Vitamin D Receptor Polymorphisms in Type 2 Diabetes in Southeastern Iranian Patients

Reza Nosratabadi, MSc,1 Mohammad Kazemi Arababadi, PhD,2 Vajihe Akbarpour Salehabad, BSc3

1Department of Laboratory Sciences, Faculty of Medicine, Islamic Azad University, Zahedan branch, Zahedan, 2Department of Microbiology, Hematology and Immunology, Faculty of Medicine, Rafsanjan University of Medical Sciences, 3Molecular-Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Islamic Republic of Iran

Abstract

Background: The impact of several environmental and genetic factors on diabetes is well documented. The present study was aimed to examine the 2 single nucleotide polymorphisms (SNP) in intron 8 and exon 9 of the vitamin D receptor (VDR) gene in type 2 diabetic patients.

Material and Methods: In this clinical study, peripheral blood samples were obtained from 100 type 2 diabetic patients and 100 healthy controls. DNA was extracted and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed to examine 2 SNP polymorphisms within the VDR gene.

Results: Our results showed a significant difference in the TaqI evaluated genotype of exon 9 in the VDR gene, but no significant differences were seen regarding other examined genotypes and alleles.

Conclusion: Based on our results, it can be concluded that VDR and its functional polymorphism in exon 9 may play an important role in the pathogenesis of type 2 diabetes.

Keywords: VDR, polymorphism, type 2 diabetes

The frequency of diabetes mellitus is increasing globally, and it is expected that this latent disorder will affect 200 million people by 2010 and 300 million by 2025.1 Type 2, sometimes referred to as non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes, is the most prevalent type of diabetes and is often caused by decreased insulin production by the pancreas.2,3 Current studies show that several genetic and environmental parameters are associated with type 2 diabetes.4,5 The causes of the agents inducing type 2 diabetes are yet to be clarified; however, it has been suggested that diabetes is an immune-related disease.5,6 For example, in type 2 diabetes peripheral blood monocytes have been shown to produce inflammatory cytokines,7 and serum levels of interferon-γ (IFN-γ) and interleukin-17A (IL-17A) are found to be higher in type 2 diabetes both with and without nephropathy.8 Furthermore, vitamin D has crucial effects on the function of insulin and may act via a number of pathways which appear to be important in the development of type 2 diabetes.9 Recent evidence demonstrated the interaction of 1, 25-dihydroxy vitamin D (the active form of vitamin D) and its receptor (vitamin D receptor [VDR]) have a supportive and regulatory impact on the immune system.10,11 For example, overproduction of IL-17 and IFN-γ were seen in deficient vitamin D and its receptor animals.11 The immunoregulatory effects of 1, 25-dihydroxy vitamin D and polymorphisms within VDR on the immune system were also demonstrated by several investigators.10,12 The association of VDR polymorphisms in immunological disorders such as hepatitis B,13 asthma,14 multiple sclerosis,15 and type 1 diabetes16,17 are well established. Previous studies demonstrated the polymorphisms of the VDR gene have an impact on VDR expression.18 Therefore, the aim of this study was to investigate the prevalence of 2 functional single nucleotide polymorphisms (SNP) within intron 8 and exon 9 of the VDR gene and correlate this with the occurrence of type 2 diabetes.

Material and Methods

Subjects

Peripheral blood samples were collected from 100 type 2 diabetic patients and 100 healthy controls. The patient and control groups were selected from within the Rafsanjan population and had similar medical and demographic characteristics including sex, age, and socioeconomic status (Table 1). Assessment of socioeconomic conditions were measured based on the level of education (diploma, weak; under graduate, moderate; and post graduate, high) and monthly income (under $250, weak; $250-$1000, moderate; and more than $1000, high). The study protocol was approved by the ethical committee of the Rafsanjan University of Medical Sciences and written informed consent was also obtained from all of the participants. Fasting blood sugar, urine albumin level, blood pressure, and clinical presentations were assessed 3 times during a period of 6 months for each patient and the control group. The bias factors such as infections, allergic
conditions, nephropathy, retinopathy, and smoking were excluded from the study.

Genomic DNA Extraction

To extract genomic DNA, peripheral blood was collected in ethylenediaminetetraacetic acid (EDTA), and genomic DNA was extracted using a commercial kit (Bioneer, Daejeon, South Korea) according to the manufacturer’s instructions. Extracted DNA was aliquoted for each sample and stored at -20°C for further analysis.

Detection of Polymorphisms

Vitamin D receptor gene polymorphisms within intron 8 and exon 9 were analyzed as previously described. In brief, primers were designed to flank a known Taq1 polymorphism carried within exon 9 and a known ApaI polymorphism with intron 8 of the VDR gene. Amplicons were subjected to restriction digestion with the appropriate enzyme, and the products were separated on an agarose gel. Alleles were scored according to the fragment patterns. Alleles digested by Taq1 or ApaI were scored as T and A alleles, respectively, and alleles not digested by Taq1 or Apa1 were scored as t and a alleles, respectively.

Statistical Analysis

The differences in genotypes and alleles were analyzed by Pearson’s chi-square test and Fisher’s exact test, respectively, and P values of less than 0.05 were considered significant.

Results

Polymorphisms within the VDR were scored according to polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of exon 8 and intron 9. Evaluation of the polymorphism within exon 9 of the VDR gene polymorphisms by Taq1 restriction digestion showed the prevalence of TTT genotype was 4 in type 2 diabetic patients and 18 in controls (Table 1). Our results also revealed the frequency of Ttt genotype was 63 and 35 in type 2 diabetic patients and the controls, respectively (Table 2). The value for the t/t genotype was 33 in type 2 diabetic patients and the controls (Table 2). Our results demonstrated the genotype differences were significant in the diabetic group when compared to the control group (P<0.001) (Table 2). The differences between groups regarding the frequency of T and t alleles were not significant (P=1.000) (Table 2).

As is shown in Table 2, the differences between the groups regarding the frequency of the A/A, A/a, and a/a genotypes were not significant. Significant differences were also not seen regarding ApaI evaluated alleles in the diabetic patient group when compared to controls (P=0.543) (Table 2).

No significant differences were observed between the groups regarding mean age (P=0.85), gender (P=0.9), and socioeconomic status (P=0.90) of the participants (Table 1).

Discussion

The impact of vitamin D and its receptor on insulin action and the development of type 2 diabetes and its inflammatory complications, such as nephropathy, is in urgent need of additional research to help elucidate the mechanisms of disease progression. It seems that immune-related factors play important roles in the etiology and pathogenesis of type 2 diabetes. In this study, the patient and control groups were matched for sex, age, and socioeconomic status. Although our findings indicated a significant difference between the Taq1 evaluated genotype of exon 9 within the VDR gene between the diabetic group and controls, a significant difference was not seen within Taq1 evaluated alleles. The alleles’ different distribution between patients and controls led to a significant difference in the frequency of polymorphisms.
Polymorphisms and type 2 diabetes. In addition, Malecki and colleagues also failed to find any relationship between these polymorphisms and type 2 diabetes; therefore, it can be concluded that VDR polymorphisms are important in the pathogenesis of type 2 diabetes. On the other hand, Bid and colleagues using TaqI polymorphisms in type 2 diabetic patients, and Malecki and colleagues also found no association between FokI, BsmI, and TaqI polymorphisms with type 2 diabetes. In addition, Malecki and colleagues also found no association between FokI, BsmI, and TaqI polymorphisms and the disease. One reason for the discrepancy between our results and these studies could be explained by the genetic differences in populations studied or their exposure to environmental factors. For example, several studies showed that stress is the main reason for inducing type 2 diabetes; therefore, it is possible that environmental factors influence the effects of polymorphisms.

Based on our study and the results from similar works, it can be concluded that VDR polymorphisms are associated with type 2 diabetes. Our research team proposes that the role of other VDR polymorphisms in type 2 diabetes should be evaluated by other researchers, simultaneously to increase the scope and power of the study.

Finally, type 2 diabetes is very complex and is associated with several environmental and genetic factors which must be taken into consideration when evaluating different population groups. Clearly, larger studies need to be completed using more parameters within the study design to evaluate the independent role of each factor in its relationship to the disease.

Acknowledgements: The authors of this article would like to acknowledge the diabetic patients and healthy controls who contributed to this research. This work was supported by a grant from the Islamic Azad University, Zahedan branch.