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ASSOCIATION OF +45(T/G) POLYMORPHISM IN THE ADIPONECTIN GENE WITH TYPE 2 DIABETES MELLITUS IN IRAQI PATIENTS

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ABSTRACT
Adiponectin is a 244 – amino acid-long polypeptide, that is exclusively secreted by adipocytes and acts as a hormone with anti-inflammatory and insulin sensitizing properties, the adiponectin gene contains 3 exons spans 16 kb on chromosome 3q27.45T/G polymorphism in exon 2 of the adiponectin gene which has been reported its association with insulin resistance, serum adiponectin level, and type 2 diabetes mellitus. The aim of the present study is to study the association of T45G polymorphisms of adiponectin gene with the risk of type two diabetes mellitus. Sixty six diabetic patients and Twenty five controls were enrolled in this study. Significance difference was found between genetic variation of adiponectin gene T45G SNP and the increased risk of T2DM in Iraqi population. The distribution of T45G polymorphisms of adiponectin gene in T2DM patients was higher than in controls with mean and standard error of (0.515 ± 0.06 for T2DM patients and 0.160 ± 0.07 for control).

KEY WORD: Adiponectin, Diabetes mellitus, 45T/G polymorphism.

INTRODUCTION

Diabetes mellitus is a potentially morbid condition with high prevalence worldwide. It was affected millions of people all over the world, thus the disease constitutes a major health concern (Macedo *et al*., 2002). Type 2 diabetes mellitus (T2D) is a heterogeneous disease that results from the interplay between environmental and genetic risk factors. The hallmark of diabetes is chronically elevated blood glucose concentrations (Bowden, 2006).

Adiponectin is a 244 – amino acid-long polypeptide, collagen like protein that is exclusively secreted by adipocytes and acts as a hormone with anti-inflammatory and insulin sensitizing properties by several mechanisms through which adiponectin may decrease the risk of type-2 diabetes mellitus, including suppression of hepatic gluconeogenesis, stimulation of fatty acid oxidation in the liver, stimulation of fatty acid oxidation and glucose uptake in skeletal muscle, and stimulation of insulin secretion (Kadowaki *et al*., 2006). The adiponectin gene contains 3 exons spans 16 kb on chromosome 3q27 (Hu *et al*., 1996).Various single nucleotide polymorphisms (SNPs) have been identified in the adiponectin gene. One of these SNPs, 45T/G, in exon 2 of the adiponectin gene, has been frequently reported in association with insulin resistance, serum adiponectin level, and type 2 diabetes mellitus(Hara *et al*., 2002).

MATERIALS & METHODS

This study was conducted during the period from November 2012 to May 2013 in the University of Baghdad/Institute of Genetic Engineering & Biotechnology for post Graduate Studies. This study includes sixty six diabetic patients. Patients included 24males and 42 females, with age range 40-74 years and Twenty five controls. The patients were selected from Baquba general hospital. T2DM was defined as excess of a hemoglobin A1C ≥6.5%. Patients with any medical condition or patients suffering from chronic kidney disease were excluded from the study. Control subjects were apparently healthy, non-T2DM individuals free of any medical complications. Control subjects with history of T2DM, HbA1C>5.8% excluded from this study.

Anthropometric measurements

Anthropometric measurements were obtained by same person. Height and body weight were measured without shoes. Body mass index (BMI) was calculated as weight/height 2 kg/m2).

Genotyping

T45G SNP T>G substitution at +45 in exon 2 (T45G) of adiponectin gene was chosen. Genotyping was carried out by PCR amplification of peripheral blood genomic DNA extracted using Blood genomic mini spin kit (Geneaid) followed by restriction enzyme digestion as was used in previous study (Ranjzad *et al*., 2011). For T45G polymorphism analysis, DNA was amplified using the forward primer, 5′- GAAGTAGACTCTGCTGAGATGG-3′ and the reverse primer, 5′-TATCAGTGTAGGAGGTCTGTGATG-3′. PCR was performed in a 25µl total volume, Primer forward 0.6µl (10PM), Primer reverse 0.6µl (10 PM), Template DNA3 µl, (3- 6µg/ml) and 12.5 master mix. A total of 35 PCR cycles with denaturation at 94oc for 25 sec., annealing for 40 sec at 61 ^{o}C and extension at 72 ^{o}C for 30 sec. were conducted. An initial DNA denaturation at 95^{o}C was carried out for 3 minutes and final extension at 72 ^{o}C were carried out for 3 minutes each. 10 µl of amplified products was mixed with 0.4 *Sma1* enzyme, 2ul of enzyme buffer and 7.6 free nucleases deionized distilled water then incubate for 3 hours in 25 ^{o}C (Li *et al*., 2012). All enzyme digestion mixture was loaded to the well in 2% agarose gel

stained with 0.5 µg /ml ethidium bromide, at 100 V for 15 min then 50 volt for 50 min in 1 X TBE buffer. Then visualized under UV light using ultraviolet transillumenater A (100-1000) DNA ladder was used and the gel was photographed by a digital camera. The absence of polymorphism TT homozygote yielded the 372 bp uncut fragment only, the presence of polymorphism TG heterozygote yielded the 372, 216 and 156 bp fragments (Ranjzad *et al*., 2011)(Figure 1).

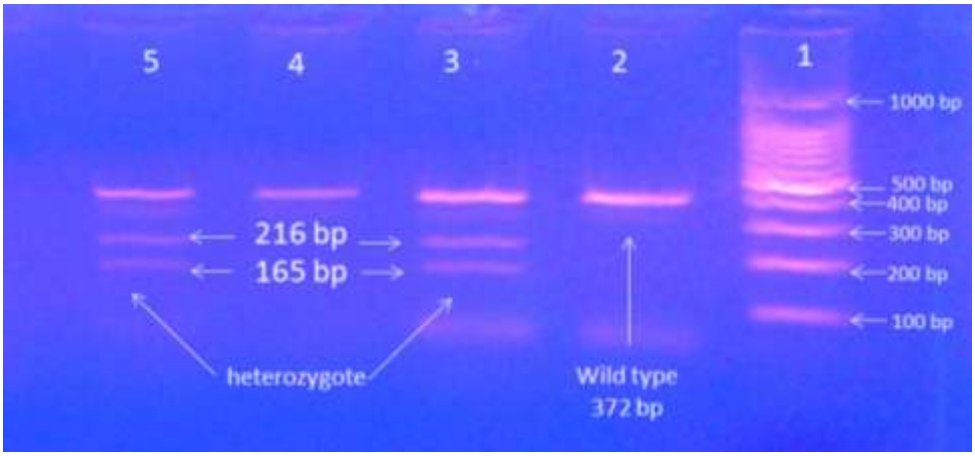

FIGURE 1: Restriction enzyme digestion (*Sma1*) of PCR product from wiled type and heterozygote individual. Lane1: DNA ladder (100-1000). Lane2, 4: Wild type 372 bp TT genotype Lane 3, 5: heterozygote TG heterozygote 372, 216 and 156 bp. The (RFLP) products were electrophoresis on 2% agarose gel at 100 voltages for 15 min. then at 50 volt for 40 min.

Statistical Analysis

The Statistical Analysis System- SAS (2010) was used to analyze the data and t test was applied to compare the significant differences between means of the studied parameters,

RESULTS & DISCUSSION

In the present study the association of T45G polymorphisms of adiponectin gene with the risk of type two diabetes mellitus was examined. The results of this study support the association between genetic variation of adiponectin gene T45G SNP and the increased risk of T2DM in Iraqi population. In this study there is a significant difference between + 45 T/G Polymorphism and diabetic. The distribution of T45G polymorphisms of adiponectin gene in T2DM patients higher than in controls with mean and standard error of (0.515 ± 0.06 for T2DM patients and 0.160 ± 0.07 for control) (Table 1). Furthermore individuals with the TG genotype in the patient group were significantly higher than those with the TT genotype with mean and standard error of (10.06 ± 0.64 for TG genotype and 9.13 ± 0.12 for TT genotype). The polymorphism of T45G SNP was significantly related with the risk of developing T2DM in this studied population. Same result has been obtained by other studies done by Hara *et al.* (2002) and Nakatani *et al*. (2005). On European population by Gable *et al*. (2007), on Spanish population by Gonza´lez-Sa´nchez *et al.* (2005), Also in Chinese people, the T45G was the only polymorphism significantly associated with the risk of persistent hyperglycemia at 5-year follow-up, and haplotypes formed by the addition of other SNPs did not confer greater association (Tso *et al*., 2006).The positive association of this study was in line with other Arab population studies in Tunis (Mtiraoui *et al*., 2012) and in Jordan (Al-khateeb *et al*., 2013).

TABLE 1. Compare between patients and healthy group in study parameters

Parameters	Mean ± SE		T-test value
	Patients (No. 66)	Control (healthy) (No. 25)	
BMI (kg/m²)	28.29 ± 0.58	27.01 ± 0.61	2.024 NS
HbAIC (%)	9.59 ± 0.23	4.58 ± 0.10	0.744 *
+ 45 T/G (Polymorphism)	0.515 ± 0.06	0.160 ± 0.07	0.225 *

* (P<0.05), NS: Non-significant.

Result of this study directly contradicts the study on Korean population as reported by Lee *et al*. (2005). They genotyped 427 non-diabetic controls and 493 type 2 diabetic patients for SNPs 45T/G of adiponectin gene and also measured plasma adiponectin concentrations in Koreans. No statistically significant differences were found in frequencies of SNPs 45 comparing control with type 2 diabetic subjects. They concluded that 45T/G of the adiponectin gene may not be important determinant of type 2 diabetes or insulin resistance in Korean subjects. In other study, the 45T/G SNP failed to show an association with type 2 diabetes and BMI as in a study on Italians by Nannipieri *et al*. (2006), French population based study by Vasseur *et al.* (2002), Italian by Cesari *et al*. (2007),

Iranian by Mohammad zadeh and Zarghami (2009). This difference in the results between different studies can be explain by that the genetics underlying of type 2 diabetes is multifactorial and complex in nature (McCarthy, 2004). Multiple genetic and environmental factors contribute to individual risk of developing type 2 diabetes. Some have only a marginal and modest effect when considered individually, and the combination of these factors is responsible for disease risk (Hattersley and McCarthy, 2005). Results of this study revealed that the differences between the genotypes and the BMI were not significant. These results have been obtained by other studies (Al-Daghri *et al*., 2012, Karani *et al*., 2008).

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