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Original Research

Association of Vitamin D Receptor Gene Polymorphism in Patients with Type 2 Diabetes in the Kashmir Valley

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ABSTRACT

Objectives: Approx 1 billion people across various ethnic and age groups have vitamin D deficiency. The high prevalence of such a deficiency is an imperative public health issue because hypovitaminosis D is an autonomous risk factor for mortality in the general population. Beyond bone integrity and calcium homeostasis, it is involved in numerous physiologic and pathologic processes. The role of vitamin D in the pathogenesis and prevention of type 2 diabetes mellitus has sparked universal interest.
Methods: This hospital-based case-control study was designed to study the association between 25-hydroxy vitamin D (25[OH]D) levels and the vitamin D receptor (VDR) gene polymorphism with diabetes and to evaluate their roles as risk factors for diabetes. 100 cases and controls were taken. 25(OH)D levels were analyzed by the chemilumenescence method using a Siemens ADVIA Centaur analyzer. Genomic DNA was extracted and Taq-1 and Bsm-1 genotyping in the VDR gene was done by using the polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP).
Results: 25(OH)D levels of patients with diabetes were significantly lower than those of controls (19.26±0.95 ng/mL vs. 25.49±1.02 ng/mL; p=0.001). 25(OH)D levels were found to be inversely associated with glycated hemoglobin percentages in cases (r^2=0.74). The results suggested that the single nucleotide polymorphisms Taq-1 t(T) allele and b (G allele) in Bsm-1 might be a susceptibility allele for diabetes in the Kashmiri population.
Conclusions: VDR gene polymorphisms appear to be an important genetic determinant in the progression of diabetes. Considering the important predisposition risk factor, we observed that Taq-1 and Bsm-1 were strongly associated with diabetes in northern Indians. But requires further study as a probable genetic risk marker for diabetes.

Mots clés :
polymorphismes génétiques
polymorphismes de longueur des fragments de restriction
diabète de type 2
récepteur de la vitamine D
chimiluminescence
réaction en chaîne par polymérase

RÉSUMÉ

Objectifs : Environ 1 milliard de personnes de divers groupes ethniques et groupes d'âge ont une carence en vitamine D. La forte prévalence de cette carence constitue un problème de santé publique d'une importance cruciale puisque l'hypovitaminose D est un facteur de risque indépendant de mortalité en population générale. Outre l'intégrité des os et l'homéostasie du calcium, elle touche de nombreux processus physiologiques et pathologiques. Le rôle de la vitamine D dans la pathogenèse et la prévention du diabète sucré de type 2 a suscité l'intérêt dans le monde entier.

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Méthodes : La présente étude cas-témoins en milieu hospitalier a été conçue pour étudier l'association des concentrations de la 25–hydroxyvitamine D (25[OH]D) et des polymorphismes du gène du récepteur de la vitamine D (RVD) avec le diabète et pour évaluer leurs rôles comme facteurs de risque du diabète. Les patients ont représenté les cas; les volontaires en bonne santé ont représenté les témoins (N=100). Des analyses hématologiques et biochimiques systématiques ont été réalisées. La méthode de chimiluminescence réalisée au moyen de l'analyseur ADVIA Centaur de Siemens a permis l'analyse des concentrations de la 25(OH)D. L'ADN génomique a été extrait du sang périphérique par la méthode en kit. Le génotypage de Taq-1 et de Bsm-1 dans le gène du RVD a été effectué à l'aide des techniques de réaction en chaîne de la polymérase et du polymorphisme de longueur des fragments de restriction (PCR–RFLP, de l'anglais *polymerase chain reaction-restriction fragment length polymorphism*) chez les cas et les témoins.

Résultats : Les concentrations sériques de la 25(OH)D chez les patients diabétiques étaient significativement plus faibles que chez les témoins (19,26±0,95 ng/ml *vs* 25,49±1,02 ng/ml; p=0,001). Chez les patients diabétiques, les concentrations de la 25(OH)D étaient inversement associées aux pourcentages de l'hémoglobine glyquée (r^2=0,74). Dans l'étude actuelle, nous avons déterminé la distribution des polymorphismes du gène, Taq-1 et Bsm-1, chez les personnes diabétiques et chez les groupes témoins. Nos résultats indiquaient que les fréquences génotypiques et alléliques des polymorphismes de nucléotide simple Bsm-1 diffèrent de manière significative entre les groupes diabétiques et les groupes normaux (p=0,0001). Les résultats indiquaient que l'allèle t(T) des polymorphismes de nucléotide simple Taq-1 et b (allèle G) de Bsm-1 seraient des allèles de prédisposition au diabète dans la population cachemirienne.

Conclusions : Les concentrations de la 25(OH)D étaient significativement plus faibles chez les personnes diabétiques. De plus, les concentrations de la 25(OH)D étaient inversement associées aux concentrations de l'hémoglobine glyquée. Les polymorphismes du gène du RVD semblent être des déterminants génétiques importants de l'origine et de la progression du diabète. Compte tenu du facteur de risque important de prédisposition, nous avons observé que Taq-1 et Bsm-1 étaient fortement associés au diabète chez les Indiens du Nord. Nos données montrent que les polymorphismes du gène du RVD pourraient être associés au risque de diabète. Par conséquent, d'autres études sont nécessaires pour démontrer s'ils sont des marqueurs génétiques probables du risque de diabète.

Introduction

Vitamin D, the sunshine vitamin, has been recently implicated in a plethora of medical illnesses. Diminution in the incidence of rickets after fortification of foods with vitamin D led physicians to believe that vitamin D-related health disorders had come to an end. But regrettably, rickets appears to be a mere drop in the vast ocean of disorders resulting from vitamin D deficiency (1). Apart from its conventionally understood role in bone health and calcium homeostasis, vitamin D is believed to have an effect on the body's endocrine system, immune system, cardiovascular system, neuropsychologic functioning and neuromuscular performance and is also believed to act as a potent antioxidant, protecting against free-radical damage, as well as being an inducer of cellular differentiation, protecting against carcinogenesis (2,3). Accumulating research suggests that circulating concentrations of vitamin D may be inversely related to the prevalence of diabetes, to the concentrations of glucose and to insulin resistance (4–7). Worldwide, some 382 million people are estimated to have diabetes. About 80% live in low- and middle-income countries. If these trends continue, by 2035, some 592 million people, or 1 adult in 10, will have diabetes. This equates to approximately 3 new cases every 10 seconds, or almost 10 million per year. The largest increases will take place in the regions where developing economies are predominant (8).

Diabetes is a multifactorial disorder; identification of modifiable risk factors is of colossal importance to curtail this pandemic. Studies of associations between insulin secretion and serum 25-hydroxy vitamin D (25[OH]D) have been inconsistent. Several studies have demonstrated a link between vitamin D and the incidence of diabetes. A number of mechanisms have been proposed to explain the inverse relationship between vitamin D and diabetes. Vitamin D deficiency has long been reported to be a risk factor for metabolic syndrome and diabetes. Higher plasma vitamin D levels have been shown to be related to a lower risk for the development of diabetes in high-risk patients (9). Also, some other studies have shown that vitamin D may play a functional role in glucose tolerance through its effects on insulin secretion and insulin sensitivity (10). The presence of a correlation between vitamin D receptor (VDR) polymorphisms and diabetes-associated metabolic parameters, including fasting glucose levels, glucose intolerance, insulin sensitivity, insulin secretion and calcitriol levels, has been reported by observational studies. Among such VDR polymorphisms, Fok-1, Taq-1, Bsm-1, EcoRV and APA1 are suspected to alter the activity of the VDR protein (11).

Therefore, we hypothesized that vitamin D deficiency may be prevalent in patients with diabetes and that vitamin D may be related to glucose control in this group of patients. The 3′ untranslated region of the VDR gene includes Bsm-1 and Taq-1. The potential effects of VDR polymorphisms on disease susceptibility have been investigated. Hence, we aimed to investigate the prevalence of 2 functional single nucleotide polymorphisms (Taq-1 and Bsm-1) of the VDR gene and correlate this with the occurrence of diabetes.

Methods

A case-control study was conducted in 100 age- and sex-matched individuals who had clinically confirmed diabetes (American Diabetes Association 2010 criteria) that was diagnosed by a senior endocrinologist and in 100 healthy volunteers as controls for a period of 1 year (April 2015 to April 2016). All the cases were recruited from the outpatient and inpatient departments of the Department of Internal Medicine of the Government Medical College Srinagar (GMC Sgr) and the associated Sri Maharaja Hari Singh (SMHS) hospital and were of Kashmiri ethnic origin (India). Chronic illnesses that potentially alter vitamin D metabolism, use of medications that affect bone metabolism (vitamin D supplements) and pregnant or breast-feeding women were excluded from the study.

Pursuant to physical examinations, records of clinical histories, including those of hypertension, obesity, hypercholesterolemia, stroke or transient ischemic attacks, smoking and other relevant details were maintained. A questionnaire was used to assess demographic details, including dietary patterns, extent of exposure to sun and use of sunscreen. Body mass indexes and complete demographic profiles were recorded.