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Research paper

Adiponectin gene polymorphisms as a predictor for development of type 2 diabetes mellitus in Iraqi population

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) incidence is increasing globally and nationally. The etiology of the disease includes environmental and genetic factors. Polymorphism of adiponectin gene was found to be implicated in the pathogenesis of T2DM in numerous populations.

Methods: A case-control study was conducted to assess the association of rs2241766 and rs822395 SNPs of adiponectin gene (*ADIPOQ*) with T2DM in Iraqi population. The study included 400 patients with T2DM and 400 healthy individuals served as a control group. Serum lipid concentrations, insulin level and the index of insulin resistance (HOMA) were measured. The genotyping of *ADIPOQ* for rs2241766 and rs822395 SNPs was performed by PCR-RFLP.

Results: The genotype distribution of rs2241766 SNP indicated a significant increase of carriers of the homozygous GG (OR: 5.04, CI95%: 2.27–11.19, *P*: 0.0001) and heterozygous TG (OR: 1.7, CI95%: 1.22–2.39, *P*: 0.002) variants when compared with those of the wild type, suggesting a risk factor of 2 and 5 to develop the disease. The minor allele frequency (MAF) G was observed to be significantly (*P*: 0.0001) higher in patients (22%) when compared with the control group (11.74%). Results of rs822395 SNP failed to exhibit a significant difference. Changes of BMI, cholesterol, triglycerides, insulin and insulin resistance index values in the diabetic patients seemed to be parallel with the presence of MAF of rs2241766 SNP.

Conclusion: The rs2241766T > G SNP of adiponectin gene is a risk factor for the development of T2DM in Iraqi population and directs the changes of serum lipid concentrations as well as insulin resistance.

1. Background

Type 2 diabetes mellitus (T2DM) is a metabolic disease frequently results from insulin resistance (IR), it has been found that about 80% of diabetic patients may suffer from IR. Globally, the epidemic of T2DM continue to increase, in 2010, the prevalence was 6.5% (285 million patients) while, in 2030 it may be 7.7% (439 million patients) (Costa et al., 2017). Nationally, the prevalence of the disease in Iraq in 2012 was 10.9% (International Diabetic Federation IDF, 2012). Thus, a serious rise in the number of type 2 diabetic patients was evident in our country. The seeking of the etiology of the disease is an essential mission to improve the management plans in our community. It has been found that the risk of developing T2DM is influenced by the pattern of genetic changes as well as the environmental factors (Cornelis et al., 2009). Adiponectin is an adipocytokine secreted frequently from adipocytes. It is engaged in numerous metabolic functions. Principally, it is an insulin sensitizing agent involved in regulation of blood glucose level and metabolism of lipids. It also has anti-inflammatory as well as anti-atherogenic properties. Adiponectin is encoded by the adiponetin gene (*ADIPOQ*) locates on chromosome 3q27.3 (Devadrita et al., 2011). Low levels of adiponectin have been found to be tightly combined to T2DM, obesity and cardiovascular disease. Polymorphisms in *ADIPOQ* were demonstrated to be involved in changes of adiponectin levels. However, sometimes such polymorphisms, has no impact on the adiponectin concentration, but still associated with the metabolic diseases (Asma and Mohd, 2015).

There are 683 single nucleotide polymorphisms (SNPs) mapped within the adiponectin locus in human (www.ncbi.nlm.nih.gov), 33 of these SNPs were cited in the PubMed (Breitfeld et al., 2012). Several studies have shown associations of *ADIPOQ* polymorphisms with

Abbreviations: ADIPOQ, adiponectin gene; DM, diabetes mellitus; HOMA-IR, homeostasis model assessment of insulin resistance; IDF, International Diabetes Federation; MAF, minor allele frequency; SNPs, single nucleotide polymorphisms; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus

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diabetes mellitus, obesity and cardiovascular diseases (Han et al., 2011). Studies on Arabic Bahraini (Al Hannan et al., 2016) and Egyptians (Motawi et al., 2015) populations showed significant association of rs2241766 and the occurrence of T2DM. In Iraqi Arabic population, it has been observed an association of the rs266729 SNP of *ADIPOQ* with T2DM in Iraqi population (Kaftan and Hussain, 2015). In the present study, the rs822395 and rs2241766 SNPs of *ADIPOQ* were examined for the association with T2DM in Iraqi Arabic population in order to identify peoples at high risk to develop the disease. Such attempt is critical in the improvement of the plans of T2DM management.

2. Methods

2.1. Study subjects

A case control study was carried out on 800 individuals, divided into type 2 diabetic patients (400) and healthy control group (400). The research was done in the department of Biochemistry, University of Kufa, College of Medicine.

2.1.1. Patient group

It contained 400 patients with T2DM (230 male and 170 female), randomly selected from the diabetic center in Najaf Governorate in Iraq. The patient ages were 56.5 ± 7.5 year (Mean ± SD). Patients were diagnosed by specialist physicians.

Inclusion criteria:

1. Patients who were diagnosed by specialist physicians as having T2DM.
2. Fasting glucose level was > 126 mg/dl (7.0 mmol/l) with symptoms of T2DM.

Exclusion criteria:

1. Those diagnosed with T1DM.
2. Those under insulin treatment.
3. Those treated with antihyperlipiaemic medicines.

2.1.2. Control group

The control group contained 400 apparently healthy individuals (220 male and 180 female). Ages of individuals of the control group were 58.9 ± 8.6 year (Mean ± SD). They were selected randomly from relatives of patients and other volunteers. They were free from symptoms and signs of any chronic diseases such as DM, cardiac diseases, heart diseases, hypertension, renal diseases or others.

All cases completed detailed questionnaire included the essential information, i.e., age, sex, family history, medicine history, and any other relevant information. Weight, height and BMI were measured for all participants and the BMI values were calculated. Najaf is a holy city in the middle of Iraq, and thousands of people visit it each day, the diabetic center in Najaf is one of the largest centers in Iraq and recruits patients from all cities of Iraq therefore, our study population could be representatives of the Iraq Arabic population.

Informed consent was taken from all participants. Kufa Medical College Ethical Committee has approved the study protocol.

2.2. Biochemical measurements

Biochemical measurements including fasting: blood sugar (FBS), total cholesterol, triglycerides, HDLc, LDLc and VLDLc were achieved by spectrophotometric techniques with the use of enzymatic procedures. Insulin was measured by ELISA.

2.3. Genotypic measurements

Blood samples of T2DM and control groups were collected in EDTA-anticoagulant tubes. DNA was extracted from whole-blood samples with the use of genomic DNA extraction kit (Promega, USA). DNA concentration and purity were measured by UV BioDrop, UK. Genotyping of adiponectin Gene for rs822395 and rs2241766 SNPs were carried out with the use of PCR-restriction fragment length polymorphism (RFLP) using thermo cycler (Tprofessional, Biometra, Germany). Primer sequences were followed as described by Bangshun et al. (2011). For rs2241766 SNP, the primers were:

Forward: 5′GCA GCT CCT AGA AGTAGA CTC TGC TG3′
Reverse: 5′GCA GGT CTG TGATGAAAGAGGCC3′.
For rs822395 SNP, the primers were:
Forward: 5′TGATCGCACCTATTAGTGGAGGAAT3′
Reverse: 5′TCCAGAATATGTGAAAGCCCCAGAG3′ for.

Amplification was performed in a total volume reaction of 25 μl which contained 12.5 μl GoTaq Green Master Mix (Promega, U.S.A), 1.5 μl of each primer, 3.5 μl of nuclease free water, and 6 μl genomic DNA solution as template. The PCR reaction program of the protocol of rs2241766 SNP were; 95 °C for 4 min followed by35 cycles of 94 °C for 35 s, 55 °C for 40 s, 72 °C for 30 s and a final extension at 72 °C for 10 min. The conductions for rs822395 SNP were; 95 °C for 4 min followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min and a final extension at 72 °C for 10 min. The amplification product of rs2241766 SNP was 390 bp, it was digested with 10 U of *Sma*I restriction enzyme and analyzed on 2% agarose. For rs822395 SNP, the amplicon size was 208 bp, it was digested with 10 U of *Hin*fI restriction enzyme and analyzed on 3% agarose.

2.4. Statistical analysis

Student *t*-test and ANOVA were used to compare phenotypic data between control and T2DM groups using SPSS v. 20.0 software (SPSS Inc., Chicago, IL). Genotypes and alleles frequency were tested for Hardy–Weinberg equilibrium by x^2 test using online software web-Assotest (www.ekstoem.com). Genetic power was calculated using online software (osse.bii.a-star.edu.sg) (OSSE), genotypes and alleles in control and T2DM groups were tested by Multinomial logistic regression analysis with and without adjustment for sex, age and BMI using SPSS.

3. Results

The present study included 400 type 2 diabetics as well as 400 healthy individuals served as a control group. Biochemical measurements and genotyping of *ADIPOQ* for rs822395 and rs2241766 SNPs were carried out on the recruited individuals. Results of rs2241766T > G SNP exhibited one band (390 bp) for the wild type (TT), two bands (217,173 bp) for the homozygous (GG) and three bands (390, 217,173 bp) for the heterozygous (TG) genotype carriers respectively (Fig. 1). Results of rs822395 A > C SNP exhibited one band (208 bp) for wild type (AA), one band (187 bp) for homozygous (CC) and two bands (208,183 bp) for heterozygous (AC) genotype carriers respectively. However, due to a small size, the third band of a 21 bp size could not be observed on the agarose gel (Fig. 2). Results of rs2241766 SNP was evident to be consistent with Hardy–Weinberg equilibrium while, rs822395 SNP did not do so. The genetic power was found to be 97.3% for rs2241766 and 14.5% for rs822395.The genotype distribution of rs2241766 SNP indicated significant increases of carriers of the homozygous GG (OR: 5.04, CI95%: 2.27–11.19, *P*: 0.0001) and heterozygous TG (OR: 1.7, CI95%: 1.22–2.39, *P*: 0.002) variants when compared with those of the wild type, suggesting a risk factor of 2 and 5 for carriers of the hetero- and homozygous variants respectively. The minor allele frequency G was observed to be significantly (*P*: 0.0001) higher in patients (22%) in comparison with the control group (11.74%) (Table 1). On the other hand, results of rs822395 SNP failed to exhibit significant differences (Table 2). Changes of BMI cholesterol, triglycerides, insulin and the insulin resistance index values seemed to