlation was found between **SPX** and **AMH** (r=0.13, p=0.51) in fertile group, while moderate significant negative correlation in non-fertile (r= -0.44, p=0.02) Figure (2). On the other hand, **SPX** levels had a negative correlation with **Insulin** and **HOMA-IR** in both fertile and non-fertile groups Figure (3,4). Spearman rank correlation matrix presenting correlation coefficient between the studied parameters in Fertile group. **SPX** levels had no significant correlation with other routine tests. While in the non-fertile group, a positive moderate correlation had been observed between **SPX** and **hip** (r= 0.329, p= 0.077) as well as a highly significant positive correlation between **SPX** and **NRG-4** (r= 0.853, p<0.0001).

Figure 2. Scatter plot of Spexin and AMH correlation in Fertile and Non-Fertile groups. A regression line was added to ease the interpretation

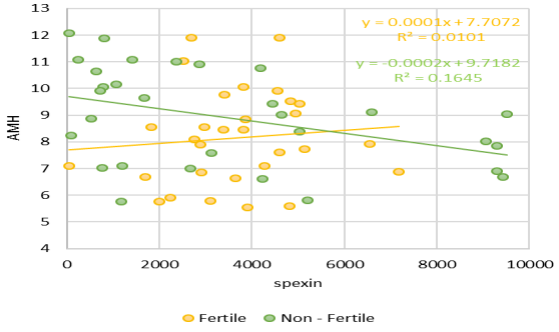


Figure 3. Scatter plot of Spexin and HOMA-IR correlation in Fertile and Non-Fertile groups. A regression line was added to ease the interpretation

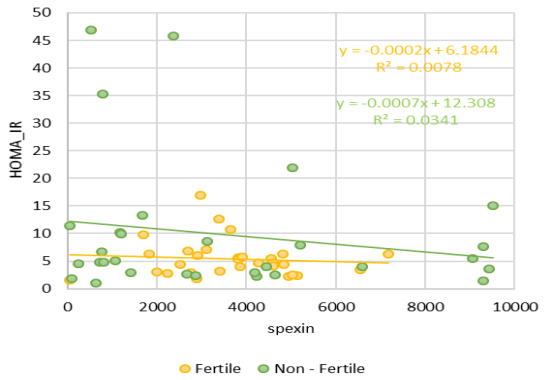


Figure 4. Scatter plot of Spexin and Insulin correlation in Fertile and Non-Fertile groups. A regression line was added to ease the interpretation

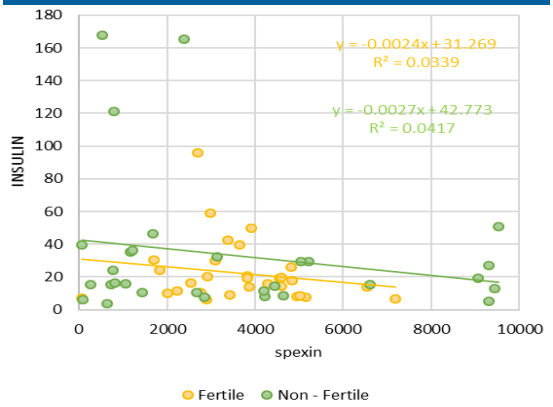
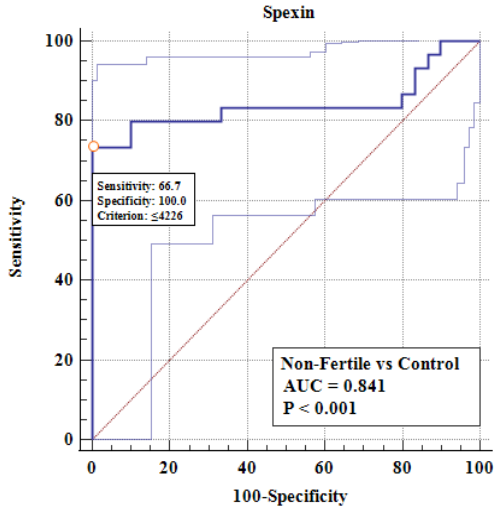
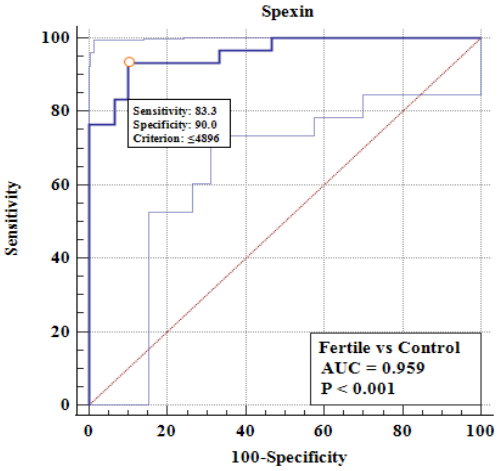
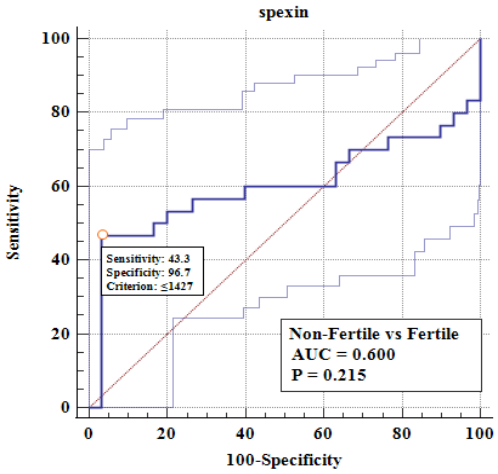


Figure 5. ROC curve analysis of selected study markers showing their discriminating power and best criterion values of highest sensitivity and specificity as differentiating Non-Fertile from Fertile, Fertile from control, and non-fertile from control subjects. AUC was added to the plot for comparison







Discussion

Infertility carries a significant social, economic, and health implications that affect eight to twelve percent of young-aged couples[8]. Male factor and oligo-ovulation were the most common underlying etiology[9]. Polycystic ovarian syndrome (PCOS) is considered one of the most prevalent etiologies for female ovulatory dysfunction which affects six to twelve percent of women of reproductive age[10,11]. With the development in the lifestyle of humans, the percentage of PCOS has increased dramatically[12]. A hypothalamic-pituitary-ovarian axis imbalance as well as disorders that negatively alters the body`s metabolism are considered as a description of PCOS[13]. Hyperinsulinemia and insulin resistance are still one of the pathogeneses of PCOS in about 70% of women. However, PCOS is still not clear fully[14,15]. Some researchers believe that combining several factors (which can make a pregnancy high-risk) and biological molecules found in blood would be an applicable technique for predicting PCOS risk and reducing its complications. This amalgamation would allow for high-efficiency surveillance and early clinical intervention[16]. In this study and an extension of the same strategy, the clinical usefulness of certain biomarkers was evaluated by exploring their value in differentiating fertile PCOS from non-fertile PCOS which is important in following up the progress of PCOS and could serve in the prediction of PCOS infertility.

Obesity is one of the most common phenomena among women, especially in childbearing age, which is mainly associated with hormonal changes that directly affect the body's metabolic metabolism, resulting in problems in women's fertility. The effect of obesity on the pathogenesis of PCOS and then on fertility in women can be explained from the scientific facts which said that: obesity is associated with high concentrations of insulin and androgen[17]. Androgen will convert to estrogen because of the excess of adipose tissue and this, in turn, affects gonadotropin production by negative feedback on the HPO axis[18]. Accordingly, these hormonal changes will lead to ovarian dysfunction and menstrual disorders[19,20]. Therefore, BMI greatly affects the fertility of the mother and reduces the chances of success in childbearing as well as increases the morbidity of the mother and fetus. For these reasons, a limit must be placed on BMI for fertility treatment[21,22].

For the first time, spexin levels are evaluated in fertile and non-fertile Iraqi women with PCOS. Compared with the control group, spexin levels showed a highly significant decrease in PCOS, but this decrease was not significant when comparing fertile and non-fertile women. In addition, it was found that the concentration of LH, Testosterone, FBS, insulin, and HOMA-IR was high in non-fertile women compared to fertile women as a result of polycystic ovaries.

Spexin levels were found to be negatively correlated with insulin resistance and weak positive association with LH and testosterone in non-fertile. The similar results between spexin and LH while the negative association with testosterone. In other words, our findings in the present study strongly support the observations indicating that serum spexin levels are affected in PCOS patients depending on the hormonal disturbance's status. Our results are in the line with the results were done by[23] which clarified the relationship between spexin and hormonal changes in patients with polycystic ovaries. The central and peripheral gonadal axis is mainly regulated via feedback mechanisms influenced by gonadal steroids[24]. It is implied that spexin plays a role in the regulation of the hypothalamic-pituitary-ovarian axis via downregulation of LH secretion[25]. The genetic spexin expression in the central nervous system was influenced by female hormones in fishes[26,] implicating a potential role of spexin in reproduction and endocrine. Trudeau implicates the role Spexin, as an inhibitory neuropeptide in their study that regulates LH in male gonads[27]. These results suggest that spexin played a role in regulating reproductive function, especially inhibiting the LH secretion.

Anik G. results were in concordance with current results that demonstrated lower values in PCOS and negative correlation with HOMA-IR where it showed that Spexin levels were significantly lower in the PCOS group compared with the control and negatively correlated. As well as spexin levels were independently and inversely associated with testosterone and weakly associated with LH secretion. These findings suggest the enrollment of spexin in PCOS via its effects on LH secretion[28].

On the other side and as mentioned in the above information, the level of spexin has an inverse correlation with insulin and insulin resistance. Hyper-insulinemia together with high LH levels had been accused of the hyper-androgenemia from theca cells of PCOS subjects[24]. The role of spexin in insulin regulation was clarified in mice as spexin reduced insulin secretion in response to glucose load[29].

Regarding anti-Mullerian hormone (AMH), highly significant differences have appeared in this study between PCOS groups and the control group. These findings were supported by other researchers[30-32]. Another study found that AMH receptors can affect GnRH-dependent luteinizing hormone (LH) secretion by increasing its levels[33]. Additionally, moderate negative correlation between AMH and HOMA-IR in the fertile group and a weak positive correlation in the non-fertile group. Skalba, et al. found a moderate positive correlation between AMH and HOMA-IR in PCOS patients[34]. However, a negative correlation between AMH and HOMA-IR has also been reported in PCOS patients[35]. Both AMH and LH showed a positive correlation. These observations suggest the possibility of a direct effect of both insulin and LH on AMH secretion or may reflect the fact that both insulin and LH contribute to ovarian androgen secretion[36]. Serum spexin lev-

els were a weak positive correlation with AMH in fertile group and a negative correlation in the non-fertile group. This is the first study looking at the relationship between Spexin and AMH in both groups. Based on what we clarified in the preceding paragraphs, we believe this is an indirect effect, in which a drop in spexin levels in PCOS causes a rise in LH levels, which leads to an increase in the production of AMH.

In this study, the receiver operating curve analysis (ROC) was used to assess the diagnostic accuracy of Spexin, AMH, and HOMA-IR in both cases of the polycystic ovarian syndrome. According to ROC analysis, Spexin had the highest specificity (100.00%) for distinguishing between non-fertile and control groups, as well as (96.67%) for distinguishing between non-fertile and fertile PCOS groups (Figure 5).

Conclusions

The significant association of Spexin with LH, IR, and androgens suggests its possible role in PCOS pathophysiology, additionally, Spexin showed a highly significant screening value and reliable diagnostic methods to differentiate PCOS cases versus control, as well as good diagnostic behavior to diagnose infertile cases but with less screening value.

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