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Association of Insulin Receptor Substrate-1 Gene Polymorphism with Insulin Resistance in Type 2 Diabetes Mellitus in Iraqi Population

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Abstract
A glycine to arginine substitution (GGG↔AGG substitutions) in codon 972 (Gly 972 Arg) is the common polymorphism of the IRS-1 gene. This polymorphism interfere with the interaction between IRS-1 and PI3-kinase. It participate in the development of insulin resistance and diabetes by impairing the ability of insulin to activate the IRS-1/PI3-kinase/Akt signaling pathway. The present study was designed to evaluate the association of insulin receptor substrate-1 gene G↔A (Gly 972 Arg) polymorphism with insulin resistance in type 2 diabetes mellitus in Iraqi population. To achieve this aim, 103 of type 2 diabetic patients and 57 apparently healthy control group were subjected to the study. The results of present study show that the heterozygous genotype (GA) of insulin receptor substrate-1 gene G↔A (Gly 972 Arg) SNP was significantly increased (OR=9.14, CI 95% 1.13-75.53, $P < 0.05$) the risk of type 2 DM by nine folds with respect to those of wild genotype (GG). The allele frequencies of G and A were 92.93% and 7.07% for the insulin resistant type 2 diabetic patients group and 99.04% and 0.96% for the control group respectively. Also, the results revealed that no significant differences in clinical characteristics between wild genotype (GG) and heterozygous genotype (GA). The study concluded that insulin receptor substrate-1 gene G↔A (Gly 972 Arg) SNP are associated and involved in the pathogenesis of insulin resistant type 2 diabetes mellitus.
Keywords: Diabetes Mellitus, Insulin resistance, IRS-1, Gly 972 Arg

1. Introduction

Type 2 diabetes mellitus is a non autoimmune, heterogeneous and polygenic metabolic diseases characterized by hyperglycemia, resulting from impairment of insulin secretion and/or action (1). Insulin resistance, the reduction of insulin sensitivity by insulin responsive tissues lead to decrease the ability of insulin to inhibit the production of glucose by the liver and decrease peripheral glucose utilization. Consequently blood glucose level would be rise in insulin resistance and increase the secretion of insulin to overcome insulin resistance (2).

Insulin promotes different metabolic effects by binding to the insulin receptor and stimulating its intrinsic tyrosine kinase. Tyrosine kinase phosphorylat tyrosine residues of a different proteins such as insulin receptor substrate (IRS) (3). Several genetic polymorphisms of IRS-1 gene and their effects on insulin action have been identified. A glycine to arginine substitution (GGG↔AGG substitutions) in codon 972 (Gly 972 Arg) is the common polymorphism of the IRS-1 gene (4). In human, the IRS-1 gene is localized on chromosome 2. The IRS are essential for insulin action and therefore it important for the regulation of the hepatic glucose production and lipid metabolism. Any defect in this complex system leads to impairment in the insulin signaling pathway resulting in insulin resistance and type 2 diabetes (5, 6).

1.1. Aims of the study

The aim of this study to evaluate the prevalence and association of insulin receptor substrate-1 gene G↔A (Gly 972 Arg) SNP in insulin resistant type 2 diabetic patient in Iraqi population.

2. Materials and Methods

2.1. Materials

2.1.1. Subjects

The study included type 2 diabetic patients and control group. All samples were collected from February 2013 till May 2013. The work was carried out in the biochemistry department laboratory in College of Medicine/University of Kufa. The study was performed on 103 of type 2 diabetic patients and 57 apparently healthy control group. Any subject suffered from problems such as, renal dysfunction, heart diseases, hypertension, patient on insulin therapy and drug dependency such as glucocorticoid were excluded from the current study.

2.1.2. Blood Sampling

Five milliliters of blood was taken from all subjects by vein puncture in fasting status and the blood was divided into two parts, the first part include three milliliters of blood placed in plain tube for estimation of insulin, glucose, total cholesterol, HDL-cholesterol, TGs, VLDL-cholesterol and LDL-cholesterol concentrations. The

second part for gene analysis includes two milliliters of blood will be collected in EDTA containing tube.

2.2. Methods

Serum glucose, total cholesterol, triglycerides (TGs) and HDL-cholesterol concentration determined by spectrophotometric methods, while serum insulin concentration determined by enzyme linked immunosorbant assay (ELISA) method. Insulin resistance was evaluated by homeostatic model assessment (HOMA) method.

Genotyping of IRS-1 gene G↔A (Gly 972 Arg) was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The DNA extracted from frozen blood by genomic DNA mini kit (Geneaid) (7). The DNA was amplified by PCR. A 221 bp DNA fragment containing the polymorphic site G↔A (Gly 972 Arg) of IRS-1 gene was amplified by using specific primers (forward primer 5'-GCA GCC TGG CAG GAG AG-3' and reverse primer 5'-CTC ACC TCC TCT GCA GC-3') (8). The PCR products were digested with *Bst*OI (Bioneer /Korea). The wild genotype (GG) remains uncut (221 bp) whereas the homozygous genotype (AA) is digested into 190 and 31 bp fragments. The heterozygous genotype (GA) contained three bands sized 221, 190 and 31 bp. The restriction digestion products were analyzed on 3% agarose gel electrophoresis.

2.3. Statistical Analysis

The results of phenotypes data were expressed as mean ± SD. Student's t-test was used for the evaluation of data. Genotype data expressed as odds ratio (OR), confidence interval (CI) 95%. Statistical analyses were performed with SPSS (version 20). P-value less than 0.05 was considered to be statistically significant.

3. Results

Fasting glucose, insulin, HOMA and total cholesterol, TG, VLDL-cholesterol and LDL-cholesterol levels were found to be elevated significantly ($p < 0.001$) in type 2 diabetic patients when compared to those of the control group. However HDL-cholesterol was observed to be lowered significantly ($p < 0.05$) during comparable evaluation as shown in table 1.

Results indicated that 92 (89%) out of 103 type 2 diabetic patients were insulin resistant when they were evaluated by the HOMA method. However 5 (9%) out of 57 healthy individuals were observed to be insulin resistant when they were analyzed similarly, hence these patients were excluded from the current investigation.

The analysis of results indicated that the IRS-1 gene G↔A (Gly 972 Arg) SNP genotype frequencies of wild genotype (GG) and heterozygous genotype (GA) were 85.87% and 14.13% in the insulin resistant type 2 diabetic patients and 98.08% and 1.92% in the control group respectively. The homozygous genotype (AA) were absent in insulin resistant type 2 diabetic patients and the control group. The heterozygous genotype (GA) was found to significantly increase (OR=9.14, CI 95% 1.13-75.53, $P < 0.05$) the risk of type 2 DM by nine folds with respect to those of the wild genotype (GG) after adjustment for age, sex and BMI. No significant variations were obtained when the analysis was carried out without adjustment as shown in table 2. The allele frequencies of G and A were 92.93% and 7.07% for the insulin resistant type 2 diabetic patients group and 99.04% and 0.96% for the control group respectively as shown in table 3. Finally, the results of present study reveal that no significant differences in clinical characteristics (fasting glucose, insulin, HOMA, total-cholesterol, HDL-cholesterol, TG, VLDL-cholesterol, LDL-cholesterol and BMI) between wild genotype (GG) and heterozygous genotype (GA).

4. Discussion

Whereas many different genes have been projected as diabetogenes, this study focused on the IRS-1 gene. IRS-1 is the major substrate that contribute in insulin action in insulin sensitive tissues. Defects in IRS-1 which is main substrate tyrosine phosphorylation characterizes insulin resistance associated with diabetes (9). The IRS-1 is an endogenous substrate of the insulin receptor that play an essential role in the insulin signaling pathway and it expressed in insulin sensitive tissues. Subsequent to the binding of insulin to its receptor, the intrinsic tyrosine kinase activity of the insulin receptor β subunit is activated, thus catalyzing the phosphorylation of specific tyrosine residues on the IRS-1 protein (10).

This phosphotyrosine residues on IRS proteins become good targets for the p85 subunit of PI3-kinase. The activated PI3-kinase activate Akt, it has multiple biological function of insulin including glucose transport, translocation of glucose transporter protein to the plasma membrane and glycogen synthesis (11, 12). The Gly 972 Arg polymorphism is found close to the C-terminus of IRS-1 and it positioned between two potential tyrosine phosphorylation sites, this sites represent the binding sites for the p85 subunit of PI3-kinase (13).

The Gly 972 Arg polymorphism did not change the expression level of IRS-1 or the degree of insulin stimulated tyrosine phosphorylation of IRS-1, but the Arg 972 polymorphism interfere with the interaction between IRS-1 and PI3-kinase, may be by altering the tertiary structure of IRS-1 (14, 15). For this explanation,

the Arg 972 IRS-1 polymorphism may participate in the development of insulin resistance and diabetes by impairing the ability of insulin to activate the IRS-1/PI3-kinase/Akt signaling pathway, consequently leading to defects in glucose transport, glucose transporters translocation and glycogen synthesis.

The current results are in consistence with the results of Laura *et al.* (16) and Fulden *et al.* (17) studies in which the IRS-1 gene G↔A (Gly 972 Arg) SNP was found to be increased risk of type 2 DM. On the other hand it differed from those described by Kozarova *et al.* (18) and Baroudi *et al.* (19) who did not find an association between IRS-1 gene G↔A (Gly 972 Arg) SNP and type 2 DM.

5. Conclusions

Insulin receptor substrate-1 gene G↔A (Gly 972 Arg) SNP is associated and involved in the pathogenesis of insulin resistant type 2 diabetes mellitus in Iraqi population with a nine folds increase of the risk of the disease.

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Table 1: Mean Fasting Serum Glucose, Insulin, HOMA, and Lipid Profile Concentration in Type 2 Diabetic Patients and the Control Group

Parameter	Subjects	Mean ± SD	Range	P-value
Glucose (mmol/L)	Control	4.83 ± 0.68	3.2-5.9	< 0.001
	patient	9.94 ± 2.95	7.1-20.2	
Insulin (µIU/mL)	Control	8.24 ± 2.98	1.56-15.27	
	patient	15.82 ± 7.79	5.47-44.36	< 0.001
HOMA	Control	1.80 ± 0.68	0.33 - 3.37	
	patient	7.12 ± 4.29	2.16 - 28.45	< 0.001
Total Cholesterol (mmol/L)	Control	4.20 ± 0.72	2.66-5.39	
	Patient	5.04 ± 0.92	3.81-6.91	< 0.001
HDL Cholesterol (mmol/L)	Control	1.04 ± 0.26	0.58-1.79	
	Patient	0.96 ± 0.22	0.52-1.54	< 0.05
Triglycerides (mmol/L)	Control	1.25 ± 0.52	0.49-2.27	
	Patient	2.05 ± 0.94	0.56-3.97	< 0.001
VLDL Cholesterol (mmol/L)	Control	0.56±0.23	0.22-1.02	
	Patient	0.92 ± 0.42	0.25-1.78	< 0.001
LDL Cholesterol (mmol/L)	Control	2.59 ± 0.77	1.16-4.19	
	Patient	3.15 ± 1.01	1.15-5.39	< 0.001

Table 2: Genotypes Distribution of Insulin Receptor Substrate-1 Gene G↔A (Gly 972 Arg) SNP in Insulin Resistance Type 2 Diabetic Patients and the Control Group

Genotype		Type 2 DM	Control	Unadjusted OR (95% CI) P-value	Adjusted OR (95% CI) P-value
GG	No.	79	51	Reference	Reference
	%	85.87%	98.08%		
GA	No.	13	1	8.39 (1.06-66.12) P < 0.05	9.14 (1.13-75.53) P < 0.05
	%	14.13%	1.92%		
AA	No.	0	0	-	-
	%	-	-		
Total	No.	92	52	-	-
	%	100%	100%		

Table 3: Allele Frequency of Insulin Receptor Substrate-1 Gene G↔A (Gly 972 Arg) SNP in Insulin Resistance Type 2 Diabetic Patients and the Control Group

Allele		Type 2 DM	Control	OR (95% CI)	P-value
G Allele	No.	171	103	Reference	Reference
	%	92.93%	99.04%		
A Allele	No.	13	1	7.83 (1.00-60.74)	< 0.05
	%	7.07%	0.96%		
Total Allele	No.	184	104	-	-
	%	100%	100%		

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