Studying Cytotoxic T-lymphocyte- Associated Antigen-4 (CTLA-4) gene Polymorphism in a Sample of Iraqi Women with Polycystic Ovarian Syndrome

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Abstract:

Polycystic ovarian syndrome (PCOS) considers as the most common disorder among women during reproductive age. Its common features involve hyperandrogenism, chronic anovulation, and weight gain. Till now, the pathogenesis of PCOS stay unknown, and there is evidence considered PCOS as a low-grade inflammatory disease. Polycystic ovarian syndrome is associated with a variety of endocrine and metabolic disturbances.

The present study was designed to detect the role of (CTLA-4) gene polymorphism (rs733618) with PCOS. A total of 60 PCOS patients and 30 healthy women, matching in average age and body mass index (BMI), were enrolled in this study. Patients with PCOS were attend to AL - Nahrain University High Institute for Infertility Assisted Reproductive Technology, in Baghdad between Septembers to December/2018. Blood samples were aspirated from both groups to detection (CTLA-4) gene polymorphism (rs733618) by tetra-primer amplification-refractory mutation system based on real time polymerase chain reaction (ARMS-qPCR). The obtained results revealed normal genotyping for both groups. The result of current study confirms that there is no role of (CTLA-4) gene polymorphism (rs 733618) in PCOS.

Key words: polycystic ovary syndrome, tetra-primer amplification- refractory mutation system (ARMS-qPCR), Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) gene polymorphism.

دراسة دور التعدد الشكلي للجين المستضد- ٤ المقترن مع الخلايا اللمفاوية التائية السامة لشريحة من النساء العراقيات المصابات متلازمة تكيس المبايض

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الخلاصة:

متلازمة تكيس المبايض هي اضطراب شائع عند النساء في سن الانجاب, تتميز بفرط افراز هرمون الاندروجين وقلة الخصوبة وزيادة الوزن. طريقة تدهور متلازمة تكيس المبايض لاتزال مجهولة الى حد كبير, وهناك أدلة تعتبر متلازمة تكيس المبايض كمرض التهابي منخفض الدرجة, حتى الان اقتصرت الرعاية الطبية للمريضات اللاتي يعانين من متلازمة تكييس المبايض على التحكم في الأعراض المتعلقة بها كزيادة الوزن ,البثور,الصلع الرجالي النمط ,انقطاع الدورة الشهرية والعقم. ترتبط متلازمة تكيس المبايض بمجموعة متنوعة من اضطرابات الغدد الصماء والتمثيل الغذائي.

تم تصميم هذه الدراسة للتحري عن دور التعدد الشكلي للجين المستضد-٤ المقترن مع الخلايا الليمفاوية التائية السامة (CTLA-4) عند مريضات متلازمة تكيس المبايض عند المقارنة مع مجموعة السيطرة المتكونة من النساء السليمات .

تضمنت الدراسة مشاركة ٦٠ إمرأة مصابة بمتلازمة تكيس المبايض و٣٠ إمرأة سليمة, وكانت المجموعتان متطابقتان من حيث متوسط العمر و معدل كتلة الجسم في هذه الدراسة. تم جمع العينات من مريضات تكيس المبايض اللاتي حضرن للمعهد العالي لتشخيص العقم والتقنيات المساعدة على الأنجاب – جامعة النهرين / بغداد للفترة من أيلول الى كانون الاول من عام ٢٠١٨. عينات الدم التي جمعت من المجموعتين تم استخدامها للكشف عن التعدد الشكلي للجين (CTLA-4) بأستخدام تقنية نظام الممانعة للتضخيم رباعي البواديء المعتمد على تفاعل البلمرة ذو الوقت الحقيقي (ARMS- qPCR). كشفت النتائج التي تم الحصول عليها من دراسة تعدد الاشكال الجينية للجين CTLA-4 عن نمط جيني طبيعي لكلا المجموعتين.

النتائج التي تم الحصول عليها من دراسة التعدد الشكلي ل CTLA-4 gene بينت أنه لايوجد أي دور للتعدد الشكلي ل gene (CTLA-4) (rs 733618) في متلازمة تكيس المبايض.

الكلمات المفتاحية: متلازمة تكيس المبايض, تقنية نظام الممانعة للتضخيم رباعي البواديء المعتمد على تفاعل البلمرة ذو الوقت الحقيقي, التعدد الشكلي للجين المستضد-٤ المقترن مع الخلايا الليمفاوية التائية السامة.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy of reproductive system of women, it is a heterogeneous condition with several signs and symptoms and a controversial diagnosis that differs between countries depending on the used criteria, like National Institutes of Health (NIH) criteria, Androgen Excess Society (AES) criteria, and Rotterdam 2003 criteria[1]. Nowadays, the proportion of women with the syndrome in the world is more than 10% in young women and increasing continuously [2].

Increased evidences confirming relationship of PCOS with obesity and insulin resistance (IR), which is an important cause of systemic complications like type 2 diabetes (DM2) and heart disease, and dermatological complications which associated with increase androgen level in plasma that lead to hirsutism, acne androgenic alopecia[3,4], metabolic syndrome and autoimmune diseases[5]. Reproductive system complications lead to infertility in some cases [6], and all these complications lead to psychological complications like depression, anxiety and selflessness.

Cytotoxic T-lymphocyte- Associated Antigen-4 (CTLA-4) gene is one of the most important negative regulators of T-cell in immune response and is a strong candidate susceptibility gene in autoimmunity. The gene location is on chromosome 2 at bands q33 of human gene maps [7]. Polycystic ovarian syndrome may result from reduce capability of T-reg generation, where CTLA4 is a specific marker of T-reg and there is a competition between this marker and CD28 to bind with CD80 and CD86 on surface of antigen-presenting cells, such as macrophages. T-cell activation can prevented when CTLA4 blocking the binding of CD28 on T cells and, therefore, loss of CTLA4 in natural T-reg cells may possibly affect the immune response and occurrence of PCOS, where the gene encoding CTLA-4 may responsible for severe autoimmunity [8,9].

The aim of study:

The current study was designed to detect role of (CTLA-4) gene polymorphesim in PCOS patients.

Subjects, Materials and Methods:

This study was conducted in Al-Nahrain University/ High Institute for Infertility Assisted Reproductive Technology-Baghdad, after taking approval by the scientific committee in the College of Pharmacy-Mustansiriyah University.

Ninety Iraqi women were participated in this study throughout a period from September to December /2018. Sixty women were already diagnosed with

PCOS and the other thirty were healthy apparent. Proceeding to enrollment of participants, history information like age (ranged from 15 to 35 years), BMI (less than 30 i.e. not obese women), concomitant diseases, other treatment were taken after achieving diagnoses by a consultant gynecologist based on abdominal ultrasound and depending on Rotterdam 2003 Criteria for PCOS, as shown in table (1) below.

Table (1): Rotterdam criteria for PCOS diagnosis [10].

NO.	Criteria
1	Oligomenorrhea and/or anovulation.
2	Clinical and or biochemical signs of hyperandrogenesim.
3	Polycystic ovaries by ultrasound.

Exclusion criteria include all subjects with any medication known to interfere with results of the present study, subjects with autoimmune diseases, DM1 or 2 and all subjects with any acute illness or infectious disease.

The body mass index was determined by dividing the individual weight (in kilogram) by the squared value of height (in meter) [11].

Blood samples were collected from subjects, placed in 1.5 mg/ml ethylene diamine tetra acetic acid (EDTA) containing tubes for DNA extraction. Then, all blood samples storage at - 20 °C until the time of assay. The assay done by tetra–primer amplification refractory mutation system based on real time polymerase chain reaction technique (ARMS-qPCR), which is a simple and effective method for detection of any mutation involve single base [12].

Statistical Analysis:

Statistical Analysis System (SAS-2012) program was used to show the mean and standard error of mean (M± SE). Dependent t-test was used to compare between mean values. The probability considered significantly differ when $P<0.05$, highly significant difference when $P<0.01$, and non-significant (NS) when $P>0.05$ [13].

Results:

Demographics and Baseline Characteristics:

The results recorded matching in mean ages and BMI between two groups. The mean ages of patients group were 25.25 ± 0.63 years, their BMI mean was 24.26 ± 0.39 kg/m^2. The mean ages of control group were 25.60 ± 0.96 years, there BMI mean was 24.80 ± 0.51 kg/m^2, as illustrated in table (2).

Table (2): Demographics and baseline characteristics.

Group	Mean ± SE	
	Age (year)	BMI (kg/m^2)
Control	25.60 ± 0.96	24.80 ± 0.51
Patients	25.25 ± 0.63	24.26 ± 0.39
T-test	2.237 ^{NS}	1.328 ^{NS}
P-value	0.756	0.427

NS= Non-Significant difference ($P>0.05$).

Detection of Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) gene polymorphism.

Distributions of genotype and allele frequencies of rs773618 showed that all subjects enrolled in the current study were expressed as TT genotype (wild type) in their genome, that mean there is no significant difference between patients and control group, as in table (3).

Table (3): Genotypes and alleles frequencies of rs733618 polymorphism in patients and control group.

SNPs (rs)	Genotype	patients	control	total	%	allele frequency in patient	allele frequency in control
733618	TT	60	30	90	100	(T)120	(T) 60
	TC	0	0	0	0	(C) 0	(C) 0
	CC	0	0	0	0	(C) 0	(C) 0

SNP= single nucleotide polymorphism, T= thymine, C= cytosine.

The melting curve of TT genotype (wild type) for patients and control groups were illustrated in figure (1-a) and figure (1-b), respectively.

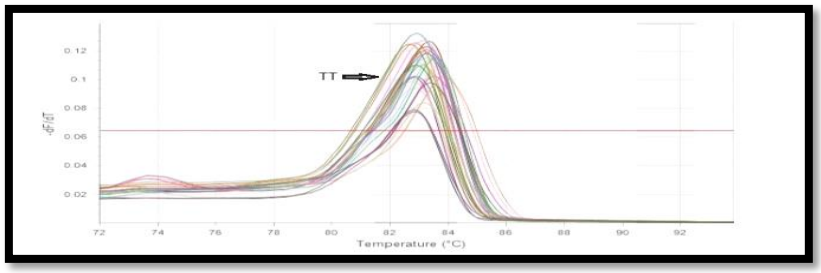

Figure (1-a): Melting curves of TT genotype for 30 subjects from patients group by tetra– primer amplification refractory mutation system based on real time polymerase chain reaction technique.

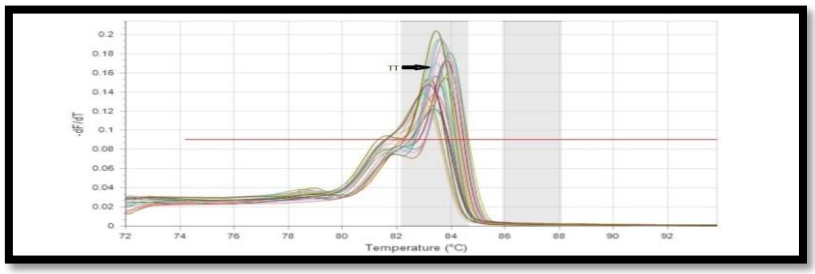

Figure (2): Melting curves of TT genotype for control group by tetra– primer amplification refractory mutation system based on real time polymerase chain reaction technique.

Discussion:

Polycystic ovary syndrome (PCOS) considers the most common cause of chronic hyperandrogenism, anovulation and infertility in young women [14]. Considerable evidence suggests that PCOS has diverse causes, arising as a complex trait with contributions from both heritable and environmental factors that affect ovarian steroidogenesis [15]. Insulin resistant leading to hyperinsulinuma, in part related to coexistent obesity, is the most common non-steroidogenic factor in polycystic ovaries, where this complex interaction generally mimics an autosomal dominant trait [16].

In the current study, no significant difference between patients and control group in both the genotypic and allelic frequency distribution was reported. All women (patients and control group) participating in this study carry normal homozygous genotype (TT) i.e. T- allele distributed 100% in population of the

study, as shown in table 4 and figures 1-a, 1-b and 2. This mean there was no association between (CTLA-4) gene polymorphism (rs733618) and PCOS.

The results of the present study didn't agree with other study performed that CTLA-4 gene polymorphism (rs733618) have a role in PCOS because the results revealed a significant difference between the two groups (patients and control) in genotype and allele distribution. The single nucleotide polymorphism (SNP) of CTLA-4 gene in that study has three genotypes (CC, CT, TT) with significant differences in the genotype and allele frequencies for both patients and control group [17]. There are many factors that could explain the discrepant results from different studies, including different population characteristics (sample size and ethic differences, inclusion and exclusion criteria), genetic diversity between two populations enrolled in studies, which in turn gets for various reasons, including random external forces can cause genetic drift sometimes, lead to random fluctuations in the numbers of alleles in a population. Migration of peoples, changing the place of living for period of time and intermarriage between different peoples, all these factors lead to share in gametes carrying alleles among peoples that can stimulate the existing proportion of alleles in the destination population, distribution of human and environmental causes also effect on genetic makeup over population. On the other hand, addition of a new allele to a population makes it more able to survive in sometimes or makes it less able to survive or can cause diseases, while in other situations have no effect at all [18,19].

Further studies suggested a significant role for (CTLA-4) gene polymorphism in autoimmune diseases and different types of cancer associated with CC genotype in patients by affecting the immune response because C allele-bearing (CTLA-4) gene has a high ability to control the activation of T-cells more than T allele-bearing CTLA-4 [20,21].

From that, it's suggested that this SNP doesn't have a role in PCOS, Other SNP in the same gene or other gene may play a role in PCOS-Iraqi patients, In the current study, the obtained results declared no correlation between CTLA-4 gene polymorphism and PCOS.

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