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The Relation of the SHBG Gene Polymorphism (rs1799941) with PCOS in a Group of Iraqi Women

Rusul Hisham[1], Dr. Adel Fawzy Shehab[2] and Hadeel Abdel-Hadi Omair[3]

[1]Master's Student, Department of Biology, College of Science, University of Tikrit, IRAQ.

[2]Professor, Department of Biology, College of Science, University of Tikrit, IRAQ.

[3]Assistant Professor, Department of Biology, College of Science, University of Tikrit, IRAQ.

[1]Correspondence Author: rusul.h.taha4434@st.tu.edu.iq

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is the most common and complex endocrine disorder that affects women of childbearing age. However, the causes of PCOS are still unknown, however, there is strong evidence supporting the role of genetics in causing it, because PCOS has a strong familial predisposition. More than one gene contributes to the heterogeneous phenotype and clinical and biochemical presentation. Patients with PCOS may complain of irregular menstruation, unwanted hair growth in multiple areas of the body, acne and scalp hair loss, unexplained weight gain, and infertility. This study explores the polymorphism of the SHBG gene locus (rs1799941) in Iraqi women with PCOS that may cause the onset of this disease. Genomic DNA was extracted from blood samples of women with and without PCOS. The SHBG gene was amplified by Tetra_ARMS PCR technology, after which the PCR product was migrated onto the agarose jells at a concentration of 2%. Three genotypes appeared, the homozygous (normal) homozygous GG which is represented by (404 + 270 bp) genotype, the heterozygous (GA) which is represented by (404 + 270 + 210) bp and the homozygous mutant AA genotype. (Which is represented by the bundle (404 + 210 bp). Among the 70 women with PCOS included in the molecular study, (4) of them were carriers of the normal homozygous genotype GG, (44) of them were carriers of the heterozygous genotype GA and (22) were carrying the mutated genotype AA. In the current study, the (OR) value of the mixed GA genotype appeared (12.833) and this indicates that the mixed genotype is a risk factor for the disease, while the (OR) value of the AA mutant genotype was (9.250), and this indicates that the mutant genotype is Homozygous is a risk factor for the disease. The frequency of the A allele was higher in the infection group compared to the control group, and this indicates that the A allele is responsible for the disease association. These results indicated that the locus SHBG gene polymorphism (rs1799941) is associated with PCOS in Iraqi women.

Keywords- PCOS (Polycystic Ovary Syndrome), SHBG (Six Hormone Binging Globulin)

I. INTRODUCTION

PCOS is a common reproductive disorder that affects up to 10% of women in childbearing age [1]. Common symptoms of PCOS are irregular menstruation (lack of ovulation), hyperandrogenism and hirsutism with acne and polycystic ovaries [2]. PCOS is often associated with metabolic abnormalities, including obesity, insulin resistance, non-alcoholic fatty liver disease (NAFLD), and high blood pressure increased, as well as an risk of type 2 diabetes and cardiovascular disease [3]. The etiology of PCOS is still not clear, it is likely to be multifactorial. However, many evidences indicate a complex interaction between genetic and environmental factors that have a significant impact in the pathogenesis of PCOS [4]. Previous observations support the familial assembly of PCOS and thus suggest a genetic basis for the syndrome [5]. Several candidate genes have been proposed, including the SHBG gene [6]. The SHBG gene is a β-globulin that consists of a homologous glycoprotein produced by hepatocytes, and is mainly associated with the steroidal sex hormones [7]. Some clinical observations and published reports indicated an inverse relationship between the level of

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this hormone and polycystic ovary syndrome. Low levels of SHBG increase the biological activity of androgens, which in turn cause ovarian diseases and affect the ovulation process and the phenotypic characteristics of polycystic ovary syndrome [8]. The gene is located on chromosome 17, specifically in the p12-p13 region [9]. It encodes 373 amino acids that regulate the biological activity of sex steroids by binding to androgens, especially testosterone, and estrogen [10]. The level of the hormone varies between individuals, so its level during puberty in women is higher than in men due to the presence of estradiol, its concentration in men's plasma is half its concentration in women's plasma despite the high affinity of this protein to link with male hormone and this is due to the direct relationship between its production rate and the amount of estrogens. As an increase in the concentration of this protein leads to an increase in the ratio of free estradiol to free male hormone [11].

II. MATERIALS AND METHODS

The study included (70) women with PCOS compared with (30) non-affected women who were used as a control group, their ages ranged between (16-40). (5) ml of venous blood was withdrawn during days (2-6). From the days of the menstrual cycle and it was divided into two parts. The first section: - Withdrawal (2) ml of blood was placed in an ED anticoagulant tube and the samples were kept in supercooling (- C) for the purpose of using it in DNA extraction and molecular studies. As for the second section, (3) ml of blood was withdrawn and placed in a gel, and the samples are kept in supercooling (86C) for the purpose of various-biochemical tests. The levels of serum hormones on day 3 of the menstrual cycle including SHBG globulin and testosterone were measured using the attached steps of the EISA ready-made kit from BIOLABO. The DNA was extracted from the blood according to the directions and instructions of the kit supplied by Geneaid Company. The DNA samples resulting from the extraction are transferred onto a 1% agarose gel for the purpose of ensuring the presence and safety of the DNA.

Detection of the SHBG gene polymorphism for the locus (rs1799941) using PCR_Tetra_ARMS technique.

The principle of this technique is that it duplicates the gene segment to be studied using four specialized primers, two of which restrict the region of heterogeneity, while the other two are designed to detect the mutant allele and the second is designed to detect the wild allele by PCR. The four primers were designed for the purpose of detecting the polymorphism of the SHBG gene for the locus (rs1799941) as shown in Table (1).

Table 1: Primers used for detection of SHBG gene polymorphisms for the locus (rs1799941)

IF41	TAACCCTCCACCGCCCACCCA
IR41	AATGTGTAGAGGCAGGCAGCCTGGC
OF41	CAGGCCCTAGAGGAGGAGAGGGGAGA
OR41	GGTGGGGAGAACAGGTCTCAGGGC

PCR reactions were applied using the program in Table (2):

Table 2: PCR reaction program

Numbers of Cycles	Time	Temperature	Phase
1	4 minutes	94	Initial metamorphosis
35	45 second	94	metamorphosis
	1 min	66	Initiating link
	1 min	72	Elongation
1	7 minutes	72	Final elongation

Statistical Analysis

Through the Chi-square test, it is determined whether the population is significant or not. The statistical program SPSS-Verison18 was also used for the two groups of women and healthy ones in order to identify the probability of risk factors for PCOS, as well as the probability of any genotypes or allelic recurrence over the risk factors for each group.

III. RESULTS

The results of the current study showed that 71.5% of the affected women suffer from irregular menstruation, and 70% of them suffer from hirsutism. About 50% of them suffer from acne, 41% suffer from baldness in the front of the head and 14% of them have She suffers from infertility. PCOS patients also had a higher body mass index than the control group. While the results of measuring hormone levels in the affected women were lower, we notice a decrease in the level of the hormone SHBG in the affected women compared to the uninfected women, but the decrease is small due to the small sample size. While the levels of the hormone were the male masculinity of women with PCOS is higher than that of non-affected women, as shown in Table (3).

Table 3: Concentration of biochemical variables in infected women compared with the control group and the value of (t) and (p) for each variable

Probability Value P & t value	*The control Group Mean ±SD*	*Infected Women Mean ±SD*	*Variables Parameters*
-5.57 , 0.0002	0.876±0.079	0.376±0.042	sex hormone-binding

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			with globulin SHBG
9.61 , 0.0005	21.33±0.014	0.478 ±0.024	Male Hormone Testo

Results of Electrophoresis in agarose gel

DNA was extracted from all blood samples under study, then electrophoresis was performed to ensure the presence and integrity of the DNA bundles on the prepared 1% agarose gel. The bundles are examined under the ultraviolet device as shown in Figure (1).

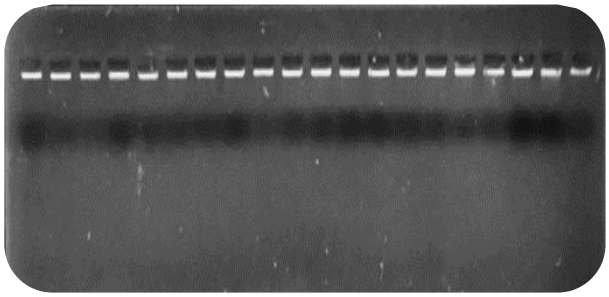

Figure 1: Results of electrophoresis on 1% agarose gel for extracted DNA samples.

Molecular characterization of the SHBG gene polymorphism at the (rs1799941) locus.The results of TARMS-PCR electrophoresis on 2% agarose gel were shown to detect the SHBG gene for the locus (rs1799941), showing the homozygous (GG) genotype (404+270 bp) and the heterozygous GA (GA). It is represented by the (404+270+210) bp band and the homozygous mutant (AA) genotype is represented by the (404+210 bp) band with the 100bp Marke as shown in Figure 2.

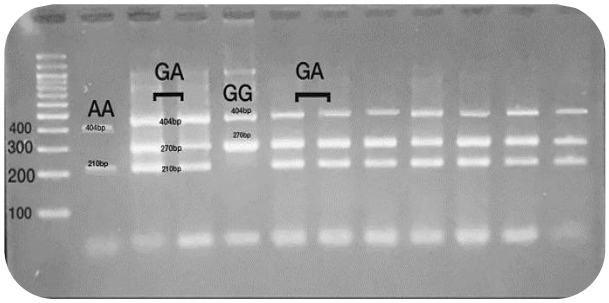

Figure 2: Electrophoresis Results of PCR product on 2% agarose gel.

The results of our current study showed that the observed number of women with PCOS with homozygous normal genotype GG is (4) and frequency (0.057), while the observed number of women with heterozygous GA genotype is (44) and frequency (0.628). As for the affected women with a homozygous genotype of the AA mutant, the observed number was (22) and the frequency (0.314), if the frequency of the G allele was (0.37), while the frequency of the A allele was (0.63).

IV. DISCUSSION

The aim of this study was to determine whether there is an independent relationship between PCOS and SHBG gene polymorphisms. Using the data collected from 70 women with PCOS, we found that the SHBG gene polymorphism of the locus (rs1799941) was associated with PCOS, which indicated that the SHBG gene might be a candidate gene [12]. In this study, it appeared that the levels of SHBG hormone in the serum of women with PCOS whose value was ng/ml (0.042 ± 0.376) was lower than its level in women of the control group, which amounted to ng/ml (0.079 ± 0.876). A useful biomarker for the diagnosis of PCOS. This study agrees with previous studies confirming the association of low levels of SHBG with PCOS It contributes to the symptoms of hyperandrogenism, such as hirsutism and acne [13] [14]. Women who have low levels of SHBG are more likely to develop hyperandrogenism as a result of dysregulation of androgen access to target tissues, as it works to regulate biological activities [15]. A low serum SHBG level is significantly associated with obesity, insulin resistance, type 2 diabetes, and abnormalities in glucose metabolism, because excess insulin in the blood inhibits the synthesis of the hormone from liver tissue. Low SHBG levels are also associated with fatty liver disease in women with PCOS. Reducing hepatic SHBG production leads to an increase in free androgens in the bloodstream, causing a host defect in the ovary [16]. While a significant increase in the male hormone concentration was observed in women with PCOS whose value was ng/ml(0.024±4780.) compared with the group of uninfected women whose value was ng/ml(0.014±2130.), these results agreed With several studies confirming that the rise in male hormone leads to excess androgen production in the ovaries, which leads to the emergence of androgenic symptoms such as excessive hair and irregular menstruation, in addition to a defect in the secretion of ovarian hormones in affected women [17]. The increased secretion from the ovaries and adrenal glands is a major source of androgen elevation in women with PCOS [18]. The concentration of male hormone is greatly affected by the concentration of SHBG because only 1-2% of testosterone in the circulatory system is free and 65% of it is bound to SHBG and the rest is bound to albumin, which can be in women with PCOS with low levels of the hormone SHBG elevated levels of free testosterone [19]. A polymorphism of the SHBG gene of locus rs1799941 that could be implicated in the pathogenesis of PCOS has been studied in several clinical settings. Since the SHBG gene is located on chromosome 7 specifically in the P12 region, this region of chromosome 7 is considered a fragile site within the human genome that contains multiple Alu sequences. These sequences are involved in chromosomal recombination activities [20].

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This gene is a 5'UTR G-A polymorphism located within the proximal promoter sequence, an important region in transcriptional regulation in exon 1 that encodes a polypeptide for SHBG secretion by the liver. The results of the current study showed that the group of women with PCOS who carry the normal genotype GG was (5.7%), while the percentage of affected women with the mixture GA genotype was (62.9%), and the percentage of the affected women with the mutated AA genotype was (31.4%). When comparing these percentages with the group of women without PCOS, it was found that the percentage of women with the normal genotype GG (46.7%), while the percentage of women carrying the mixed genotype GA was (40%), While the percentage of women carrying the AA genotype was (13.3%). The OR value of the mixed GA genotype was (12.833) and this indicates that the mixed genotype is a risk factor for the disease at the level of probability ($P<0.01$). Whereas, the (OR) value when comparing the affected and unaffected women who carry the mutant AA genotype was (9.250), and this indicates that the homozygous mutant genotype is a risk factor for the disease at the level of probability ($P<0.01$). And we noticed that the frequency of the A allele is higher in the infected group compared to the control group, and this indicates that the A allele is responsible for the association of the disease. It indicates that the mutated allele A represents a risk factor in patients at a certain level ($0.01>P$), while the G allele has a protective role from the disease. This result agrees with a study [21] conducted on Bahraini women, which indicated that the role of SHBG as a candidate gene for PCOS has an important and effective role in the pathophysiology of PCOS. In another study, which indicated that there is a relationship between this gene and obesity for young people without diabetes, it was found that the percentage of people carrying the AA genotype is more compared to other genotypes, and the OR value of the mutant allele was (2.54) [22]. The current study demonstrated the effect of SHBG gene polymorphisms on hormone levels in women with PCOS. The current study showed the effect of SHBG gene polymorphisms on hormone levels in women with PCOS, as there was a significant difference ($P < 0.05$) in the level of SHBG hormone for affected women who carry the AA genotype, whose value was ng/ml (0.088 ± 0.562) compared with the level of SHBG gene. The hormone SHBG in women carrying heterozygous genotype GA and normal genotype GG, whose value was recorded as ng/ml (0.055 ± 0.386) and ng/ml (0.158 ± 0.342), respectively. This result agreed with a study that confirms a significant and independent association of this gene with SHBG levels [23], the level of this hormone increased in women with PCOS who carry the AA mutated genotype than it is in women with GG genotype. The AA genotype was found to be associated with higher levels of SHBG compared to the GG genotype in both postmenopausal men and women. The results of the study showed that there were significant ($P < 0.05$) differences in the level of testosterone hormone when comparing between the level of hormones ng/ml (0.089 ± 0.541)' ng/ml (0.041 ± 0.499), ng/ml (0.031 ± 0.461) among the affected women who They carry the normal genotype GG, mutants AA and mixture GA, respectively.

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