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Research Article

THE ASSOCIATION OF RESISTIN GENE –420C/G (RS1862513) SINGLE NUCLEOTIDE POLYMORPHISM WITH TYPE TWO DIABETES MELLITUS IN IRAQI POPULATION

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ABSTRACT

Background: Worldwide studies have shown polymorphisms within the resistin gene like–420C/G (rs1862513) to verify the effect of these polymorphisms in the occurrence of T2DM. Aim: To study the association of –420C/G with T2DM in Iraqi population. Methods: The study consisted of 400 T2DM patients and 400 healthy control individuals. Genotyping of –420C/G polymorphism is achieved by PCR–RFLP. DNA was extracted from whole blood and genotyping was carried out by specific primers to amplify fragments for digestion with restriction enzyme. Bbs I restriction enzyme used for digestion followed by electrophoresis on agarose gel. Various statistical analyses were used to analyze the data. Results: The genotype and allele frequencies of rs1862513 SNP revealed insignificant differences among the homo (GG) (OR= 1.42 ,CI 95%;0.95-2.11;P=0.08), hetero (CG) (OR= 1.07 CI 95%; 0.78-1.45, P= 0.7), dominant (OR = 1.42, CI 95%; 0.96-2.11, P= 0.08) and recessive (OR = 1.37, CI 95%; 0.96-1.98, P= 0.08) models. Furthermore, the minor allele frequency (G) was insignificantly increased (P= 0.21) in T2DM when compared with the control group. Conclusion: Resistin gene (rs1862513) polymorphism was exhibited non-significant association with T2DM in Iraqi population.

Keywords: Diabetes mellitus, polymorphism, gene, resistin, RFLP

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion by pancreas and increased glucose production by liver [1]. Several characteristic symptoms may be present with T2DM such as polyuria, polydipsia and polyphagia [2]. Insulin resistance is the most pathological condition at which the cells in the target organs are unable to respond to normal serum insulin level [3]. T2DM making up about ninety percent of the diabetic subjects [4]. The etiology of type 2 diabetes mellitus clarify involvement of complex interactions between environmental and genetic factors [5]. There are a number of genes identified as having an effect on developing the disease including resistin gene (RETN) [6]. the common variants single-nucleotide polymorphisms +299 G/T (rs3745367) and -420 C/G (rs1862513) studied in different populations to verify the susceptibility of individuals to T2DM [7,8,9,10,11,12,13]. Resistin is a circulating adipocytokine produced by white adipose which cause both insulin resistance[14]and metabolic syndrome [15]. This study aimed to estimate the association of -420 C/G SNPs with T2DM in Iraqi population.

MATERIALS AND METHODS

This Study was included 800 subjects. The patient population included 400 subjects with type 2 diabetes mellitus, patients age ranged between 40-60 years, they randomly selected from who attended the Diabetic Center at Al-Sadder Medical City, AL-Najaf, Iraq during their routinely visiting periods for clinical examination and regular checking glucose level. Ethical Committee of Al-Kufa Medicinal College accepted the work protocols.

Inclusion criteria includes

1. Fasting blood glucose (FBG) level ≥ 126mg/dl.
2. The symptoms of diabetes (polyuria, nocturia , polyphagia, weight loss).
3. The selection of type 2 diabetes depend on physicians diagnosis in this study.

Exclusion criteria includes

1. subjects younger than 40 years old.
2. They have type 1 Diabetes or type 2 diabetes but need insulin injection.
3. They have heart failure, cardiomyopathy or congenital heart disease.
4. They have an autoimmune disease (Rheumatic arthritis RA, cancers, infections, sever renal or liver disease, pregnancy or currently using glucocorticoid therapy.
5. Smoking or alcoholism

A 400 of apparently healthy individuals (without Diabetes mellitus) were selected and included in the study with an age range 40-60 Years.

The practical part of the study was carried out in laboratory of Clinical Laboratory Sciences department / faculty of Pharmacy / Al-Kufa University. Peripheral blood samples of T2DM and control groups were collected in EDTA- tubes, and then DNA was extracted from whole-blood samples using the genomic DNA extraction kit (Favorgene). Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (BioDrop,U.K)

Genotyping analysis was accomplished by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) for resistin gene using thermocycler (Biometra, Germany). The primers provided by (OneAlpha, U.S.A) and the sequence of primers used was : reverse 5′-TGG GCT CAG CTA ACC AAA TC-3′ and forward 5'-TGT CAT TCT CAC CCA

GAG ACA-3′. Amplification was contained 12.5 µl GoTaq Green Master Mix, (Promega Corporation, Madison, WI), 1.5 µl of each primer (1 Mm final concentration) , 3.5 µl nuclease free water, and 6 µl of DNA template ,the performed total volume is 25 µl. Cycling conditions : initial denaturation 95°C for 7 min followed by 35 cycles of 95°C for 30s, 64°C for 30s, 72°C for 1 min, and a finally the extension step of 72°C for 10 min. The PCR product was digested with 1 µl of restriction enzyme (Bbs I) (Biolab) and run on 3.5% agarose gel.

Statistical analysis

Genotype and allele frequencies (G) % were compared using the $\chi 2$ statistics or the fisher's exact test. A p-value ≤ 0.05 was considered statistically significant. The Hardy Weinberg equilibrium (HWE) was tested by using the chi-square. Odds ratios were calculated by logistic regression.

RESULTS

The PCR product –420C/G (rs1862513) i.e, the amplicon is of 533 bp was digested by restriction enzyme Bbs I. The product of digestion were analyzed by 3.5% agarose gel electrophoresis. Results show (533bp), two (323 bp,210 bp) and three (533 bp,323 bp,210 bp) bands for those with homozygous wild type (CC), homozygous (GG) and heterozygous (CG) genotypes respectively.

The genotype and allele frequencies of rs1862513 SNP give insignificant differences among the homozygous (GG) (OR= 1.42 ,CI 95% ;0.95-2.11;P=0.08), heterozygous (CG) (OR= 1.07 CI 95%; 0.78-1.45, P= 0.7), dominant (OR = 1.42, CI 95%; 0.96-2.11, P= 0.08) and recessive (OR = 1.37, CI 95%; 0.96-1.98, P= 0.08) models . Furthermore, the minor allele frequency (G) was insignificantly increased (P= 0.21) in T2DM when compared with the control group

Table 1: Result of genotyping and frequency of alleles of RETN (rs1862513) in studied groups

C/G)	Control	Type 2 DM	OR (CI95%) P- value.
Co dominant			
CC(Ref.)	164	150	.
CG	173	168	1.07 (0.78-1.45) 0.7
GG	63	82	1.42 (0.95-2.11) 0.08
Dominant			
GG+CG	236	250	1.42 (0.96-2.11) 0.08
Recessive			
CC+CG (Ref.)	337	318	--
GG	63	82	1.37 (0.96-1.98) 0.08
Additive			
2(GG)+ CG	299	332	1.22 (0.92-1.59) 0.16
Frequency of G allele %	%37	41%	1.21 (0.98-1.66) 0.21

Table 2: The relation between Resistin (rs18621513 C>G) gene polymorphism genotypes and the investigated parameters in patients group under the dominant model.

Clinical Characteristic	Genotype M ± SD		P-value
	CC (n=150)	CG+GG(n=250)	
FBS (mg/dl)	224.74±48.23	227.57±47.78	0.56
Insulin (µIU/ml)	32.56± 2.47	32.74± 2.71	> 0.05
HOMA	18.07± 1.56	18.38± 1.72	0.07
Resistin (ng/ml)	18.9±13.3	19.5± 12.9	0.65
Cholesterol (mg/dl)	227.98±26.72	233.83±24.28	<0.05
HDL (mg/dl)	50.36± 9.40	48.67± 10.26	0.10
Triglycerides (mg/dl)	196.71±49.13	204.07±54.29	0.17
VLDL (mg/dl)	39.34±9.82	40.81± 10.85	0.17
LDL (mg/dl)	138.28±33.75	144.35±37.68	0.10
BMI (kg/m2)	30.30 ± 2.93	30.31± 2.97	0.97

DISCUSSION

The determination of genetic variants that have risk to T2DM is a center of attention of research to perceive the etiology and pathogenesis of this disorder as well as related pathological consequences. Such attempt may improve the plans of protection, diagnosis and treatment of Iraqi society. Advances in genetic technology such as the development of genome-wide association studies (GWAS) have enabled the identification of a number of genes associated with the occurrence of T2DM. In the current study, the −420C/G SNP of the resistin gene is assessed in diabetic patients in attempt to explore the etiology of the disease in Iraqi population hoping to improve the management plans. Results revealed insignificant variation of the genotype distribution of the −420C/G (rs1862513) SNP of resistin gene in patients and the control group. Moreover, the minor allele frequency (G) % was found to be non-significantly altered in the two investigated groups. These results suggested the absence of the association of this SNP with the occurrence of the disease in our population. These findings are consistent with Suriyaprom K, et al and Conneely ,et al [7,16] . The analysis of the changes in serum lipid concentration in relevance to the genotype distribution under the co-dominant model exhibited slight elevation in cholesterol levels parallel with the presence the G alleles of -420C/G SNP in the resistin gene. Changes appeared to be more profound with significant difference when the analysis was achieved under the dominant model. However, , VLDL, and LDL were not elevated in carriers of the G allele when they were compared with those of the reference allele. These results suggested that resistin gene polymorphism -420 C/G is implicated in elevating serum cholesterol concentration and the G allele could be considered as a risk factor for the development of atherosclerosis and ischemic heart disease. It is not easy to explain why the G allele is enhancing the rise of serum cholesterol concentrations .

In the literature, there were very little data regarding the changes of serum lipid concentrations or other metabolites in relevance to resistin polymorphisms. It has been found that there was no association between metabolic syndromes, obesity and serum triglyceride and the −420 G allele [17] .The association of the −420G allele with high glucose concentration were reported in Finish population [18] China [19] and Europe [20]. However, these results were not reported in Scandinavian population [21]. Differences in the current results from those reported previously may belong to the ethnic diversity or may related to the mechanism by which resistin can modulate these metabolites
In the Conclusion the –420C/G (rs1862513) polymorphism of the resistin gene is not associated with T2DM in Iraqi population.

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