Association of Genetic Polymorphism of Insulin Receptor Substrate 1 (IRS-1) with Polycystic Ovary Syndrome Pathogenicity in Iraqi Women

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Abstract

Background: Insulin receptor substrate 1 (IRS-1) is an intracellular signaling adapter protein that integrates and coordinates multiple biologically key extracellular signals within the cell, is also a key central receptor in insulin signaling, and plays a focal role in maintaining essential cellular capabilities, e.g., survival, development and digestion system. IRS1 is essentially found in the cytoplasm But localization in nucleus may occur in some cell types and under certain stimuli.

Materials and Method: A total of 104 healthy control and 215 Iraqi women have Polycystic ovary syndrome (PCOS) aged 20–40 years who was admitted to conducted a prospective clinical study at kerbala gynecology teaching hospital, it was measured the genotype distribution of the rs2943641 T to C substitution of IRS1 and the effects of genotypes on Polycystic Ovary syndrome Pathogenicity in Iraqiwomen,

Results: Analyses were conducted to assess the association between the SNP rs2943641 [TT (Wild type), TC (heterozygous type), and CC (mutated type)], with the pathogenesis of PCOS according to logistic regression results. This survey demonstrated that there was no significant association between different alleles for this SNP with the pathogenesis of PCOS

Conclusion: Genetic polymorphism with IRS-1may be associated with metabolic disturbance but not Polycystic Ovary syndrome in Iraqiwomen.

Keywords: *Polycystic Ovary Syndrome, Pathogenicity, Genetic Polymorphism, Insulin Receptor Substrate 1.*

Introduction

Polycystic ovary syndrome (PCOS) is highly prevalent hormonal disorder among reproductive-aged women. Its clinical manifestations are heterogeneous(1). at a recent time, they are different studies have proven that genetic factors are associated with the occurrence of PCOS, although many candidate genetic mutations and single nucleotide polymorphisms (SNPs) have been studied for poly cystic ovary(2), the molecular technique subordinate with PCOS, and the mode of inheritance for PCOS was unclear, in addition to the scarcity of sources on the subject of insulin receptor substrate 1 (IRS1) with PCOS, It was also considered as novel candidate gene for PCOS(1). The IRS1 is the most important intermediate in insulin signaling and plays a crucial role in maintaining the essential function of the cell, so any polymorphism in IRS gene acts as a competitive inhibitor of the insulin receptors. polymorphism in IRS1 gene lead to

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susceptibility to insulin resistance and PCOS, Molecular scanning of the IRS1 gene has showed substitutions of several amino acid[3]. Insulin Receptor Substrate 1 (IRS1) polymorphism significantly decrease insulin-dependent receptor tyrosine autophosphorylation and increase Insulin-independent receptor serine phosphorylation markedly[3]. These serine phosphorylation inhibit normal receptor signaling and make the primary defects in insulin-stimulated glucose transporters (GLUT4) production[4], Decreased glucose uptake may result from suppressed insulin signaling or impaired glucose transporter (GLUT) 4 trafficking. In adipocytes of women with PCOS that decrease insulin responsiveness[5].

Materials and Method

Sample Collection: A total of 104 healthy control and 215 Iraqi women with PCOS, aged 20–40 years old have been recorded the PCOS subjects had at least two of the following signs : 1) chronic oligoanovulation 2) hirsutism or increased serum total testosterone levels; and 3) polycystic ovarian morphology at ultrasound, according to the Rotterdam consensus. Conference criteria (6). The protocol was approved by the local Ethics Committee, and all women gave written informed consent.

Polymerase chain reaction: The human genomic DNA extracted from whole blood by using genomic DNA extraction kit (G-DEX llb Introne, korea), according to the manufacture company, the purity and concentration of DNA obtaining was determinate by nanodrop apparatus (biobase, china), Polymorphism of IRS1 gene was detected by amplification refractory mutation system (ARMS) polymerase chain reaction (PCR),nucleotides primers were designed through Primer-BLAST allows users to design new target-specific primers according to the websites (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi) it is also prepared by (Bioneercom.Korea) company as the following (Table 1)

Optimization of PCR reaction was recorded as initial denaturation for 3 min at 94 °C, followed by 35 cycles consist of second denaturation for at 94 °C 30 seconds, 45 second at 56 °C, first extension 55 seconds at 72 °C, then last extension at 72 °C for five minutes. The amplification of insulin receptor substrate 1. was run electrophoresis in the 1.5% concentration of agarose gel at 70 V for 60 min after stained with 2 µL ethidium bromide, the product size was visualized under Ultraviolet.

Statistical Analysis: Statistical analysis were used by software SPSS program version 20, Test for Hardy-Weinberg equilibrium in controls and allelic or genotypic association in cases versus control were evaluated by Chi – square (x^2) test. This analysis was performed for all genotypes in this study using Hardy-Weinberg equilibrium online calculator.To assess the predictability of PCOS, logistic analysis of SNP was applied, this yielded odds ratio (OR) also the 95% confidence interval of the OR was calculated which is good estimator for the significance of the OR; when the value of "one" included within interval, this is an indicator that the OR is not significant. All statistical procedures and tests were applied under a level of significance (P- value) of less than 0.05 to be considered as significant difference or correlation.

Table 1. ARMS PCR primer nucleotides used in this study with product size

Primers		Sequence	Product size (bp)
Primers sequences of IRS1 rs2943641 Alleles T > C	O-F	TGGTTCTGTAACTGGGTG	537
	O-R	AGTTGAAGTAGCCATCTTTC	537
	Allele T	ATCAGGGCTAATAGTTAGAAGA	387
	Allele C	GTTGGAAATGAGAGGAACC	190
O-F: Outer forward; O-R: Outer Reverse			

Results and Discussion

PCOS is a polygenic endocrine and metabolic disorder. The prevalence of PCOS has grown rapidly. Several genetic polymorphisms have already been enrolled in the pathogenesis of PCOS[7]. The IRS-1 gene has been considered to be a candidate gene for the etiology of metabolic diseases such as type 2 DM,

PCOS, and obesity. The presence of polymorphisms of the IRS-1 gene has been documented to be associated with the development of IR. The IRS-1 gene located on chromosome 2q36 is the substrate for the insulin tyrosine kinase receptor, responsible for insulin signaling. The protein is expressed in multiple cells and tissues sensitive to insulin. Binding of insulin to its receptor activates phosphorylation of cytosolic substrates of IRS-1[8]. IRS-1 activation is a first step in the insulin signaling pathway, and functional studies of polymorphism in the IRS-1 gene showed weak insulin signals through the PI3-kinase pathway[9].

Analyses were conducted to assess the association between the SNP rs2943641 with the pathogenesis of PCOS according to logistic regression and (Figure 1) . This survey demonstrated that there was no significant association between different alleles for this SNP with the pathogenesis of PCOS, (Tables 2 and 3).

The exact cause of PCOS is unknown, but several studies suggest a strong genetic component that is affected by gestational environment and lifestyle factors, or both[10]. Thus, numerous genetic variations have been related to the presence of PCOS in different populations[11]. In the present study, we investigated the possible association between the single nucleotide polymorphisms (SNPs) (rs2943641) of the IRS1 gene and susceptibility to PCOS in Iraqi women, (TT wild type, TC heterozygotes, and CC mutated form for the two SNPs). The frequencies of SNP rs2943641 variant observed in our study were not significantly different between PCOS and healthy control women (11.5% vs. 12.1%, P= 0.368) as shown in table (2). Our data reviled that the IRS-1 polymorphism (rs2943641) is not associated with increased susceptibility to PCOS in Iraqi populations. However, we cannot exclude the possibility that other genetic polymorphisms of the IRS1 family are associated with PCOS and might be clinically useful as markers to assess the disease risk, as polymorphism of Gly972Arg that could play a contributory role in the pathophysiology and risk of PCOS [12]. But C allele of rs2943641 is associated with increased hyperinsulinemia and impaired insulin sensitivity[13].

Table 2. Distribution of SNP rs2943641 in the healthy control group and polycystic ovary syndromegroup.

Variables	Control	PCOS	p-value
Number	104	215	-
SNP1 (rs2943641)			
TT (Wild type)	24 (23.1%)	65(30.2%)	0.368
TC (Heterozygotes)	68 (65.4%)	124 (57.7%)	
CC (Mutant)	12 (11.5%)	26 (12.1%)	

Table 3. Logistic analysis of SNP rs2943641 to predict polycystic ovary syndrome pathogenesis.

SNP1 (rs2943641)	OR (95%CI)	p-value
TT (Wild type)	1.0 (reference value)	-
TC	0.67 (0.39 – 1.17)	0.161
CC	0.80 (0.35 – 1.83)	0.598

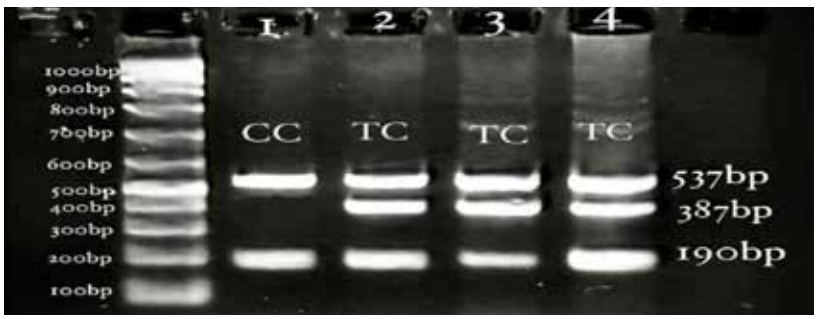

Figure 1. ARMS-PCR amplification of IRS1 gene T> C showing the outer primer 537 bp in size, T allele is 387 bp in size while C allele is 190 bp in size.

Conclusion

Polycystic ovary syndrome is associated with hyperinsulinemia and insulin resistance that affected by insulin receptor substrate 1 (IRS1) protein, this protein is an important intermediate in insulin signaling and plays a key role in maintaining the basic function of the cell, so any polymorphism in IRS1 genes acts as a competitive inhibitor of the insulin receptor. IRS1 rs2943641 polymorphism may associated with susceptibility to insulin resistance but not poly cystic ovary syndrome in Iraqi women.

Ethical Clearance: Informed consent was obtained from all participants, Data were collected in accordance with declaration of Helsinki of the World Medical Association, 2013, all other ethical issues were approved by the authors from the University of Kerbala

Conflict of Interest: Authors Declared none.

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