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A study of hormonal changes and genetic frequency of ABO blood groups and Rh factor (RH) in women with polycystic ovary syndrome in Sebha city

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دراسة التغيرات الهرمونية والتكرار الجيني لفصائل الدم ABO وعامل الريزوسي (RH) لدى النساء المصابات بمتلازمة تكيس المبايض في مدينة سبها.

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Abstract
Polycystic ovarian syndrome (PCOS) is defined by hormonal imbalances that impact several organ systems, resulting in diverse health concerns such as menstruation abnormalities, infertility, hirsutism, acne, and obesity. This study aimed to ascertain the hormonal alterations and genetic distribution of ABO blood types and Rh factor in women with PCOS. One hundred ten blood samples were taken, categorised into two groups: the PCOS group (60 samples) and the control group (50 samples), with ages ranging from 20 to 50 years. Luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), and thyroid-stimulating hormone (TSH) levels were quantified via enzyme-linked immunosorbent assay (ELISA). Blood types were identified, and the genetic frequency was determined for the PCOS cohort. The PCOS group had a statistically significant reduction ($p<0.05$) in FSH levels and an elevation in LH levels relative to the control group. Conversely, no significant differences were observed in TSH and PRL levels between the PCOS group and the control group. The results revealed that the phenotypic frequency of blood group O was the highest, whereas that of blood group AB was the lowest. Moreover, the Rh+ phenotype exhibited greater prevalence than the Rh- phenotype, with the allele frequency of blood types as follows: IO > Ia > Ib > Iab. The findings indicated a correlation between blood types and the prevalence of PCOS. In conclusion, according to the findings of this study, hormonal abnormalities were identified in women with

PCOS, characterized by a considerable decrease in FSH levels and a rise in LH levels. Additionally, the data suggested that women with blood group O+ are more likely to developing PCOS.

Keywords: genetic frequency, Hormonal disorders, Polycystic ovary syndrome, ABO blood groups system, Rh factor.

الملخص

تتميز متلازمة تكيس المبايض (PCOS) باختلال في مستويات هرمونات الجسم، مما يؤثر على العديد من أجهزة الجسم، ويؤدي إلى مضاعفات صحية متنوعة، بما في ذلك اضطرابات الدورة الشهرية، والعقم، وكثرة الشعر، وحب الشباب، والسمنة. هدفت هذه الدراسة إلى تحديد التغيرات الهرمونية والتكرار الجيني لفصائل الدم ABO والعامل الريزوسي لدى النساء المصابات بمتلازمة تكيس المبايض. جمعت 110 عينة دم، وتم تقسيمها إلى مجموعتين: مجموعة متلازمة تكيس المبايض (60 عينة) ومجموعة ضابطة (50 عينة)، تراوحت أعمارهن بين 20و50 عامًا. تم قياس مستويات هرمون الملوتن (LH)، والهرمون المنشط للحويصلة (FSH)، وهرمون البرولاكتين (PRL)، والهرمون المنشط للغدة الدرقية (TSH) باستخدام المقايسة ا المناعية المرتبطة بالإنزيم (ELISA) ، كما تم تحديد فصائل الدم وحساب التكرار الجيني لمجموعة متلازمة تكيس المبايض. أظهرت نتائج مجموعة متلازمة تكيس المبايض انخفاضًا كبيرًا ($p<0.05$) في تركيز مستويات FSH وزيادة في مستويات LH مقارنةً بالمجموعة الضابطة. من ناحية أخرى، لم تكن هناك فروق كبيرة في مستويات TSH ومستويات PRL بين مجموعة متلازمة تكيس المبايض والمجموعة الضابطة، بالإضافة إلى ذلك أشارت النتائج إلى أن تكرار النمط المظهري لفصيلة الدم O كان الأعلى، بينما كان تكرار النمط المظهري لفصيلة الدم AB هو الأقل. علاوة على ذلك، كان النمط المظهري Rh+ أكثر انتشارًا من النمط المظهري Rh-، وكان التكرار الاليلي لفصائل الدم كما يلي: IO> Ia >Ib >Iab. اشارات النتائج أيضًا إلى وجود علاقة بين فصائل الدم وحدوث متلازمة تكيس المبايض. في الخلاصة وطبقا لنتائج هذه الدراسة لوحظت اضطرابات هرمونية لدى النساء المصابات بمتلازمة تكيس المبايض تميزت بانخفاض كبير في مستويات FSH وزيادة في مستويات LH، إضافة إلى ذلك أشارت النتائج إلى أن النساء ذوات فصيلة الدم O+ هن أكثر عرضة للإصابة بمتلازمة تكيس المبايض.

الكلمات الدالة: التكرار الجيني، الاضطرابات الهرمونية، متلازمة تكيس المبايض، نظام فصائل الدم ABO ، العامل الريسوس.

1. Introduction

Polycystic Ovary Syndrome (PCOS) is a common endocrinopathy occurring in reproductive age women. *Hyperandrogensim* polycystic ovaries, chronic anovulation and metabolic disorders are the common features in POCS. (Yang and chen, 2024). PCOS is considered a multifactorial disorder influenced by environmental, hormonal, genetic, and metabolic factors (Fahs et al., 2023), which may lead to several diseases, including obesity, metabolic syndrome, insulin resistance, type 2 diabetes, and infertility (Stener-Victorin et al., 2024). The prevalence of PCOS ranges from 4% to 20%, depending on diagnostic criteria. The principle symptoms of this syndrome include menstrual irregularities, acne and excessive amounts of androgenic hormones (Kamble et al, 2023). Among the common causes of this syndrome are hormonal imbalances, with estrogen and androgen hormones being particularly implicated in the development of PCOS (Xu et al, 2024) . Additionally, elevated levels of prolactin hormone and increased luteinizing hormone, when accompanied by elevated insulin levels, can lead to an increase in testosterone hormone production.

Many studies have indicated a relationship between ABO blood groups and various diseases. A study by Qurtam (2013), revealed a significant correlation between the prevalence of different types of diabetes and their association with ABO blood groups. The study found that individuals with blood group O are more susceptible to diabetes. Similarly, another study

demonstrated that the majority of people suffering from allergies were of blood type O (Hamad, 2016). Conversely, a previous study showed that individuals with blood type O are more resistant to anemia, while those with blood types A, B, and AB are more prone to it (Harvey, 2004). The development of blood cancer, cardiovascular diseases, infections and haematologic disorders, cognitive disorders, circulatory diseases, metabolic diseases, and malaria have all been linked to the antigens of the ABO blood group systems that are found on the surfaces of red blood cells and other cells in the body (Abegaz, 2021). Additionally, the study found a link between stomach cancer and blood group A (Abegaz, 2021). Additionally, some research findings have indicated a connection between blood type O and the likelihood of polycystic ovarian syndrome (Pal et al., 2014). This study intends to ascertain the genetic frequency of ABO blood groups and the Rh factor, as well as assess hormonal changes in women with polycystic ovary syndrome in the city of Sabha, because there are currently insufficient studies on the frequency distribution of these factors in women with the condition.

2. Materials and methods

2.1Study design and subjects:

Cross-sectional study was conducted and 110 blood samples were collected from women attending the infertility centre in Al-Manshiya at Sabha city during the period from October to November 2021. The samples were divided into two groups: the first group included 60 samples from women with polycystic ovary syndrome, and the other group included 50 samples as control group. Aged ranged from 20 to 50 years.

2.2Sample Collection and Preparation

The samples were collected from venous blood and divided into two parts. One part was placed in plain tube and then centrifuged for 5 minutes to obtain the serum for hormone analysis, the other part was placed in EDTA tube which was used to determine the blood groups for the ABO system and the Rh factor.

2.3 Measurement of hormone levels:

The measurement of hormone levels, including PRL, FSH, LH, and TSH, were conducted using the Enzyme-Linked Immunosorbent Assay (ELISA) as described by (Uotila et al, 1981). In principle, this method was utilized to identify antibodies in the control solution that bind to antigens in the sample, forming an antigen-antibody complex. The complex migrates through a nitrocellulose medium and interacts with the antibodies present on the strip. The sample on the strip contains larger quantities of antigens, resulting in a substantial amount of immune complexes. These immune complexes, in turn, generate an immunofluorescent signal on the antibody indicator.

2.4 Determining of blood groups and the genetic frequency of ABO and Rh system

Blood groups were determined using the slide method, according to Barbara (1993). The frequencies of phenotypic patterns and genetic frequencies of ABO blood groups and the Rh were determined using the Hardy-Weinberg Equilibrium (Griffith et al., 2008; Anees et al, 2007). The Hardy-Weinberg principle states that in a large randomly mating population, the gene frequencies remain constant from generation to generation in the absence of selection, migration and mutation. (Mandal, 2002).

2.5 Statistical analysis.

In the tables, the results were presented as mean ± standard deviation (SD), and in the graphs, as mean ± standard error (SEM). IBM's SPSS (Statistical Package for Social Sciences) version 20 was used to analyse the data. The variable's normalcy was assessed using the Shapiro-Wilk

test. Accordingly, the significant difference between groups was estimated using the T-test. The observed genotypic and allelic frequency distributions of the blood group antigens were compared to those predicted by the Hardy–Weinberg equation using the chi-square test. When $p<0.05$, the group differences were deemed significant.

3. Results:

3.1 Body Mass Index (BMI) indicator:

The results of this study showed no statistically significant differences ($p = 0.93$) in the average of body mass index between PCOS group (30 ± 9) and the control group (29.9 ± 6.8).

3.2 LH and FSH hormone levels:

The results, as shown in Figure 1, revealed an increase in LH hormone levels and a decrease in FSH hormone levels in PCOS group compared to the control group. However, these changes of LH hormone levels were not statistically significant. On the other hand, significant differences were observed in the levels of FSH hormone between PCOS group compared to the control group.

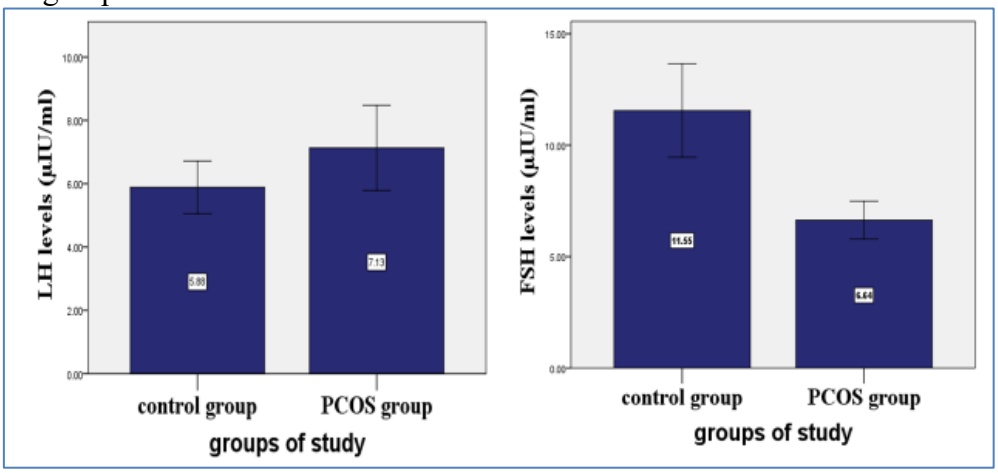

Figure 1. The levels of LH and FSH hormones in the in PCOS and control groups.

The results are expressed as mean ± standard error.

3.3 Results of TSH and PRL hormone levels:

PCOS group showed a decrease in PRL and TSH hormone levels compared to the control group. However, no statistically significant differences were found between study groups (Figure 2).

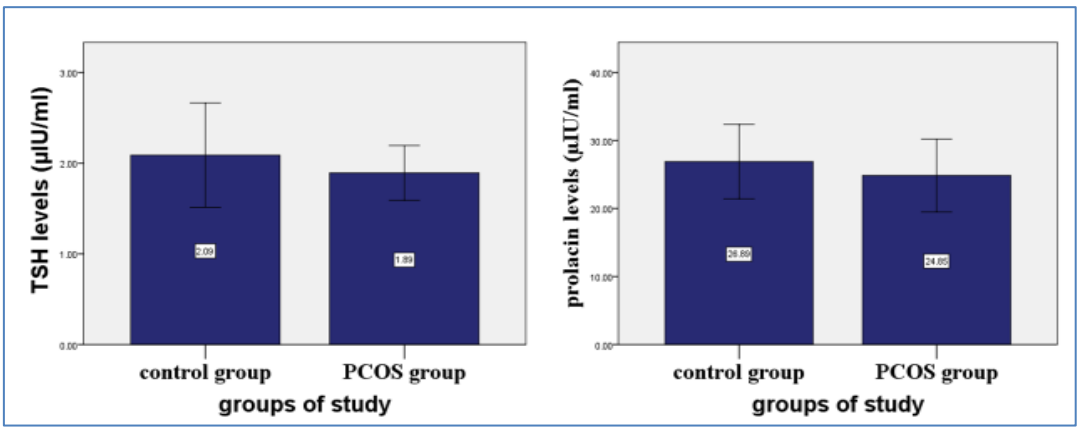

Figure 2. The levels of TSH and PRL hormones among PCOS and control groups.

The results are expressed as mean ± standard error.

Table 1. illustrates the phenotypic of ABO blood groups and Rh factor in women with PCOS and control groups.

PCOS group		ABO blood groups and Rh factor
Percentage	**Number**	
%31.7	19	A
%15	9	B
%5	3	AB
%48.3	29	O
%100	60	**Total**
%90	54	Rh+
% 10	6	Rh-
%100	60	**Total**

3.4 Phenotype of ABO blood groups and Rh factor:

The results shown in Table (1) indicated that the phenotype of blood group O had the highest frequency (48.3%) in the PCOS group compared to other blood groups frequency. Following the phenotype of blood group, A which had a frequency of 31.7%, while blood group B had a frequency of 15%. The phenotype of blood group AB had the lowest frequency of 5%. These results also revealed the predominance of the Rh-positive phenotype compared to the Rh-negative phenotype in the PCOS group

Table 2. illustrates the phenotype and genotype frequencies of ABO blood groups and Rh factor among PCOS group.

Expected requency (EF)	Observed frequency (OF)	Number	Genotype frequency	Genotype	Phenotype
0.3339	0.32	19	$p^2 + 2pr$	$)I^aI^a \; and \; I^aI^o$	A
0.1639	0.15	9	$q^2 + 2qr$	$I^bI^b \; and \; I^bI^o$	B
0.0462	0.05	3	2qp	I^aI^b	AB
0.4761	0.48	29	r^2	I^oI^o	O
1	1	60	$(p+q+r)^2$		**Total**
0.69	0.9	54	P=1-q	DD,Dd	Rh^+
0.31	0.1	6	q=1-p	Dd	Rh^-
1	1	60	P+q=1		**Total**

3.5 Frequency of phenotype of ABO blood groups and Rh factor:

The frequency of the observed phenotype of ABO blood groups and Rh factor was calculated for PCOS group Table (2). The results of current study showed that the frequency distribution of blood group O was the highest compared to the other blood groups, followed by the frequency of blood group A, B, and AB respectively. Thus the frequency distribution of ABO blood groups indicated O >A > B > AB . These results also refer to the frequency distribution of the Rh-positive phenotype was higher compared to the Rh-negative phenotype.

3.6 Frequency of allele and genotype of ABO blood groups and Rh factor:

The expected allele frequency of ABO blood groups and Rh factor (Table 2) were calculated using the method described by Ahmed et al., 2009, as follows:

The expected relative frequency of blood group A (P) is calculated as P = 1 - √(B + O).

The expected relative frequency of blood group B (q) is calculated as q = 1 - √(A + O).

The expected relative frequency of blood group O (r) is calculated as r = √O.

The expected relative frequency of blood group AB is calculated as 2pq.

$$= 0.2P = 1-\sqrt{\mathbf{0.1639 + 0.4761}}$$

$$=0.1q = \ 1-\sqrt{\mathbf{0.3339 + 0.4761}}$$

$$0.69=r= \quad \sqrt{\mathbf{0.4761}}$$

The expected relative frequency of Rh-negative (Rh-) is calculated as q = √(Rh-).

The expected relative frequency of Rh-positive (Rh+) is calculated as p = 1 - q.

$$q = \sqrt{\mathbf{0.1} = \ 0.31}$$

$$p = 1 – 0.31= 0.69$$

Since ABO blood groups in human are determined by multiple alleles including three alleles of ABO blood groups: I^a, I^b, and I^o, represented by p, q, and r respectively. p represents the genetic frequency of blood group A, q represents the genetic frequency of blood group B, and r represent the genetic frequency of blood group O. Accordingly, the frequency of ABO genotype can be expressed using the Hardy-Weinberg Equilibrium equation as follows:

$$(P + q + r)^2 = p^2 + 2pq + q^2 + 2pr + r^2 = 1.$$

Similarly, the frequencies of the RhD blood group can be represented by the alleles D and d, which are represented by p and q respectively, where p represents Rh+ and q represents Rh-. The genetic frequency of the Rh factor is represented by the equation

$$(P + q)^2 = p^2 + 2pq + q2 = 1$$

where p^2 represents the frequency of the DD genotype, 2pq represents the frequency of the Dd genotype, and q^2 represents the frequency of the dd genotype.

Based on the results shown in Table (2), the genetic frequencies for blood groups follows the same phenotype frequencies. Therefore, the allele frequency for blood groups as follows:

$$I^{AB} > I^B > I^A > I^O.$$

The results also indicate that the frequency of the p allele, responsible for the Rh^+ phenotype, is higher compared to the q allele, responsible for the Rh^- phenotype.

To find the relationship between blood groups and polycystic ovary syndrome (PCOS), a chi-square test was used to determine if there were significant differences between the observed and expected genetic frequencies (Chakraborty, 2010) [17]. The calculation is as follows:

$$\text{Chi-square } (\chi^2) = \Sigma ((OF - EF)^2 / EF)$$

Where:
χ^2: represents the chi-square value.
Σ: represents the summation.
OF: represents the observed frequency.
EF: represents the expected frequency.
X^2 calculation represents the calculated chi-square value.
X^2 table represents the tabulated chi-square value.

$$X^2 = (O - E^2 / E + (O1 - E)^2 / E + (O2 - E)^2 / E + (O3 - E)^2 / E (O4 - E)^2 / E$$

- α = 0.05, which represents the significance level.

$$\boldsymbol{X^2 = \frac{(0.32 - 0.3339)^2}{0.3339} + \frac{(0.15 - 0.1639)^2}{0.1639} + \frac{(0.05 - 0.0462)^2}{0.0462} + \frac{(0.48 - 0.4761)^2}{0.4761}}$$

Based on the results of the chi-square test, it was found that the calculated chi-square value (13.28) is higher than the tabulated chi-square value (1.77). This indicates there were statistically significant differences ($p < 0.05$) between the observed frequencies and the expected frequencies of the ABO blood groups.

12.Discussion

Polycystic ovary syndrome (PCOS) is a chronic and complex disorder, commonly observed in women of reproductive age, and it affects teenage girls as well (Shermin et al., 2019). It is accompanied by abnormal hormonal changes (Almhana, 2011). The results of the current study showed no significant differences in the average body mass index (BMI) among the group of women with PCOS compared to the control group. These findings were inagreement with a previous study conducted in Iraq of 35 women with PCOS, which indicated that the most affected women have weight gain (55%) and 44% of them suffered from obesity (Almhana, 2011).

Similarly, the study by Shermin et al. also revealed that the most of women with PCOS are at a three-fold increased risk of obesity compared to control group. The prevalence of obesity and weight gain was found to be around 80%, accompanied by an increase in the average body mass index (BMI). It was found that excessive weight or obesity has a negative impact on ovulation rate and that elevated levels of androgen and luteinizing hormone (LH) negatively affect treatment response (Conway et al., 2014). Therefore, it is recommended to measure the BMI of women with PCOS in order to control and prevent excessive weight gain (Conway et al., 2014; Teede et al., 2018).

Previous studies have indicated that polycystic ovary syndrome (PCOS) is accompanied by hormonal changes, including luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels (Khmil et al., 2020). The results of this study demonstrated that the women with PCOS had increased levels of LH compared to control group. These findings are consistent with the results of the study conducted by Almhana , which included 35 women with PCOS and they found a significant increase in LH levels compared to control group. Previous studies conducted on PCO syndrome has been observed that LH hormone excessively increased, while the increase in FSH hormone was very minimal or may not change (Clamn et al., 2002). This is consistent with the findings of the current study, where no elevation in FSH levels was observed in women with PCOS, and FSH levels were within the normal range. In normal physiological conditions, FSH levels are usually higher than LH levels, whereas in women with PCOS, it has been found that LH levels are higher than FSH levels (Clamn et al., 2002).

Furthermore, studies conducted by Khmil et al. (2020) and Nath et al. (2019), on a group of women with fertility issues and PCOS have shown an increase in LH hormone levels and a decrease in FSH hormone levels compared to the control group. Additionally, the current study's results indicated there were no significant changes in PRL hormone levels among the group of women with PCOS compared to the control group. These findings were consistent with a previous study conducted in China on a sample of 2052 women with PCOS, which showed a decrease in prolactin hormone levels in affected women compared to the control group (Yang et al., 2020). On the other hand, the current study's results were inconsistent with a previous study conducted in Iraq on 103 samples of women with PCOS and 50 samples of the control group, which observed a statistically significant increase in PRL hormone levels in women with PCOS compared to healthy women (Omear et al., 2014).

The results of this study revealed no statistically significant differences in the average levels of TSH hormone among women with PCOS compared to control group. These findings were consistent with a previous study conducted on 90 samples of women, including 60 affected women and 30 healthy women, which showed no significant differences in TSH hormone levels between the two groups of study (Salman and Jasim, 2016). On the other hand, this study demonstrated a significant increase in the levels of T4, T3, FSH, and LH hormones (Salman and Jasim, 2016). Another study conducted in India by Nath et al. (2019) , to assess the changes in the LH/FSH hormone ratio among women with PCOS and to evaluate the effect of PRL and TSH hormones on the rate of LH/FSH ratio elevation, the results indicated a significant increase in the LH/FSH ratio among over 70% of women with PCOS. Additionally, there was a statistically significant increase in TSH hormone levels between the group of women with PCOS and the healthy women. This study reported that thyroid dysfunction is one of the most common endocrine disorders, and PRL hormone levels are inversely associated with TSH hormone levels in women with PCOS (Salman & Jasim, 2016).

The results of the current study showed that blood group O was the highest frequency among women with polycystic ovary syndrome (PCOS) compared to other blood groups, followed by blood group A and B, and finally blood group AB. Additionally, the frequency of the Rh+ phenotype was higher than the frequency of the Rh- phenotype. The results also indicated that the frequency of the p allele, responsible for the Rh+ phenotype, was higher than the frequency of the q allele, responsible for the Rh- phenotype. Furthermore, the genetic frequency of blood group O was the highest among women with PCOS, followed by blood group B, and then blood group A, while blood group AB had the lowest frequency compared to other blood groups. These findings were consistent with a study conducted by Jassim (2012), which conducted to determine the relationship between diabetes, high cholesterol, and high blood pressure with the

ABO blood group system. The study by Nishi et al. (2012), also demonstrated that there were association of blood groups with Hypertension, which found hypertensive patients have highest incidence for the disease in blood group O while the lowest incidence in group AB. In Rh+ ve individuals have, highest incidence was found in blood group O and lowest in AB blood group, on the other hand, individuals with Rh-ve, which have highest incidence were found in group O and the lowest in group AB, this study indicated that the disease was found to be statistically associated with blood group. Moreover, the previous study reported that there was a relationship between ABO blood group alleles and susceptibility to various diseases such as cardiovascular diseases, blood cancer, and thrombosis (Tregouet, 2009).

The results of the current study also revealed that the genetic frequency for blood groups was consistent with the phenotypic frequency, explaining the role of genetic structure in displaying the phenotype. Based on this, the allelic frequency for blood groups was as follows: IO> Ia > Ib > Iab . These findings were consistent with previous studies conducted in different regions of the world, including studies by Yassin (2013) in South and East Nigeria, Yan et al. (2005) in China, and Hussain (2001) in Pakistan, which all showed the genetic frequency of blood groups as IO> Ia > Ib > Iab . Furthermore, the chi-square test indicated significant differences between the observed and expected genetic frequencies, suggesting a relationship between blood groups and the occurrence of PCOS.

11.Conclusion

Through this study, we deduced that the imbalance in hormone levels or hormonal disorders in women with fertility problems are likely due to Polycystic Ovary Syndrome (PCOS). This was observed by the increase in LH hormone levels and decrease in FSH hormone levels and prolactin. Therefore, hormonal imbalance in women affected by this syndrome can lead to fertility issues and infertility. Consequently, hormone analysis tests are considered necessary and one of the most important diagnostic tools to determine the prevalence of infertility among women of reproductive age. Additionally, it was found that women with PCOS who have blood group O+ are more susceptible to developing this syndrome. Furthermore, the results of this study also showed that the genetic frequency for blood groups was consistent with the phenotypic frequency, explaining the role of genetic structure in displaying the phenotype.

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