Vitamin D deficiency, vitamin D receptor gene polymorphisms and cardiovascular risk factors in Caribbean patients with type 2 diabetes

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Abstract

Aim. – The prevalence of diabetes in the French West Indies is three times higher than in mainland France. We aimed to assess the associations between vitamin D deficiency, vitamin D receptor (VDR) gene polymorphisms and cardiovascular risk factors in Caribbean patients with type 2 diabetes (T2D).

Methods. – In this cross-sectional study of 277 patients, 25-hydroxyvitamin D was measured by radioimmunoassay. FokI, BsmI, ApaI and TaqI single nucleotide polymorphisms (SNPs) of the VDR gene were genotyped. Analysis of covariance and logistic regression were performed.

Results. – The study included 76 patients of Indian descent and 201 patients of African descent. The prevalence of vitamin D deficiency (<20 ng/mL) was 42.6%. When patients were classified into groups with (G1) and without (G2) vitamin D deficiency, there were no significant differences in age, systolic blood pressure, low-density lipoprotein cholesterol and HbA1c, although body mass index was significantly higher in G1. Vitamin D deficiency was significantly associated with increased diastolic blood pressure and triglyceride levels, and reduced high-density lipoprotein cholesterol (P<0.05). Prevalence of vitamin D deficiency was decreased in patients carrying the f allele of FokI (OR: 0.52; P= 0.02) and the aa genotype of ApaI (OR: 0.46; P = 0.05). BsmI and TaqI SNPs were not associated with vitamin D deficiency.

Conclusion. – The rate of vitamin D deficiency was high in our T2D patients, and was associated with the VDR gene FokI and ApaI polymorphisms and cardiovascular risk profile. Measurements of vitamin D may help to detect T2D patients with cardiovascular risk, and VDR polymorphisms might explain why vitamin D deficiency is so frequently seen in some T2D patients.

Keywords: Cardiovascular risk factors; Type 2 diabetes; Vitamin D deficiency; Vitamin D receptor gene polymorphisms

Résumé

Carence en vitamine D, polymorphismes du gène du récepteur de la vitamine D et facteurs de risque cardiovasculaire chez des diabétiques de type 2 aux Antilles. La prévalence du diabète aux Antilles françaises est trois fois plus élevée qu’en France métropolitaine.

Objectif. – Évaluer l’association entre carence en vitamine D, polymorphismes du gène du récepteur de la vitamine D (VDR) et facteurs de risque cardiovasculaire chez des caribéens diabétiques de type 2 (T2D).


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**Résultats.** — La prévalence de la carence vitaminique D (<20 ng/mL) était de 42,6%. Chez les patients classés en deux groupes selon le statut carencé G1 ou non G2 en vitamine D, il n’était noté aucune différence significative selon l’âge, la pression artérielle systolique, le LDL-cholestérol, l’HbA1c. L’IMC était significativement plus élevé dans le groupe G1. La carence vitaminique D était significativement associée à une pression artérielle diastolique et des triglycérides élevés, un HDL-cholestérol bas (P < 0,05). La prévalence de la carence en vitamine D était plus faible en présence de l’allèle F (SNP FokI OR 0,52 ; P = 0,02) et du génotype aa (SNP ApaI OR 0,46 ; P = 0,05). Il n’a pas été mis en évidence d’association significative entre le statut vitaminique D et les polymorphismes BsmI ou TaqI du gène VDR.

**Conclusion.** — La carence en vitamine D est fréquente chez les caribéens diabétiques de type 2, chez qui elle associée à un profil à risque cardiovasculaire (diminution du HDLc, augmentation de l’IMC et des triglycérides). Le dosage systématique de la vitamine D permettrait de détecter les DT2 à risque cardiovasculaire élevé. L’étude des polymorphismes du gène VDR pourrait expliquer pourquoi cette carence est plus fréquente chez certains diabétiques.

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*Mots clés : Risque cardiovasculaire ; Diabète de type 2 ; Vitamine D ; Polymorphismes du gène du récepteur de la vitamine D ; Antilles françaises*

1. **Introduction**

   L’île de la Guadeloupe (French West Indies), the overall prevalence of diabetes is high; it was estimated to be 8.1% in 2009 [1,2]. Type 2 diabetes (T2D), obesity and dark skin are associated with a high risk of vitamin D deficiency [3–8]. This deficiency is now considered a public-health matter because it has been associated with greater risks of other morbidities, such as cardiovascular disease and cancer [9,10]. Vitamin D is activated after binding to its specific cytosolic receptor (VDR) [11]. The VDR gene is located on chromosome 12q12-q14 in humans [12], and four adjacent restriction fragment length polymorphisms for BsmI, ApaI, FokI and TaqI at the 3′ end of VDR have been previously identified. Their associations with vitamin D levels and several diseases have also been investigated [13–16]. However, the prevalence of vitamin D deficiency and its relationship to VDR polymorphisms in Caribbean patients with T2D is not well known. The aim of the present study was to assess the prevalence of vitamin D deficiency in Caribbean T2D patients, and the associations between vitamin D deficiency, VDR polymorphisms and cardiovascular risk factors in such a population.

2. **Patients and methods**

   Our cross-sectional study included 277 T2D patients with dark skin, and was conducted in the diabetes department of the University Hospital of Guadeloupe. Exclusion criteria included patients with a previous history of kidney or inflammatory disease, pregnant women and those taking vitamin D replacement therapy. The study was approved by the regional ethics committee. All patients gave their written informed consent to take part in this study of cardiovascular and metabolic risks.

   2.1. **Measurements**

   The patients were interviewed by physicians who followed a standard questionnaire. Height, weight and waist circumference (WC) were measured. Body mass index (BMI) was calculated as weight/height² (kg/m²), and obesity was defined as a BMI ≥ 30 kg/m². Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were estimated using automated sphygmomanometers. Blood samples were obtained from all participants after overnight fasting. Low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDLC), triglyceride (TG) levels, fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) were evaluated. Plasma concentrations of 25-hydroxyvitamin D (25OHD) were measured using a direct radioimmunoassay (DiaSorin SA, Antony, France). Vitamin D deficiency was defined as plasma 25OHD levels less than 20 ng/mL. Participants were then classified into two groups according to whether they were vitamin-D-deficient (group G1) or not (group G2).

   Genomic DNA was extracted from peripheral blood samples using a QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany). Four single nucleotide polymorphisms (SNPs) in exon 2 (FokI), exon 9 (TaqI) and intron 8 (ApaI and BsmI) of VDR were analyzed by a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method.

   2.2. **Statistical analysis**

   Data for categorical variables are presented as numbers (percentages) and data for continuous variables as means (standard deviations). Associations with cardiovascular risk factors (hypertension [SBP and DBP], BMI, WC, FBG, HDL-C and LDL-C, TG levels), demographic factors (age, gender, obesity) and allele frequencies of the four SNPs were estimated. Each polymorphism was tested for departures from the Hardy–Weinberg equilibrium using the chi-square test. Subjects were classified into groups G1 and G2 according to the presence and absence, respectively, of vitamin D deficiency (defined as 25OHD < 20 ng/mL). Statistical methods included the Chi² test and analysis of covariance (ANCOVA, adjusted for age, gender and BMI when indicated) for comparisons between groups. Adjusted logistic regressions were performed for all covariates that were significant (P < 0.20) in the univariate and multivariate analyses. SPSS version 18.0 statistical software (SPSS Inc, Chicago, IL) was used for the analyses.
Vitamin D status of the whole study population. 25OHD: 25-hydroxyvitamin D; CV: cardiovascular. Data are expressed as means (SD) unless otherwise stated.

3. Results

A total of 277 patients with T2D and a mean age of 64 ± 11 years were studied; 62.1% were women. Average BMI was 28.9 ± 5.6 kg/m² with a WC of 75.9 ± 13 cm. In this population, 76 patients (27.4%) were of South Indian (Indian) descent and 201 (72.6%) were of African (Afro-Caribbean) descent. Antidiabetic treatment was prescribed in 97.5% of these T2D patients, and the mean HbA1c was 7.26 ± 1.69%. The prevalence of preexisting cardiovascular disease (coronary disease, stroke, peripheral artery disease) in the study population was 31%, while the prevalence of vitamin D deficiency was 42.6% and, for vitamin D insufficiency (vitamin D levels < 30 ng/mL), 89.9%. Taking the study population as a whole, 25OHD levels were inversely correlated with BMI \((r = -0.15; P = 0.01)\). Considering only those who were obese (37.5%), 25OHD levels were significantly decreased (21.6 ng/mL) vs. 19.8 ng/mL in the rest of the sample population \((P = 0.001)\), and vitamin D deficiency was recorded in 52.5%.

The vitamin D status of all patients according to their clinical characteristics is summarized in Table 1, and the clinical characteristics of groups G1 and G2 are compared in Table 2. BMI was higher in G1 than in G2 (29.8 vs. 28.3 kg/m², respectively; \(P = 0.02\)). There was no significant difference between the two groups in terms of age, SBP, FBG, LDL-C or HbA1c. Vitamin D deficiency was significantly associated with higher DBP and TG levels, and lower HDL-C levels \((P < 0.05)\). The results of VDR genotyping were available for 270 patients. Carrying the aa genotype of the Apal SNP or the f allele of the FokI SNP was significantly associated with a low risk of vitamin D deficiency (Table 3), whereas the BsmI and TaqI allelic variants were not significantly associated with vitamin D deficiency status.

25OHD levels differed depending on the VDR polymorphism: vitamin D levels were significantly lower in the presence of the bb genotype of the BsmI SNP compared with the BB or Bb genotypes \((18.9 \text{ vs. } 21.2 \text{ ng/L, respectively}; P = 0.02)\). Also, the prevalence of vitamin D deficiency was significantly lower in patients with the aa genotype of the Apal SNP compared with the AA/Aa genotypes \((28\% \text{ vs. } 46\%, \text{ respectively}; P = 0.02)\) and in carriers of the f allele of the FokI SNP \((Ff \text{ or } ff \text{ genotype})\) compared with the FF genotype \((33\% \text{ vs. } 49\%, \text{ respectively}; P = 0.02; \text{ Table 4})\).

In the univariate analysis, vitamin D deficiency was significantly associated with obesity \((\text{ odds ratio } [\text{OR}]: 1.88; P = 0.01)\), HDL-C \((\text{OR}: 0.54; P = 0.05)\) and TG \((\text{OR}: 1.48; P = 0.02)\). In the multivariate analysis, the variables introduced into the model were age, obesity, DBP, HDL-C, TG and VDR polymorphisms. The adjusted ORs for vitamin D deficiency were significant for obesity \((\text{OR}: 2.31; P = 0.004)\), TG \((\text{OR}: 1.44; P = 0.05)\), and carrying the f allele of the FokI SNP \((\text{OR}: 0.52; P = 0.02)\) or the aa genotype of the Apal SNP \((\text{OR}: 0.46; P = 0.05; \text{ Table 3})\).

4. Discussion

Vitamin D insufficiency, defined as vitamin D levels less than 30 ng/mL (75 nmol/L), leads to increases in parathormone levels, and in bone disease and fractures. Cardiovascular risks appear to be associated with vitamin D levels less than 20 ng/mL, defined as vitamin D deficiency [17,18]. To assess cardiovascular risk in our T2D patients, we analyzed the groups with vitamin D levels greater to and less than 20 ng/mL. To our knowledge, this is the first such study to analyse the prevalence of vitamin D deficiency in Caribbean subjects with T2D, as well as the distribution of VDR polymorphisms in relation to vitamin D deficiency and cardiovascular risk factors. The results of the
Table 2
Characteristics of the study patients according to vitamin D deficiency status.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Vitamin D deficiency (&lt; 20 ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes = 118 patients</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>64%</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.4 (13.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.8 (6.5)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>133 (16.0)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76 (11.0)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.3 (0.3)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.7 (0.9)</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.4 (1.1)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.4 (1.8)</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>7.4 (1.8)</td>
</tr>
<tr>
<td>Vitamin D levels (ng/mL)</td>
<td>15.7 (3.5)</td>
</tr>
<tr>
<td>FokI rare allele (ff/Ff vs FF)</td>
<td>34%</td>
</tr>
<tr>
<td>BsmI bb genotype</td>
<td>14%</td>
</tr>
<tr>
<td>TaqI rare allele (tt/Tt vs TT)</td>
<td>43%</td>
</tr>
<tr>
<td>Apal aa genotype</td>
<td>11%</td>
</tr>
</tbody>
</table>

Data are expressed as means (SD) unless otherwise stated.

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglyceride; FBG: fasting blood glucose.

The present study are of interest because they highlight, for the first time, a high prevalence of vitamin D deficiency (42.6%) in a diabetic population living in a sunny climate. In a control group of 280 subjects, vitamin D deficiency was estimated at 6.8% and vitamin D insufficiency at 54.6% (unpublished data). These values are lower than those in our present diabetic population, suggesting that diabetic patients are at greater risk of vitamin D deficiency.

The risk factors for vitamin D deficiency include extreme age, female gender, dark skin pigmentation, winter season, malnutrition, lack of sun exposure, a covered-up style of clothing and obesity [7]. The patients in the present study all had high levels of skin pigmentation. The melanin pigment in human skin competes for and absorbs the ultraviolet B photons responsible for the photolysis of 7-dehydrocholesterol to previtamin D3. Studies have reported lower mean 25OHD levels in Africans than in other ethnic groups [16]. Indeed, a previous study showed that, compared with white Americans, healthy Afro-Americans at most latitudes in North America fail to achieve optimal 25OHD concentrations at any time of year [19]. Furthermore, data from the National Health and Nutrition Examination Survey (NHANES) III showed that 53–76% of non-Hispanic blacks in the United States had vitamin D levels less than 50 nmol/L compared with only 8–33% of non-Hispanic whites [20]. In our present study, there was no light-skinned control group because, although the prevalence of T2D is high in Afro-Caribbeans living in Guadeloupe and other Caribbean islands [21], the prevalence of T2D in the Caucasian population of Guadeloupe is very low.

As in previous studies in non-diabetic subjects, lower levels of 25OHD and a greater frequency of vitamin D deficiency were noted in obese compared with non-obese subjects [21,22]. In fact, a high prevalence of obesity was previously found in the

Table 3
Distribution of 25-hydroxyvitamin D (25OHD) and of vitamin D deficiency according to VDR polymorphisms.

<table>
<thead>
<tr>
<th>Apal (n = 272)</th>
<th>AA (n = 83)</th>
<th>Aa (n = 142)</th>
<th>aa (n = 47)</th>
<th>P</th>
<th>AA/Aa (n = 225)</th>
<th>aa (n = 47)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>25OHD (ng/L)</td>
<td>21.2 (5.7)</td>
<td>21.3 (7.4)</td>
<td>23.1 (6.2)</td>
<td>0.22</td>
<td>21.2 (6.8)</td>
<td>23.1 (6.2)</td>
<td>0.08</td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
<td>45%</td>
<td>47%</td>
<td>28%</td>
<td>0.06</td>
<td>46%</td>
<td>28%</td>
<td>0.02</td>
</tr>
<tr>
<td>BsmI (n = 270)</td>
<td>BB (n = 123)</td>
<td>Bb (n = 118)</td>
<td>bb (n = 29)</td>
<td>0.05</td>
<td>BB/Bb (n = 241)</td>
<td>bb (n = 29)</td>
<td>0.11</td>
</tr>
<tr>
<td>25OHD (ng/L)</td>
<td>21.4 (6.4)</td>
<td>22.2 (6.9)</td>
<td>18.9 (6.6)</td>
<td>0.23</td>
<td>21.2 (6.7)</td>
<td>18.9 (6.6)</td>
<td>0.02</td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
<td>42%</td>
<td>42%</td>
<td>59%</td>
<td>0.23</td>
<td>42%</td>
<td>59%</td>
<td>0.11</td>
</tr>
<tr>
<td>FokI (n = 270)</td>
<td>FF (n = 156)</td>
<td>Ff (n = 95)</td>
<td>ff (n = 19)</td>
<td>0.74</td>
<td>FF (n = 156)</td>
<td>Ff/ff (n = 114)</td>
<td>0.60</td>
</tr>
<tr>
<td>25OHD (ng/L)</td>
<td>21.3 (7.0)</td>
<td>21.6 (6.1)</td>
<td>22.5 (7.0)</td>
<td>0.04</td>
<td>21.3 (7.0)</td>
<td>21.8 (6.2)</td>
<td>0.33</td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
<td>49%</td>
<td>35%</td>
<td>32%</td>
<td>0.04</td>
<td>49%</td>
<td>33%</td>
<td>0.02</td>
</tr>
<tr>
<td>TaqI (n = 262)</td>
<td>TT (n = 140)</td>
<td>Tt (n = 103)</td>
<td>tt (n = 19)</td>
<td>0.48</td>
<td>TT (n = 140)</td>
<td>Tt/tt (n = 122)</td>
<td>0.23</td>
</tr>
<tr>
<td>25OHD (ng/L)</td>
<td>20.9 (6.2)</td>
<td>22.0 (6.9)</td>
<td>21.1 (7.2)</td>
<td>0.58</td>
<td>22.0 (6.9)</td>
<td>21.9 (6.2)</td>
<td>0.32</td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
<td>46%</td>
<td>39%</td>
<td>42%</td>
<td>0.58</td>
<td>46%</td>
<td>40%</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Data are expressed as means (SD) unless otherwise stated, and are derived by ANCOVA, adjusted for age, body mass index and gender.
Caribbean population [23]. In our island, the increased adoption of Westernized lifestyles and diets, along with reduced energy expenditure such as physical activity, are contributing to obesity that, in turn, raises the risk of T2D.

The bioavailability of vitamin D in obesity is decreased because of its storage in adipose tissue [24]. Obesity is also associated with cardiovascular risk factors. In our present study population, the prevalence of obesity was 37.5%, which might explain why vitamin D deficiency was so common. Vitamin D has also been suggested to be an important mediator of cardiovascular disease [25–27]. A recent multinational, placebo-controlled, double-blind trial showed that paricalcitol, a selective activator of VDR, lowers albuminuria in patients with T2D nephropathy, a population with a high risk of cardiovascular disease. These results suggest the potential role of vitamin D deficiency in both renal and progressive vascular disease [28].

Low vitamin D levels can be associated with hypertension, atherosclerosis and diabetes, and may predispose to cardiovascular diseases with an increased risk of mortality [29]. According to our present results, vitamin D deficiency is indeed associated with a clinical profile of cardiovascular risk. However, no records of lifestyle factors (smoking habits, physical activity and alcohol consumption) were taken to allow confirmation of such a finding. Moreover, there was an inverse association between vitamin D levels and DBP. A previous study had reported that vitamin D replacement could improve DBP [29]. Our T2D patients with vitamin D deficiency had lower HDL-C and higher TG levels than those with normal 25OHD levels. However, unlike our findings, no association was found between lipid profiles and vitamin D levels in Iranians; thus, discrepancies are still found in the relationship between lipid profiles and vitamin D deficiency status [30,31].

Vitamin D is activated after binding to VDR. Recently, a genome-wide association study showed that VDR polymorphisms could contribute to the variability of 25OHD levels, but this did not appear to be linked to skin pigmentation [32]. The gene encoding for VDR is located on the 12q12-q14. A direct effect of vitamin D on adipocyte differentiation and metabolism is a possible mechanism for such an effect, as VDR is expressed in preadipocytes [33]. It has also been suggested that the underlying mechanism of the relationship between vitamin D deficiency and chronic disease is the presence of VDR in several tissues and cells, including pancreatic beta cells and adipocytes [33,34]. In addition, an association between VDR polymorphisms and body weight and insulin secretion has also been reported [6,13]. In our present study population, the genotypes at FokI and Apal SNPs of VDR were associated with vitamin D deficiency. Also noted were significant relationships between 25OHD levels and allelic variants of FokI and BsmI SNPs. Carrying the f allele of the FokI SNP or the aa genotype of the Apal SNP might be protective against vitamin D deficiency: an earlier study found that they can affect circulating levels of vitamin D [35] and may also influence cardiovascular risk.

One limitation of our present investigation is that the study was cross-sectional and, therefore, cannot determine whether the decrease in 25OHD preceded the occurrence of cardiovascular risk factors. Nevertheless, our study has important clinical implications by highlighting the high frequency of vitamin D deficiency in Afro-Caribbean patients with T2D, despite sun exposure, that is further increased in obese T2D patients. Thus, preventing, screening for and treating vitamin D deficiency and its health consequences need to be a priority in such a population.

In conclusion, VDR allelic variations and certain cardiovascular risk factors are associated with vitamin D deficiency in our Afro-Caribbean T2D patients. However, the mecha-
nisms through which these relationships arise require further investigation.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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