Relationship of Polymorphisms of the AR Gene (Rs6152) With Polycystic Ovary Syndrome in a Group of Iraqi Women.

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Abstract

Androgens are a group of steroidal sex hormones that have an important role in regulating female fertility and ovarian function. Androgens such as testosterone and dihydrotestosterone are affected by androgen receptors (AR) found in the cytoplasm inactively activated by androgens. This study explores androgen receptor (AR) gene polymorphisms in Iraqi women with PCOS that may cause the onset of this disease. Genomic DNA was extracted from the blood samples of women with and without PCOS. The AR gene was amplified by Tetra_ARMS PCR technique, then the PCR product was migrated onto a 2% agarose gel. Three genotypes appeared, the normal homozygous (GG) genotype (250+172 bp) and the heterozygous (GA) heterozygous genotype. It is represented by the band (250+172+118bp) and the homozygous mutant homozygous (AA) genotype is represented by the bundle (250+118 bp). Of the 70 PCOS patients included in the molecular study, 13 (18.6%) were carriers of the normal homozygous GG genotype, 40 (57.1%) were the heterozygous GA genotype and 17 (24.3%) were the mutated AA genotype. In the current study, the (OR) value of the mixed GA genotype appeared (5.543), which indicates that the mixed genotype is a risk factor for the disease at a probability level ($p \leq 0.01$), while the OR value of the mutated AA genotype was (8.596).) This indicates that the homozygous mutant genotype is a risk factor for the disease at a probability level (0.01) $p \leq$. 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 disease association. These results indicated that POCS It could be due to mutations in exon 1 of the AR gene.

Keywords: androgen receptors (AR), Polycystic ovary syndrome (PCOS)

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common and complex hormonal and genetic disorders in women of childbearing age and affects about 10-15% of women around the world (McCartney et al.,2016). It was first described by Leventhal & Stein in 1935 (Stein et al.,1935) who reported 7 cases of patients with amenorrhea, infertility and hirsutism. PCOS is a clinically heterogeneous condition with poorly understood etiologies (Sheikhha et al.,2007). There are many clinical and biochemical features of PCOS, including increased levels of hyperandrogenesis, such as an increase in the level of testosterone hormone than the normal limit, which causes infertility in most cases, anovulatory and hirsutism (Leon et al.,1994). As well as insulin resistance (IR), as about 70% of them are exposed to insulin resistance that develops into type 2 diabetes (Diamanti et al.,2008) (Kollmann et al.,2014). Three criteria for diagnosing this syndrome were established by the National Institutes of Health (NIH) (1990).), and the European Society for Human Reprodaction and Embryology (ESHRE) in cooperation with the American Society for Reprodactive Medicine (ASRM) and the Society for Hyperandrogenism (AES) in 2006 (Anagnostis et al.,2018). Polycystic ovary syndrome is based on the Rotterdam criteria established by the Society (ESHRE) in cooperation with (ASRM) in 2004, and it is one of the most used criteria in the diagnosis of this syndrome, which depends on the presence of at least two phenotypic characteristics: - Anovulation (Anovulation) and hyperandrogenism (HA) The presence of cysts on the ovaries.Genetics plays a major role in the events of the syndrome, as it was found that a woman in her family has one or more individuals with PCOS, such as a mother or sister. They are more at risk of contracting it than others (Louis, (2007). Also, many studies have shown that identical twins are more likely to have polycystic ovary syndrome than non-identical twins or non-twins (Kabal, (2016). However, the way in which the syndrome is inherited is still unclear, but clinical genetic study indicated that it is autosomal dominant (Balen, (2010).then the Identification of many genes closely related to polycystic ovary syndrome, including genes responsible for the manufacture of steroid hormones (CYP19 - CYP11A - CYP17 - CYP21) or that contribute to chronic inflammation such as (TPA, PTI-1, TNF-a genes) or that are involved in the process of Reproduction and insulin resistance, including genes (FSHR, INSR, IL-6) (Prapas et al .,2009). Androgens are a group of lipophilic steroidal sex hormones that have an important role in regulating female fertility and ovarian function (Astapova et al .,2019). Regulated by the hypothalamic-pituitary hormone axis. Androgens

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such as testosterone and dihydrotestosterone are affected by the androgen receptor (AR) Human AR belongs to the nuclear receptor family of transcription factors that are ligand-activated. Androgen receptors are widely expressed in female reproductive tissues such as the endometrium. When androgen receptors are activated by androgens, they travel to the nucleus and bind to specific chromosomal DNA sequences (androgen response elements) in the regulatory regions of AR regulatory genes. Androgen-regulated proteins (Walters et al.,2018). The AR gene is located on the long arm of the X chromosome, specifically at its region (q11.2-q12), as shown in Figure (1). This gene consists of 8 exons separated by relatively long introns (Heinlein et al.,2002), this gene encodes 919 amino acids AR is isolated by heat shock proteins in the cytoplasm that help to stabilize and protect AR from degradation (Younas et al .,2019), and this gene has an important role In causing polycystic ovary syndrome, any defect in this gene leads to an increase in the level of androgen, which is one of the most common characteristics of this syndrome (Xia et al.,2012). There are two main types of AR gene polymorphisms, the triple CAG repeats and the triple GGN repeat (Vezza et al.,2020). Triple repetition length is associated with increased androgen production, acne vulgaris, and testosterone levels in the blood. In terms of the triple repeat GGN, the first study to determine the importance of the length of the triple repeat GGN on the function of AR was published in 1996 by eliminating the repeat that reduced AR activity by 30% (Gao etal.,1996). Short GGN frequency has been reported to be associated with decreased androgen activity (Sharp et al., 2019)

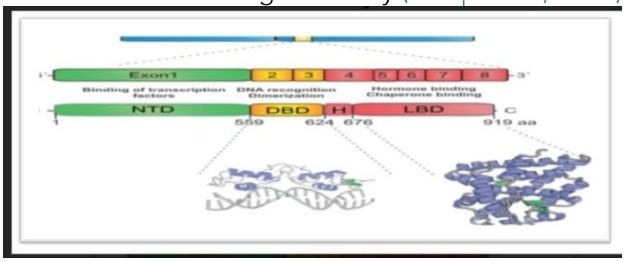

Figure 2 Genomic regulation of the androgen receptor gene AR

2. Materials and Methods

The study included (70) women with PCOS with (30) non-infected women used as a control group, their ages ranged between (16-40). (5) ml of venous blood was drawn for each woman with and without PC. The ovaries, during days (2-6) of the menstrual cycle and were distributed into two parts. The first section: - Withdrawal (2) ml of blood was placed in an EDTA anticoagulant tube, and the samples were kept in supercooling (-86 m) for the purpose of using it in the extraction of blood. DNA and molecular studies. As for the second section, (3) ml of blood was withdrawn and placed in a gel tube in order to separate the serum and keep the samples in supercooling (86-C) for the purpose of conducting various biochemical tests. Clinical and Biochemical measurement All information was recorded on the sample collection form including height and body weight. On day 3 of the menstrual cycle, serum levels of hormones including AR and testosterone were measured using the attached steps of the EISA ready-made kit from BIOLABO.

Protocol of DNA Isolation DNA

The DNA was extracted from the blood according to the instructions 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 AR gene polymorphism for the locus (rs6152) using PCR_Tetra_ARMS technique.

The principle of this technique is that it duplicates the gene segment to be studied by 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 polymerase chain reaction. The four primers were designed for the purpose of detecting the polymorphism of the AR gene at the rs6152 locus, as shown in Table (1).

Table (1) The prefixed for the purpose of detection of multi-gene AR on site rs6152	
IF52	GCAGCGGGAGAGCGAGGTAG
IR52	AAGTGGGAGCCCCCGAGTCT
OF52	CTTAAAGACATCCTGAGCGAGGCC
OR52	GAAGCTGTTCCCCTGGACTCAGAT

PCR reactions were applied using the program in Table (2 .).			
Phase	Temperature	Time	Number of cycles
Initial metamorphosis/	94	4 minutes	1
Metamorphosis	94	45 seconds	35
Initiating link	66	1 minute	35
Elongation	72	1 minute	35
Final elongation	72	7 minutes	1

3. 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.

4. Results

The results of the current study showed that 70% of the women with PCOS suffer from hirsutism, and 71.5% suffer from irregular menstruation. About 50% of them suffer from acne, 41% suffer from baldness in the front of the head and 14 % of them were infertile. The PCOS patients also had a higher body mass index than the control group, while the levels of the AR hormone in the serum of the PCOS women were lower compared with the non-affected women. While the levels of the male hormone in the PCOS women were higher compared to the PCOS patients. With uninfected 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

Variables	Infected Parameters Mean ±SD	control group Mean ±S	Probability P-value and t.-value
Androgen Receptor AR	0.168±0.01	0.872±0.09	-7.26, 0.000
Androgen Testo	0.478 ± 0.02	21.33 ± 0.01	9.61, 0.000

Results of electrophoresis in agarose gel

DNA was extracted from all blood samples under study, then electrophoresis was carried out to ensure the presence of DNA bundles on the prepared acarzo gel with a concentration of 1% under a voltage of 70 volts/cm and a current of 40 mA for one hour. illustrated in Figure (2).

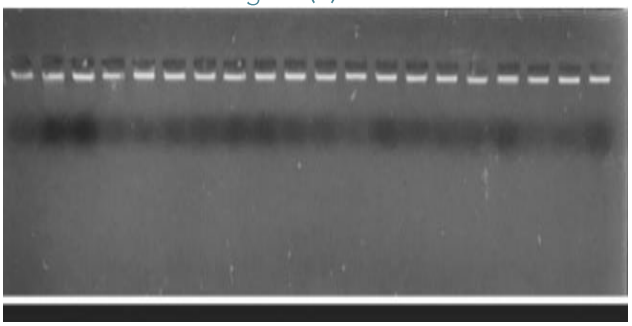

Figure 2: Electrophoresis results on 1% agarose gel for extracted DNA samples.Molecular characterization of the AR gene polymorphism of the rs6152. Locus

The results of the electrophoresis of the PCR product on agarose gel at a concentration of 2% showed that the wild allele (G) appeared at a bundle of (172) bp in size, and the mutant allele (A) appeared at a bundle of (118) bp in size. It results in three genotypes. Normal Homozygous (GG), Heterozygous (GA) and Mutant Homozyous (GG) as shown in Figure 3. The results of the two current studies shown in Table (4) showed that the observed number of women with PCOS with normal homozygous genotype GG is (13). while the observed number of women with a heterozygous GA genotype was (40, and for women with a homozygous mutated AA genotype, the number observed was (17), if the frequency of the G allele is (0.47), while the frequency of the A allele is (0.53).

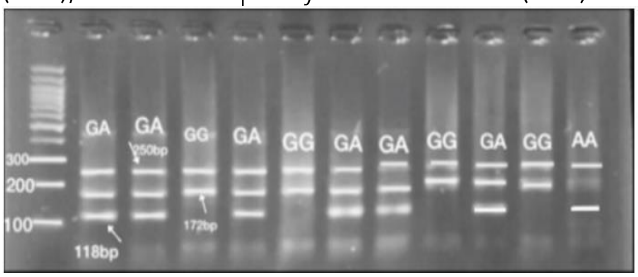

Figure 3 Electrophoresis results of the PCR product on 2% agarose gel.

Table (4) Percentage and frequency of alleles and genotypes of the androgen receptor (AR) locus (rs6152) for women with and without PCOS

genotype	infected women number (70)		healthy women number (30)		OR. value	95% CI	Statistical function (p value)
	number	(%)	number	(%)			
GG	13	18.6	22	73.3	Ref.	Ref.	-
GA	40	57.1	8	26.7	5.543	7.659 - 23.527	≤ 0.01 **
AA	17	24.3	0	0	8.596	3.238 - 50.68	≤ 0.01 **
allele	number	(%)	number	(%)	OR. value	95% CI	P value
G	66	47.2	47.2	73.33	Ref.	-	
A	74	52.8	52.8	26.67	3.083	1.591 - 5.974	≤ 0.01 **
P ≥ 0.05: Non-significant; *: Significant at p ≤ 0.05; **: Highly significant at p ≤ 0.01							

The G allele is the normal allele. The A allele is the mutant allele.

5. Discussion

It is well known that PCOS is a common endocrine disorder in women of childbearing age (McCartney et al.,2016). In this study, phenotypes such as hyperandrogenism represented by hirsutism, acne and hair loss in addition to menstrual irregularities and ovulatory dysfunction were noted as common features. For polycystic ovary syndrome. In this study, it appeared that the levels of androgen receptor hormone in the serum of women with PCOS whose value was ng/ml (0.016 ± 0.168) was lower than its level in women of the control group, which amounted to ng/ml (0.872 ± 0.096). The results of the two current studies agree with the study Walters and his group, which confirms the lack of androgen activity leads to ovarian weakness and a decrease in the rate of ovulation (Walters et al.,2012). AR is present in the cell cytoplasm in an inactive form that is activated when testosterone binds to it. While a significant increase in the male hormone

concentration was observed in women with PCOS whose value was ng/ml (0.024±4780.) compared to the control group whose value was ng/ml (0.014±2130.). These results agreed with several Studies conducted for several decades confirm that the increase in male hormone leads to the production of excess androgens 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 (Pasquali et al.,2016). The male hormone is considered one of the estrogenic hormones that has a high efficacy in causing PCOS. This hormone is produced in females by the ovaries and adrenal glands (Skarra et al.,2017), the increased secretion from the ovaries and adrenal glands is a main source of androgen elevation in women with PCOS (Dumitrescu etal.,2015). AR is expressed in granulosa cells and endometrium in polycystic ovaries compared to normal ovaries (Rice et al.,2007). Recent studies in both human and animal models emphasize the importance of AR in follicle growth and survival (Nielsen et al.,2011). Important in regulating female fertility and ovarian function. (Astapova et al.,2019). In the study we tested the association of the rs6152 (G/A) locus AR gene polymorphism and the role of the substitution mutation of the androgen receptor gene in women with PCOS. The SNP (rs6152) is located between the CAG repeat sequence that encodes this polyglutamine extension and the GGN repeat sequence of the polyglycine extension in exon 1 of the androgen receptor gene that encodes the amino domain of the androgen receptor involved in transcriptional activation (Faber et al.,1989). Our study found an association between the AR gene and PCOS. This study agreed with the results of a study that confirmed the association of polymorphisms of the AR gene with PCOS and revealed that shorter CAG(n) alleles in Exon 1 of the AR gene enhance susceptibility to PCOS by regulating AR activity. Or by causing excessive androgen t, the presence of short androgen receptors (CAG) (n) tends to appear more frequently in women with PCOS than in the control group (Livadas et al.,2014) (Rajender et al.,2013). The results of our current study indicate that the group of women with PCOS who carry the normal genotype GG was (18.6%), while the percentage of affected women with the mixed genotype GA was (57.1%) and the percentage of women with the mutated genotype AA ((24.3%). When comparing these percentages with the group of women without PCOS, it was found that the percentage of women with the normal genotype GG (73.3%), while the percentage of women carrying the mixed genotype GA was (26.7%), while the mutant genotype AA did not appear in the women The control group, the OR value of the mixed GA genotype was (5.543), and this indicates that the mixed genotype is a risk factor for the disease at a probability level (0.01 p ≤), while the OR value when comparing the affected and uninfected women who carry The AA mutant genotype was (8.596), and this indicates that the homozygous mutant genotype is a risk factor for the disease at the probability level ($p \leq 0.01$). We noted that the frequency of the A allele is higher in the infected group compared to the control group. This indicates that the A allele is responsible for the disease association. It indicates that the mutated A allele is a risk factor for patients. The current study showed the effect of the G to A substitution mutation (G1733A) in the AR gene on hormone levels in women with PCOS.) followed by women with a GA mixture genotype whose value was recorded (0.015 ± 0.176), and the lowest value of AR was recorded for women with mutated AA genotype, which was (0.103 ± 0.032). As for the effect of the gene on the levels of Testosterone hormone, a significant increase was observed at the level ($P \leq 0.05$) in the concentration of the hormone in affected women with a genetic phenotype.

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