Diabetes & Metabolic Syndrome: Clinical Research & Reviews xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Original Article

Correlation of Resistin Serum Level with Fat Mass and Obesity-Associated Gene (FTO) rs9939609 Polymorphism in Obese Women with Type 2 Diabetes

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ARTICLE INFO

Article history:
Received 25 April 2017
Accepted 11 May 2017
Available online xxx

Keywords:
rs9939609 polymorphism
FTO gene
Obesity
Type 2 diabetes
Resistin

ABSTRACT

Aims: The aim of this study was to detect any association of fat mass and obesity-associated (FTO) rs9939609 variant to metabolic and anthropometric parameters and resistin level as adipokines in Iranian obese women with type 2 diabetes mellitus.
Material and Methods: Totally, 42 diabetic and 36 non-diabetic women were selected. The PCR amplicons of FTO gene were sequenced and metabolic, anthropometric parameters and resistin level were measured.
Results: Serum resistin concentrations were not different between diabetic and non-diabetic subjects ($p > 0.05$), while resistin level in diabetic group with AA genotype was lower than that with other genotypes in the same group. In rs9939609 SNP adjusted analysis, insulin and HOMA levels were high in AA genotype. While levels of FBS and HbA_{1c} were higher in AA and AT genotypes. In diabetic group, only TG showed significant difference among three genotypes and mean of TG was higher in TA genotype. No significant correlation between resistin and anthropometric and metabolic parameters was found except for DBP in diabetic patients.
Conclusion: There was no significant association between rs9939609 and resistin serum level in type 2 obese diabetic women while percentile ranges (25^{th}, 50^{th} and 75^{th}) of resistin concentrations was high in diabetic group.

1. Introduction

Obesity is a risk factor for type 2 diabetes (T2D) and increasing insulin resistance and blood glucose levels could complicate the control of T2D [1]. Two pathophysiological defects, including pancreatic beta-cell dysfunction and insulin resistance in muscle, fatty and liver tissues, could cause T2D T2Dwhich would be affected by multiple genetic and environmental factors such as race, age, and obesity before and after birth. BMI was used for measuring the obesity level, as BMI more than 25 and 30 kg/m2 were classified as overweight and obese, respectively [2].

Many studies have been done to identify the relationship between obesity and T2D, though the molecular mechanisms are not known yet [3]. On the other hand, extensive efforts have been focused on the recognition of related genes in T2D and obesity. Very recently, FTO has been identified as a new genetic cause for obesity. Although FTO is expressed in all tissues, it seems that the highest expression is in hypothalamus which is responsible for controlling the food consumption. By the way, molecular mechanisms about the effect of FTO on obesity are not known exactly [4,5]. FTO is a polymorphic gene which is located on chromosome 16 and its molecular weight is 58 kDa [6]. Mutation of FTO gene is known as the strongest genetic risk factor for obesity in humans. Studies on the entire human genome have demonstrated a relationship among FTO gene polymorphism, T2D, and obesity. Moreover, relationship between FTO and obesity was confirmed by

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http://dx.doi.org/10.1016/j.dsx.2017.05.004
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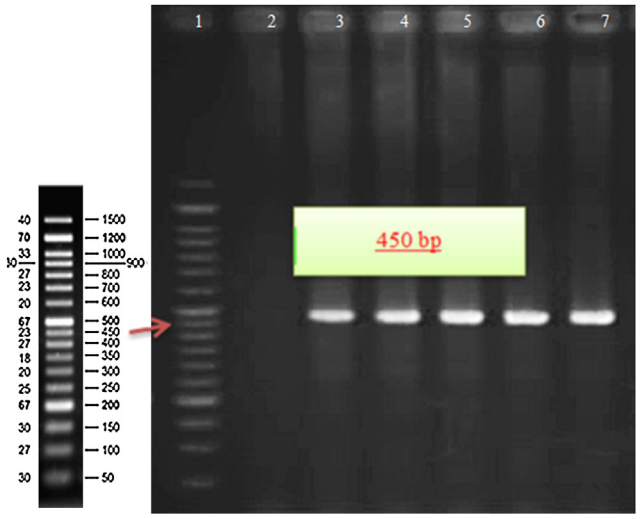

Fig. 1. Amplification of rs9939609 SNP incorporated into FTO gene (fragment size of ~450 bp), Line 1: 50 bp molecular weight ladder, and line 2–7: PCR product from samples.

detection of SNP rs9939609 polymorphism [7]. Overexpression of FTO could cause a series of metabolic defects such as increasing phosphorylation of protein kinase B that are associated with T2D. Following phosphorylation of this protein, lipogenesis is increased and mitochondrial oxidation is decreased. Some studies have shown that an association may exist among the expression of FTO, its polymorphism, and insulin activity in subcutaneous and visceral adipose tissue [8].

The adipose tissues are secreting the resistin hormone which it is named as RELM (resistin-like molecule) or FIZZ (found in inflammatory zone). Resistin categorize into cysteine-rich C-terminal proteins family. This hormone was isolated in the form of mRNA and then it was found that its expression is suppressed by PPARγ agonists [9,10].

Resistin expression in adipocytes decreases during fasting and increases upon feeding [11]. The role of resistin in insulin resistance has not been identified in humans. It is suggested that variation in resistin gene sequences plays an important role through changes in fasting glucose and insulin levels [12]. Increasing levels of resistin are associated with obesity, T2D, coronary disease and also gastric cancer. [13–15]

It seems that serum resistin level is high in T2D with FTO polymorphism.[16,17] Therefore, it is essential to determine association between FTO gene polymorphisms and serum resistin levels in obese people with T2D in various people. The aim of this study was to detect any association of Fat Mass and Obesity associated (FTO) rs9939609 variant (mutation of allele T to allele A) with metabolic and anthropometric parameters and resistin level as adipokines in Iranian obese women with T2D.

2. Material and Methods

2.1. Subjects

The calculation of patients sample size was done using G power version 3.0.10 (power: 80% and α: 0.05). In this case-control study, a total of 78 obese women (36 non-diabetic and 42 type 2 diabetic) were selected from patients who referred to University Teaching Hospital, Tabriz, Iran, after filling out informed consent form. The inclusion criteria for selecting obese participations were: BMI >30 Kg/m2, FBS > 126 mg/dL, T2D, no family relationship between the subjects in non-diabetic group, no specific disease or chronic history, and no special diet at least in past 6 months. The exclusion criteria included the having of kidney disease and using cortisol and lipid lowering drugs.

2.2. Measurement of anthropometric indicators

Measurements of all anthropometric indicators were done according to the standard kit (Pars Azmun. Tehran, Iran). Age, blood pressure (systolic and diastolic) (SBP, DBP), BMI, and FBS (fasting blood sugar) were calculated for each subject.

2.3. Sampling

Blood samples were obtained from subjects after an 8–12-hour fasting and were transferred into tubes containing EDTA to prevent their clotting. The samples were aliquoted and stored at–20oC. DNA isolation and determination of different biochemical parameters from whole blood were performed.

2.4. Determination of biochemical parameters

Measurement of total cholesterol (TC), high-density lipoprotein (HDL), and triglyceride (TG) concentrations was done using enzymatic available kit (Pars Azmun. Tehran, Iran). Low-density lipoprotein (LDL) cholesterol was calculated by means of Friedewald equation:

LDL cholesterol = total cholesterol–HDL–TG/5 (mg/dL), Glycated hemoglobin (HbA1c) level and serum resistin were determined by NycoCard reader and enzyme-linked immunosorbent assay (ELISA: Crystal day Biotech Co., Ltd Kit), respectively.

2.5. FTO genotyping: point mutation [T (wild allele) to A (mutated allele)]

Extraction of genomic DNA was done from the peripheral blood using the standard salting-out method. To amplify the FTO rs9939609 SNP, forward primer (5' GTAGGAATACTAGGAGAGGAG 3') and reverse primer (5'GCTTAAAGTTAATGGCTTCAGG 3') were designed using oligo5 program. PCR reactions were performed in 25 μl volumes containing 40 ng of template DNA and 0.4 μmol/L of each primer using 1X PCR Master Kit (CinnaGen Co., Iran). PCR amplification was performed in the following conditions: initial denaturation at 95 °C for 2 min, 35 cycles of denaturation at 96 °C for 20 sec, annealing at 60 °C for 40 sec, extension at 72 °C for 1 min and a final extension of 72 °C for 5 min. The size of the amplified PCR products was determined by comparison with the 50 bp DNA ladder (Fermantase Co., Canada). PCR products were electrophoresed on 1% agarose gel. Subsequent analysis included multiple sequence alignment using the BLAST program (available at: https://blast.ncbi.nlm.nih.gov/Blast.cgi) and Chormas software (version 2.6.2).

2.6. Statistical analysis

Analysis of the data (parametric and non-parametric) was done using mean ± SE (standard error). Distribution of the alleles and genotypes were calculated by Hardy-Weinberg equilibrium and significant differences of frequencies were determined using Chi-square test. Spearman correlation coefficient was used to evaluate correlation of resistin level with the other parameters. Calculation of Odds ratio was done. For statistical analysis, statistical package of SPSS 17.0 was used. If the P value was lower than 0.05, results were considered statistically significant.

Please cite this article in press as: M.A. Majdi, et al., Correlation of Resistin Serum Level with Fat Mass and Obesity-Associated Gene (FTO) rs9939609 Polymorphism in Obese Women with Type 2 Diabetes, Diab Met Syndr: Clin Res Rev (2017), http://dx.doi.org/10.1016/j.dsx.2017.05.004