Physiological and Molecular Study of Iraqi Women with Polycystic Ovary Syndrome

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Abstract

Polycystic ovary syndrome is define as illness in women that a reproductive age because common endocrinopathy and lead to reproductive dysfunction also infertility. PCOS is usually alignment with insulin resistance and metabolic syndrome, it is think that PCOS is the outcome of genetic effect addition to ecological factor. In this study (100) samples which divided into 40 samples of heath women and 60 samples of women with PCOS. It diagnosed by ovarian morphology on ultrasound also test the fasting blood sugar, insulin, insulin resistance and testosterone hormone by biochemical method also used molecular method to detect the genotype of TCF_4 gene (rs290487) by RFLP-PCR technique. The results revealed that the frequency of allele T was recorded a highly significance ($P<0.01$) in patients (0.81%) while in control (0.89%), the frequency of allele C allele significant ($P<0.01$) in patients (0.19%) while in control (0.11%). We can be concluded from this study that the genetic variation in Iraqi women of gene (TCF4) was linked with PCOS while contrary in control (healthy women).

Keywords: *Ovary, Testosterone, gene, polycystic, polymorphism, Insulin resistance.*

Introduction

Polycystic ovary syndrome (PCOS) is a symptom because endocrinopathy or elevated androgens (male hormones) in females at reproductive age, with a incidence of upper 10% of women [1]. PCOS is produce to a mixture of genetic and environmental factors [2]. Rate of alterations among people may reveal that the effect of factors on the phenotype such environmental features, ethnic origin and race [3]. Many factors such as increased adiposity, mainly visceral adiposity lead to waist circumference (waist-to-hip) ratio elevation, may be related with hyperandrogenemia, glucose intolerance, dyslipidemia, and insulin resistance [4]. In actuality, about fifty percent of women with PCOS women have obesity or overweight [5]. Overweight in women suffer from PCOS align endocrine syndromes than in women with non over weight [6]. One of the most complaints in women with POCS is an ovulatory (infertility), also have risk factor as cardiovascular analogous to metabolic syndrome [7]. Stein and Leventhal who first explained PCOS since 1935 after they introduced and published it about seven women has amenorrhea, enlarged ovaries showed with polycystic shape, hirsutism, also obesity[8]. Actuality, PCOS known since 1844 via [9]. PCOS, also describe via ultrasonography (the occurrence of cyst about (2-8) mm in width or diameter, the number of it 10 or extra cysts, placed either peripherally position in core of stroma or scattered in it lead to stroma and ovarian volume increasing), this very most important diagnostic way for PCOS[10,11], the aim of study was to estimate some physiological and molecular parameters of Iraqi women with polycystic ovary syndrome

Material and Method

Experimental design: The study was directed afterward gaining ethical approval from Scientific Research Committee University of Baghdad/College of science/Department of biology, fulfillment this work in laboratories in Department of biology/College of science. The samples of study included (100) Iraqi women with PCOS the age of women about 18-45 years old, all of it diagnosed by morphology on ultrasound added to the biochemical method. They were divided into two main groups: the first group included 40 healthy women and the second included 60 women with PCOS.

Collection of Blood Sample: Blood was collected after more than 12 hours fasting by syringes from vein. Venous blood was transported into tube covering with

EDTA for extraction of DNA and other amount of blood used to measurement some physiological parameters.

Biochemical Tests: Serum was separated after clot for 15-25 minutes at room temperature by centrifugation at (3500 rpm 15 min) to measurement biochemical tests. The fasting blood glucose test determined by (biosystem kit, Spain). Serum concentrations of insulin and testosterone were assessed using an ELISA kit (DRG Instruments Mbh, Germany).

Molecular Study: Total Genomic DNA was extracted from blood sample using G-spin™ kit. DNA was stored at −70 ºC, the PCR technique used to amplify of (rs290487) in *TCF4*, Amplification of the region with forward primer 5'-AGGAGGCTGCCATATTGTTACTT-3› and reverse primer 5'-ACACCTTTCTCATTTTCAATTTCG-3›. The condition of PCR in (table 1).

Table 1: The optimum condition of amplification TCF_4 (rs290487)

No.	Phase	Tm (°C)	Time	No. of cycle
1	Initial Denaturation	94°C	3 min.	35cycle
2	Denaturation -2	94°C	35 sec	
3	Annealing	53°C	35 sec	
4	Extension-1	72°C	35 sec	
5	Extension -2	72°C	7 min.	

The PCR product was 153-bp after electrophoresed on polyacrylamide gel with red stain. It was digested for 50 min with BstUI by RFLP- PCR, reaction conditions in table (2)

Table 2: Reaction condition of Restriction Enzyme BstUI

Protocol	Volume
Product PCR	µl 5
R. E.	µl 1
Buffer	µl 4
Temperature/Time	60°C/50 min

Statistical Analysis: The program is system SAS 2012 was used to compare between difference of means such as Least significant difference test (ANOVA).

Results and Discussion

The study revealed that the differences in values of biochemical parameters as fasting blood level also hormones like insulin and testosterone, also level of insulin resistance when compare this result between women with PCOS and normal cases. The study showed high significant increasing (P≤0.01) (23.401) in weight of patients (92.20 ± 9.66) if compare with control (56.80 ± 3.10). At the same time, high significant increasing (P≤0.01) (35.333) of fasting blood level (177.20 ± 14.23) while in healthy women (92.78 ± 5.67). The differences high significant increasing (P≤0.01) (7.929) in insulin hormone concentration in serum in patient's women with PCOS (42.10 ± 1.98) while in control group (24.06 ± 2.81), insulin resistance showed high significant (P≤0.01) (4.549) in patients with PCOS (15.61 ± 1.61) that differ from healthy women (7.23 ± 1.13). Also the same result, high significant (P≤0.01) (127.91) of testosterone hormone concentration in women with PCOS (228.88 ± 98.24) while in healthy women (49.40 ± 10.78) Table 3.

The differences of genotype and allele frequency in patients with PCOS and control showed in (Figure-1). It was performed by RFLP- PCR, restriction enzyme BstUI digested products of PCR and were separated on agarose gel. Red stain was used of bands to stain in the gel

Table 3: Biochemical parameters of women with PCOS and control

Parameters	Mean ± SE		T-test	P-value
	Patients (PCOS)	Control		
Weight (kg)	92.20 ± 9.66	56.80 ± 3.10	23.401**	0.0082
FBS (mg/dl)	177.20 ± 14.23	92.78 ± 5.67	35.333 **	0.0006
Insulin (ng/ml)	42.10 ± 1.98	24.06 ± 2.81	7.929 **	0.0008
Insulin resistance	15.61 ± 1.61	7.23 ± 1.13	4.549 **	0.0028
Testosterone (ng/ml)	228.88 ± 98.24	49.40 ± 10.78	127.91 **	0.00109
** (P≤0.01).				

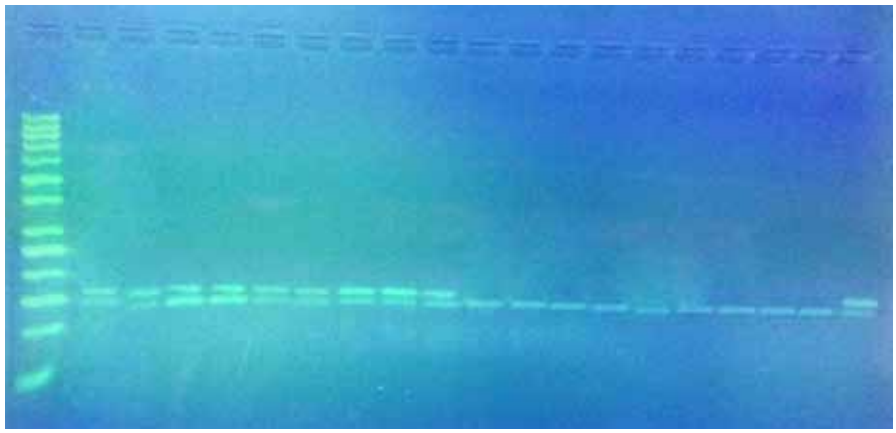

Figure (1): Electrophoresis arrangement of produce PCR of (2%). Lane's 2,3,4,5,6,7,8,9,19 heterozygous: TC genotype; Lane's 9,10,11,12,13,14,15,16,17,18 homozygous: TT genotype . M: molecular marker of DNA (50 bp) size.

The genotype variation of TCF4 (rs290487) polymorphism and allele frequency in patients with PCOS and control result revealed that the genotype TT showed high significant differences (P-value = 0.0082, χ^2 = 6.339) in patients with PCOS (61.36%) and in control (77.42%), also the genotype TC demonstrated significant differences (P-value = 0.0082 χ^2 = 6.339) in patients with PCOS (38.64%) and in control (22.58%). While the genotype CC seen no significant differences between patients and control. In addition, the frequency of allele T was recorded a highly significance (P<0.01) in patients (0.81%) while in control (0.89%), the frequency of allele C allele significant (P<0.01) in patients (0.19%) while in control (0.11%) Table-4.

Table 4: Genotype and allele frequency of TCF4 (rs 290487) gene in patients with PCOS women and control.

Genotype	Control		Patients		Chi-Square-χ^2	P-value
	No	%	No	%		
TT	48	77.42	54	61.36	6.339 **	0.0082
TC	14	22.58	34	38.64	6.339 **	0.0082
CC	0	0.00	0	0.00	NS	NS
Total	62	100	88	100	---	---
Allele	Frequency					
T	0.89		0.81		0.0082	
C	0.11		0.19		0.0082	
** (P≤0.01).						

In this study we depended on biochemical tests and ultrasonic method to determine any changes may be PCOS in women [12], the results in we study showed that increasing significantly in weight of women with PCOS versus normal women, our result agree align Dunaif et.al. result, who revealed that most of patients women with PCOS have obese contrary to normal [13]. Our results showed increasing significantly in level of blood glucose, insulin concentration and insulin resistance, Furthermore, β-cell dysfunction is one of metabolic abnormalities linked with PCOS and diabetes T2, may heritable factor of families. There are close linked both PCOS and obesity, because similar genes may causes fatness in affected patients with PCOS [14]. Also the insulin resistance addition to diabetes T2 is a common result of women with PCOS in state without obesity and insulin resistance with obese PCOS[15]. As many as the insulin resistance about 70% in women with PCOS but it nearly 10% in women with DM [16,17]. the study revealed that increasing in level of testosterone with elevated of glucose levels, insulin, and insulin resistance which may lack the sensitivity to expect risk of PCOS in women. Our study similar with this study which the women patients with PCOS suffer from hyper-insulinemia also have increasing in testosterone level, the testosterone level may greater than the normal value and middle raised level [18], but very high level of testosterone concentration is not usually in PCOS because a virility tumor [19,20]. The recent study showed that correlation between high testosterone concentration with obesity, exacting with fat in abdominal distribution[21], also insulin resistance with a elevated of glucose level rate[22]. In addition to, hyperandrogenism guide to PCOS as well as ovulatory dysfunction in vivo and vitro[23,24]. Level of insulin might increase peripheral steroidogenesis, whereas lead to destruction activity of CYP17 [25] inducing generally to testosterone overload without androstenedione and DHEAS excess therefore, hyperinsulinemia and insulin resistance raise production of ovarian testosterone in women with PCOS without effect on secretion of androstenedione[26]. In fact that, no association between adrenal androgen secretion and insulin resistance in PCOS or eumenorrhoic patients [27].

Our results revealed that the genotype and allele frequency of TCF4 (rs 290487) gene, allele T was recorded a highly significance (P<0.01) in patients (0.81%) while in control (0.89%), the frequency of allele C allele significant (P<0.01) in patients (0.19%) while in control (0.11%), while this study revealed that that there is no contribution of TCF4 gene variation of Tunisian women with PCOS [28]. The confirmation of relationship with (2) independent loci of TCF4 in a PCOS first1: relationship between insulin level and the diabetes type 2 locus; and second 2: connection through reproductive PCOS phenotype. The study recommends that polymorphism in altered loci of a vulnerability gene donate to divergent phenotypes, also showed no proof for relationship among the rs7903146/rs12255372 polymorphism and PCOS. Though, there are observing first round proof for relationship with PCOS on a loci about (100 kb) downstream in the Caucasian type 2 diabetes locus[29]. Other findings recorded that relationship among the proinsulin:insulin ratio and glucose tolerance in patients with PCOS and the TCF4 Caucasian type 2 diabetes locus that confirmation for other studies which reveal in variation of TCF4 gene with defects in insulin secretion [30], possibly by changing alteration of proinsulin to insulin in pancreatic beta-cells [31]. Lyssenko et al. recorded that the type 2 diabetes related genotype of rs7903146 besides rs12255372 gene are linked with deficiency in arginine-stimulated insulin production in patients with irregular glucose level but not healthy[32].

Conclusion

The genetic variation of gene TCF4 was linked with PCOS while contrary in control (healthy women). The genotype and allele frequency of TCF4 (rs 290487) gene, allele T was recorded a highly significance in patients when compare with control. The frequency of allele C allele lower significant in patients than in control. There are relationship between the allele frequency of TCF4 (rs 290487) gene and genetic variation with POCS.

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