Original Article

Evaluation of Two Types of Vitamin D Receptor Gene Morphism in Patients with Type 2 Diabetes and Obesity

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ABSTRACT

Background: Diabetes is the most common endocrine disorder that affects many people each year. Many people suffer from diabetes and obesity. The effects of renoprotective vitamin D and its analogues in chronic kidney diseases, including diabetic nephropathy, have been reported. The aim of this study was to investigate the rs731236 and rs2228570 polymorphisms of vitamin D receptor gene (GDR) in patients with type 2 diabetes and obesity.

Methods: In this case-control study, 120 patients with type 2 diabetes and 120 healthy controls (control group) referred to Amiralmomenin Arak Hospital were investigated during 2018. After collecting clinical data and performing laboratory tests, DNA samples were extracted from blood samples by phenol chloroform method, and polymorphisms in specimens were examined by specific genotypes. Data were analyzed by SPSS 22 software.

Results: There was no correlation between *Taq*1 and *Fok*1 polymorphisms with both healthy and healthy patients. Although the frequency of CT genotype was higher in both groups, this difference was not significant (P-value>0.05).

Conclusions: No evidence was found that VDR gene polymorphisms play a role in the risk of type 2 diabetes and obesity in Iranian subjects. Further studies in prospective cohort studies should be performed to determine the genome-wide association to evaluate the direct effect of these polymorphisms on type 2 diabetes.

Key words: Type 2 diabetes, Diabetes, Obesity, Vitamin D receptor gene (VDR), Genotype, Polymorphism

Introduction

The prevalence of diabetes is increasing worldwide, in 2010, about 200 million people are diabetic, and according to the World Health Organization (WHO), it is expected to reach about 300 million in 2025 (Arababadi *et al.*, 2012). The pathology of this disease is complex, so both environmental and genetic factors may play a role in this matter. Todays, several genes have been identified in relation to type 2 diabetes. Genetic determinants interact with environmental factors to create the final phenotype of disease (Hamman, 1992). Obesity is one of the most important causes of type 2 diabetes; although the final interaction of genes with environmental factors, including nutrition

and obesity, may be related to different gene variants. Therefore, identifying varieties of a gene not only improves the understanding of the pathophysiology of the disease but may also reveal biochemical and physiological pathways that bind different risk factors and create the potential for disease.

Based on epidemiological studies, it has been suggested that vitamin D deficiency may be a risk factor for glucose intolerance and also a risk to insulin resistance (Reis *et al.*, 2005), metabolic syndrome (Martini and Wood, 2006) and diabetes progression (Reis *et al.*, 2005).

The function of vitamin D is mediated through the vitamin D receptor, the gene that encodes the VDR is found on the chromosome 12cen-q12 and several polymorphisms in the gene have been identified including *Taq*I, *Fok*I, *Bsm*I, *Apa*I (Bid *et al.*, 2009; Uitterlinden *et al.*, 2004). Recent studies have reported that single-nucleotide polymorphisms within the VDR gene may affect the stability, quantity, and activity of the VDR protein as well as the speed of the transcription of the VDR gene (Palomer *et al.*, 2008). Genetic society studies have reported association between VDR gene polymorphisms with type 2 diabetes and obesity (Reis *et al.*, 2005). It also observed that variants of the VDR gene may have the same effect in different racial groups (Kittles and Weiss, 2003). But one of the most important determinants of the success of nutrition policies at the population level is the awareness of the prevalence of "Responsive genotypes" and the identification of those who are at risk for vitamin D deficiency.

Therefore, this study was aimed to investigate the interaction of vitamin D receptor gene polymorphisms with type 2 diabetes and obesity for the prevention and treatment of these individuals.

Materials and methods

In this case-control study, 120 patients with type 2 diabetes and 120 healthy controls (control group) n the age range of 38 to 80 years referred to Amiralmomenin Hospital in Arak, Iran were investigated during 2018.

Written informed consent was taken from all study participants and the study was approved by the Ethics Committee of faculty of Basic Sciences Dissertation of biology Islamic Azad University Central Tehran Branch.

Participants information was collected using a questionnaire including age, gender, body mass index (BMI) and medical history. Entry requirements included the absence of a medical condition or medical disorder requiring drug intervention, a history of chronic diseases, especially coronary artery disease, nephropathy, thyroid disease, and the last stage of renal or liver disease (ESRD, ESLD), non-pregnancy, and lactation.

Laboratory tests

5 mL of blood was taken from each patient after 12 hours of fasting, Blood samples were centrifuged; Samples were stored with the anticoagulant ethylene diamine tetra-acetic acid and were stored until use at 4 °C. Blood samples were collected in EDTA-containing tubes and DNA samples from the whole blood were extracted by a DNA extraction kit of the brand PrimePrepth (Genet BIO, South Korea) according to the details given in the kit for the separation of the genomic DNA. DNA was stored at -20 °C until polymorphisms were determined (Shab-Bidar *et al.*, 2011).

Determination of polymorphisms

The genotype was determined at the locations of single nucleotide VDR gene polymorphisms by the PCR-RFLP method by means of heat transfer device (Corbett Research, Gradient Palm cycler, Australia) and enzymatic digestion of the products by restriction enzymes (Fementas, Thermo Scientific, Canada). To perform RFLP on the PCR product, exposed the PCR product with the restriction enzyme, and maintained for 16 h at 37 °C. Along with the specimens, two specimens were controlled: A sample without enzyme (Positive control) and one sample without DNA (Negative control). After the required time, PCR products were analyzed by electrophoresis on a 1.5% agarose containing ethidium bromide gel and detected by gel recording and imaging apparatus gel (documentation system UVItec; UVIdoc, England).

***Fok*I Polymorphism (rs10735810):** This polymorphism was amplified by PCR using back (5'-CCCTTCATGGAAACACCTTGCTTC-3') and forth (5'-AGCTGGCCCTGGCACTGACT-3') primers according to (Deng *et al.*, 2002). And 273 bp products resulted. PCR was performed in 30 cycles at 58 °C. DNA was digested by the ***Fok*I** restriction enzyme. Genotypes of the beginning codon of polymorphism ***Fok*I** of the vitamin D receptor were CC, CT, and TT.

***Taq*I Polymorphism (rs731236):** This polymorphism was amplified by PCR using back (5'-TCATGGCTGAGGTCTCAAGGG-3') and forth (5'-CAGAGCATGGACAGGGAGCAA-3') primers according to (Deng *et al.*, 2002). And 737 bp products resulted. PCR was performed in 30 cycles at 64 °C. DNA was digested by the ***Taq*I** restriction enzyme. Genotypes of the beginning codon of polymorphism ***Taq*I** of the vitamin D receptor were CC, CT, and TT.

Statistical calculations

SPSS 22 software were used to the analysis of the characteristics of the participants and the calculation of the frequency of genotypic and allelic polymorphisms, as well as the evaluation of the relationship between different genotypes and bone density, and other statistical analyzes. Differences in the distribution of alleles and genotypes as well as in order to determine the deviation from Hardy Weinberg equilibrium were used in both healthy and healthy groups Chi square test. The relationship between different polymorphism genotypes with bone density was also analyzed by ANOVA. Charts related to these analyzes are drawn by Microsoft Excel software.

Results

49.6% (119 people) of the 240 patients examined was female and 50.4% (121 people) was male. 11.3% of people were aged 36 to 45 years old and 88.8% was older than 46 years old. The demographic data of the subjects examined is shown in Table 1.

Table1. Comparison of characteristics of T2D and control groups

	T2D (n=120)	**control** (n=120)	**Min-Max**
Age	60.55±10.46	60.27±10.52	38-80
Height	169.50±11.44	171.05±11.14	141-193
Weight	74.53±12.52	76.27±12.36	51-105

According to Table 2, no association was found between the frequency of genotypes and T and C alleles with type 2 diabetes in the studied population. It is clear that the level of significance is more than 0.05 and therefore it is concluded that there is no significant relationship between polymorphisms rs2228570 and rs731236 with the probability of diabetes type 2 and obesity.

Table 2. The results of polymorphisms *Taq*I and *Fok*I

	CC	CT	TT	P-Value
*Taq*I				0.807
T2D	20(16.7%)	54(45%)	46(38.3%)	
Control	19(15.8%)	59(49.2%)	42(35%)	
*Fok*I				0.607
T2D	2(1.7%)	70(58.3%)	48(40%)	
Control	4(3.3%)	71(59.2%)	45(37.5%)	

Discussion

In this study, the association of this polymorphism with type 2 diabetes and obesity were investigated. There was no significant difference in frequency of them in comparison with subjects in both groups.

The findings of some different studies suggest that there is a link between genotype and haplotypes of VDR polymorphism in people with type 1 diabetes (Turpeinen *et al.*, 2003). While our study has shown contradictory results in various relationships between the genetic variants of VDR and type 2 diabetes. In previous studies, the results of different *Taq*1 genotypes in the diabetic group with the control group showed a higher prevalence of tt and Tt in the diabetic population (Nosratabadi *et al.*, 2011). On the other hand, Bid *et al.*, demonstrated using *Fok*1, *Bsm*1, and *Taq*1 polymorphisms that there was no relationship between VDR polymorphisms and type 2 diabetes (Bid *et al.*, 2009). Also, other studies in Turkey and Spain failed to demonstrate this connection (Valdivielso and Fernandez, 2006; Dilmec *et al.*, 2011).

Based on our findings, there was no correlation between the frequency of genotypes and T and C alleles with type 2 diabetes in the studied population.

According to a study by Ramos-Lopez *et al.*, some haplotypes can be considered as protective against the susceptibility to type 1 diabetes in the German population, and the cause of the protection of these rare haplotypes is currently unclear, may also be related to the metabolism of vitamin D in the body (Ramos-Lopez *et al.*, 2006).

VDR gene polymorphisms in type 2 diabetic patients in the Polish population were also investigated, No association was found between the genotype frequency and allele and haplotype of the three *Bsm*I/*Apa*I/*Taq*I polymorphisms (Malecki *et al.*, 2003). In the study of the relationship between vitamin D receptor gene polymorphism (*Apa*I/*Fok*I) and micro/macrovascular complications in postmenopausal women with type 2 diabetes in Brazil, there was no significant difference in the allele frequency and genotype of two polymorphisms (Maia *et al.*, 2016).

In our study, there was no significant correlation between polymorphisms *Fok*I with the probability of diabetes mellitus type 2 and obesity. There was also no correlation between the frequency of genotypes and T and C alleles with type 2 diabetes in the studied population. There was also no correlation between the frequency of genotypes and T and C alleles with

type 2 diabetes in the studied population. In contrast, the population of Saudi Arabia, FokI polymorphism was associated with increased risk of T2D (Al-Daghri *et al.*, 2014).

The reasons for the difference between the results of the study can be explained by the genetic differences in the population studied as well as the environmental factors (Simmons, 2007; Carey *et al.*, 2009).

*Taq*1 and *Fok*1 polymorphisms have been reported to be the best and most suitable model for expression of the VDR gene mRNA.

Since there are sporadic studies with different outcomes, future studies can be done with more sample numbers and suspicious haplotypes. Also, the amount and level of 1-25 hydroxyvitamin D3 levels are not available for samples, measuring the level of vitamin D3 and its relationship with VDR haplotypes can also be an advantage in future studies.

Acknowledgments

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