Vitamin D receptor gene polymorphisms and 25(OH) vitamin D: Lack of association to glycemic control and metabolic parameters in type 2 diabetic Egyptian patients

Hala Ibrahim El Gendya, Noha Adly Sadika, Mona Youssry Helmy, Laila Ahmed Rashed

Background: Vitamin D deficiency and vitamin D receptor (VDR) gene polymorphisms have been linked to type 2 diabetes mellitus (T2DM) and its metabolic parameters, however there are conflicting results therefore we aimed to evaluate VDR gene polymorphisms (FokI, BsmI and TaqI) and vitamin D status in Egyptian patients with T2DM and to detect the associations of these polymorphisms to their metabolic parameters and glycemic control.

Methods: 50 patients with T2DM and 50 healthy age matched control subjects were enrolled. FBG, 2 h –PPG, fasting lipids, Hb A1c, calcium, phosphorus, urea, creatinine, ALT, AST were measured. BMI has calculated. Serum 25 hydroxy vitamin D (25(OH)D) has measured by ELISA. VDR gene polymorphisms detection has done by polymerase chain reaction through restriction fragment length polymorphism (PCR-RFLP) technique.

Results: Our study has shown lower mean levels of 25(OH)D in patients with T2DM (28.54 ± 10.02) in comparison with control subjects (44.65 ± 7.19), p < 0.001. Vitamin D insufficiency was more prevalent in T2DM 58% than in healthy control subjects 4%. There were statistically significant differences between patients with type 2 diabetes and controls regarding the distribution of FokI genotypes and alleles (p = 0.005) and non significant difference regarding BsmI and TaqI. Neither VDR gene polymorphisms nor 25(OH)D showed significant association with glycemic control, fasting lipids and BMI in patients with T2DM.

Conclusions: Vitamin D deficiency is prevalent in Egyptian patients with T2DM. Associations were found only between VDR FokI gene polymorphism and susceptibility to Egyptian patients with T2DM. Non significant differences in VDR gene polymorphisms distribution has found regarding glycemic control and metabolic parameters.

Original research
reported VDR gene polymorphisms linked to diabetes is VDR (FokI, BsmI, TaqI and ApaI) [15]. We aimed to evaluate different VDR gene polymorphisms as (FokI, BsmI and TaqI) and vitamin D status in Egyptian patients with T2DM and assess their associations with glycemic control and metabolic parameters.

Subjects and methods

Subjects

This case control study was conducted on 50 patients with T2DM who had attended the diabetes and endocrine clinic at Kasr Al Ainy Hospital, Cairo University and 50 healthy age matched control subjects. The study was performed from December 2016 to November 2017. T2DM was defined according to American Diabetes Association criteria [16]. All patients were diagnosed to have diabetes mellitus for at least 5 years. Patients with diabetic nephropathy, chronic liver disease, coronary heart disease, pregnancy, lactation, infections, bronchial asthma, smoking or receiving vitamin D supplementation were excluded.

All subjects were subjected to full history taking and full clinical examination. BMI classified according to the classification of WHO [17]. Patients were classified into 3 groups according to their anti-diabetic medication into group 1 using insulin, group 2 using oral-hypoglycemic and group 3 treated by both. Laboratory investigations in the form of fasting blood glucose (FBG), 2 h postprandial blood glucose (2 h –PPG), fasting lipids (total cholesterol (TC), triglycerides (TAG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), glycosylated hemoglobin (Hb A1c), calcium, phosphorus, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) were measured. Serum 25 hydroxy vitamin D [25(OH)D] was measured by ELISA kit supplied by ALPCO USA. Vitamin D receptor gene polymorphisms detection (BsmI, FokI and TaqI) has done by polymerase chain reaction and restriction fragment polymorphism (PCR-RFLP) technique.

Vitamin D deficiency has been defined according to the classification of the Endocrine Society 2011 as 25(OH)D of equal and less than 20 ng/ml, vitamin D insufficiency as 25(OH)D of 21–29 ng/ml and sufficiency as 25(OH)D ≥ 30 ng/ml [18].

Research protocols were approved by the medical ethics committee of Kasr Al Ainy Medical School, Cairo University. All participants were provided a written informed consent after the research protocols were carefully explained to them.

Detection of VDR polymorphisms

Detection of VDR (BsmI, FokI and TaqI) gene polymorphism by PCR-RFLP technique. Genomic DNA was extracted from peripheral blood leukocytes using kit supplied by (Qiagen, USA). According to manufacture instructions purity of extracted DNA was determined at 260 nm and 280 nm using Nanodrop Analyzer spectrometer [19]. VDR genotyping was done by PCR-RFLP technique using PCR Master Mix supplied by Thermodiner. FokI, BsmI and TaqI genotyping were done by amplification of PCR reaction using 25 ml total volume for each containing 10 mM trisHCl, 200 mM dNTPs and 20 pmol from the VDR DNA primer sequence.

Sequence of VDR DNA primers

<table>
<thead>
<tr>
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<th>Primer sequence</th>
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<tbody>
<tr>
<td>FokI</td>
<td>Forward: 5′-AGC TGG CCGTGG CAC TGACTC GCTT-3′</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5′-ATGGAA ACA CCT TGC TTC TTC CTC CTC-3′</td>
</tr>
<tr>
<td>BsmI</td>
<td>Forward: 5′-CAACAATGGAAAGCTAAGTAGCGGGGTACAGTG-3′</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5′-AACGAGGGAAAAGGCTAAGG-3′</td>
</tr>
<tr>
<td>TaqI</td>
<td>Forward: 5′-CAG AGC ATG GACAG GAGCAA-3′</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5′-CAC TTC GAC CAC AAG GGGGTTAG C-3′</td>
</tr>
</tbody>
</table>

The PCR cycling condition were denaturized at 94 °C for 4 min, followed by 35 cycles at 94 °C for 1 min, 68 °C for 1 min and 72 °C for 2 min with additional 10 min incubation at 72 °C after completion of the last cycle.

The PCR products for VDR were digested by Taq-1 polymerase enzyme. For the Fok1 restriction polymorphism it revealed 2 possible alleles F and f. The FF genotype yielded one band at 265 bp, the ff genotype yielded two bands at 196 bp and 69 bp and three bands at 265 bp, 196 bp and 69 bp in the heterozygous Ff. For the Bsm1 restriction polymorphism, the homozygote (BB) yielded one band at 820 bp, the bb yielded two bands at 650 and 170 bp and the heterozygote Bb yielded three bands at 820, 650 and 170 bp. For the Taq1 restriction polymorphism, the homozygous TT yielded bands of 500 bp and 210 bp. The homozygous tt yielded bands at 210 bp and the heterozygous Tt yielded bands at 290 bp. The designated PCR products were electrophoresed on 2.5% agarose gel to separate fragments [20] stained with ethidium bromide and DNA was visualized by placing the gel on an ultraviolet trans-illuminator. The ethidium bromide intercalated into the DNA and gave bright pink bands.

Statistical analysis

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Science) version 22. Data was summarized using mean and standard deviation in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. We used unpaired t test and one way ANOVA test for quantitative variables. For comparing categorical data, Chi square (2) test was performed. Exact test was used instead when the expected frequency is less than 5. Odds ratio (OR) with 95% confidence intervals was calculated. Correlation was done using Pearson correlation coefficient. P value less than 0.05 indicates significant result.

Results

Table 1 shows the demographic data of our studied groups. Group 1 included 50 healthy control subjects, 34 (68%) were females and 16 (32%) were males with their mean age (51 ± 8.2) years and group 2 included 50 healthy control subjects, 34 (68%) were females and 16 (32%) were males with their mean age (51 ± 8.2) years.

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls n = 50</th>
<th>T2DM patients n = 50</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51 ± 8.2</td>
<td>52.48 ± 6.55</td>
<td>0.322</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>30.57 ± 7.68</td>
<td>33.06 ± 6.07</td>
<td>0.075</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>94.98 ± 16.56</td>
<td>110.60 ± 9.33</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ALT (UI/L)</td>
<td>23.90 ± 6.40</td>
<td>30.52 ± 6.45</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AST (UI/L)</td>
<td>25.08 ± 6.66</td>
<td>30.78 ± 6.23</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.62 ± 0.20</td>
<td>0.63 ± 0.16</td>
<td>0.784</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>29.16 ± 4.99</td>
<td>32.78 ± 4.90</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>9.29 ± 0.49</td>
<td>8.97 ± 0.46</td>
<td>0.001</td>
</tr>
<tr>
<td>PO4 (mg/dl)</td>
<td>3.40 ± 0.42</td>
<td>3.48 ± 0.55</td>
<td>0.405</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>142.64 ± 61.19</td>
<td>158.9 ± 58.22</td>
<td>0.177</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>167.40 ± 57.47</td>
<td>204.10 ± 62.54</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL-c (mg/dl)</td>
<td>145.02 ± 40.46</td>
<td>154.08 ± 45.43</td>
<td>0.295</td>
</tr>
<tr>
<td>LDL-c (mg/dl)</td>
<td>45.22 ± 10.28</td>
<td>45.78 ± 14.52</td>
<td>0.823</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>85.94 ± 5.80</td>
<td>222.58 ± 85.89</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2hr –PPG (mg/dl)</td>
<td>129.90 ± 9.66</td>
<td>244.40 ± 87.62</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hb A1c %</td>
<td>4.76 ± 0.54</td>
<td>7.18 ± 2.04</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>25(OH)D (ng/ml)</td>
<td>44.65 ± 7.19</td>
<td>28.54 ± 10.02</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD, *P < 0.05 is significant, WC, waist circumference, BMI, Body mass index, Ca, Serum calcium, PO4, Serum phosphorus, FPG, fasting blood glucose, 2 h –PPG, 2 Hours postprandial blood glucose, HDLc, high-density lipoprotein cholesterol, LDLc, low-density lipoprotein cholesterol, Hb A1c, glycated haemoglobin, 25(OH)D, 25 hydroxy vitamin D.
included 50 type 2 diabetic patients, 40 (80%) were females and 10 (20%) were males with their mean age (52.48 ± 6.5) years with no statistically significant difference between patients with T2DM and control subjects regarding the gender (P = 0.171). Thirty five (70%) of our patients were receiving both insulin mixtard 30/70 + oral anti-diabetic drugs mainly metformin, ten (20%) of them were receiving oral anti-diabetic drugs mainly metformin + glibenclamide and only five (10%) of them were receiving insulin mixtard 30/70. The mean disease duration was 11.46 ± 5.98 years.

The mean serum 25(OH)D level was significantly lower in patients with T2DM compared to the control subjects (p < 0.001) (Table 1, Fig. 1).

By comparing vitamin D status between groups vitamin D deficiency was detected in 15 patients (30%), vitamin D insufficiency found in 14 patients (28%) where optimal levels found in 21 patients (42%) and only 4% of the control subjects found to be vitamin D insufficient with statistical significant difference (p < 0.001).

Also we found 60% of the patients treated by insulin had deficient vitamin D level, 20% had insufficient level and 20% had optimal level, while the patients treated by combined oral hypoglycemic drugs and insulin were 28.6% deficient in vitamin D level and only 20% treated by oral hypoglycemic drugs only had deficient vitamin D level. The mean serum level of 25(OH)D was lower in the group of patients treated with insulin (21.38 ± 6.56) than the group of patients treated by both oral hypoglycemic drugs and insulin (28.65 ± 10.13) or oral hypoglycemic drugs only (31.72 ± 10.01) with no significant statistical difference (p = 0.170).

With regard to the genetic distribution of Vitamin D receptor gene polymorphisms in patients with T2DM in comparison to controls we found statistically significant differences only in Fok 1 genotypes distribution (p = 0.005). There were increased frequency of both ff and Ff genotype in patients with T2DM in comparison to controls with statistical significant difference, (p = 0.016 and 0.005 respectively). Also there was increased frequency of allele f genotype in patient group with significant statistical difference in comparison to controls (p = 0.001) (Table 2, Fig. 2).

According to Bsm 1 genotypes distribution there was no statistical significant difference between patients with T2DM and controls (Table 2, Fig. 3).

Also with Taq1 genotypes distribution there were no statistical significant differences between patients with T2DM and controls (Table 2, Fig. 4).

In our study we didn’t find any significant correlations between serum 25(OH)D and FBS, 2hr PPG, HbA1c, lipid profile and BMI in our patients (Table 3a).

Also we didn’t find any statistically significant difference in VDR...
polymorphisms (Fok I, Taq1, Bsm I) distribution regarding BMI, lipid profile, glycomic control or vitamin D levels in T2DM patients (Tables 4a, 5a, 6a).

Discussion

Vitamin D has identified to play an important role on insulin synthesis, secretion and function also it has a significant role on elements of inflammation which may affect the development of T2DM [21]. Vitamin D stimulates insulin secretion from the pancreatic β cells through enhancing the intracellular Ca concentration converting the pro-insulin to insulin. Also it increases the sensitivity of cells to insulin by increasing the expression of insulin receptors and by keeping adequate supply of calcium pool [13]. Alterations of Ca supply lead to irreversible after vitamin D supplementation and the beneficial effect of vitamin D supplementation on glycemic control might needs shorter duration of diabetes which wasn’t present in their patients selection [30].

Regarding VDR gene polymorphisms and their relation to glycemic control we didn’t find any significant difference in the genetic and allelic distribution among our patients which is in agreement with other studies [23,27]. On the contrary previous studies linked Taq1 and Fok1 polymorphism to insulin secretion [31] and BsmI polymorphism to fasting glucose [32] and HOMA-IR [33] but these studies were done on healthy non diabetic subjects in contrast to our patients with long disease duration.

Also we did not find any significant correlation between vitamin D level with BMI and waist circumference in patients with T2DM. This result was in agreement with other previous studies [34,30]. On the contrary Hannemann et al. [35] found the BMI, waist circumference, waist-to-hip ratio were inversely correlated with serum 25(OH)D concentrations. The inverse relation between low vitamin D levels and metabolic syndrome was explained by the fat solubility of vitamin D which leads to its sequestration in the adipose tissue so decreases its bioavailability [30]. Also we didn’t find significant association between VDR polymorphisms (Fok1, Taq1 and Bsm1) and BMI in our T2DM patients. An Egyptian study had done on obese women reported significant association between obesity and VDR polymorphisms in vitamin D deficient cases [36]. Similarly Mackawy and Badawi found significant association between Fok1 polymorphism and waist circumference in T2DM patients with metabolic syndrome [23].

The difference between our results and other studies regarding genetic distribution of VDR polymorphisms may be due to genetic variations in the population studied or due to exposure to environmental factors. There is limitation in our study that the sample size is relatively small and the non significant association of vitamin D or VDR polymorphism with glycemic control or metabolic parameters mostly related to our selection of patients as our patients with long disease duration may not image these relations correctly, so it was better to assess the vitamin D status and genetic polymorphism and their association to metabolic parameters in patients newly diagnosed or patients with impaired glucose tolerance in comparison with healthy subjects to reconsider the causality and other relations. Therefore the relation between VDR polymorphisms and T2DM is still not completely clear and will require additional studies.

Conclusion

We concluded from our study that vitamin D deficiency was highly prevalent in our Egyptian patients with T2DM. There were statistically significant differences between patients with T2DM and controls as regard Fok1 genotypes and alleles distribution which could be a risk factor for Egyptian patients with T2DM. Neither vitamin D nor VDR risk. Regarding (Taq1, Bsm1) genotypes and alleles distribution we didn’t find significant statistical differences between patients with T2DM and controls. Similarly other previous studies [23,24,26] and a Meta analysis done on 11 reports [28] did not find any significant differences regarding VDR BsmI genotypes among T2DM and controls, also Turkish investigators didn’t find association between Taq1 polymorphism and T2DM [29].

Despite the fact that hyperglycemia is one of the main presentation of T2DM we didn’t find any significant correlation between vitamin D and glycemic control among patients with T2DM. In agreement to our result the study had done by Luo et al. where they didn’t find any significant association of vitamin D level and glycemic control even after vitamin D supplementation [30]. Luo et al. in their study had suggested that the inflammatory mediators which are released during the diabetic disorder were highly stimulated with long duration of diabetes which also associated with marked β cell dysfunction and intense insulin resistance therefore glycemic control parameters were not so much reversible after vitamin D supplementation and the beneficial effect of vitamin D supplementation on glycemic control might needs shorter duration of diabetes which wasn’t present in their patients selection [30].
(FokI, TaqI or BsmI) polymorphisms found to be associated with the metabolic parameters and glycemic control in our patients with T2DM. The possible role of vitamin D in the pathogenesis of T2DM is still not completely understood. Further knowledge and studies are needed to identify new gene polymorphisms which can play a significant role in the treatment and prevention of T2DM.

Declaration of interest

None. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcte.2018.11.005.

References