



Original research

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 Gendy^a, Noha Adly Sadik^{a,*}, Mona Youssry Helmy^a, Laila Ahmed Rashed^b

^a Internal Medicine, Faculty of Medicine, Cairo University, Egypt

^b Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Egypt



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ABSTRACT

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.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic endocrine disease characterized by pancreatic β cell dysfunction and insulin resistance [1]. It represents one of the major health problems worldwide [2] due to its chronic disabling complications which need special medical care for early prevention and treatment [3]. Several genetic and environmental factors have linked to T2DM development [4]. Vitamin D deficiency has been identified as one of the risk factors for type 2 diabetes development [5] and several evidence based studies have documented the relation between vitamin D deficiency and development of T2DM [6] where vitamin D supplementation found to be associated with the decreased risk in predisposed patients [7]. Vitamin D has identified to play a crucial role in diabetes where it regulates the insulin receptor gene [8] and controls the nuclear peroxisome proliferator activated

process (PPAR- δ) an important factor in regulating the fatty acid metabolism in skeletal muscles and adipose tissue which plays a crucial role in insulin sensitivity [9].

The biological effects of vitamin D mediated after its binding to cytosolic/nuclear vitamin D receptors (VDRs) [10] which belong to steroid hormone receptor family [11]. These receptors spread in many tissues regulating essential genes linked to chronic illness, inflammatory processes and bone diseases [12]. Activation of vitamin D through VDRs plays an important role in regulating insulin secretion from the pancreatic β cells [13]. Many VDR gene polymorphisms have been identified to be related to T2DM, insulin release [14] and metabolic parameters of obesity [6]. Till now there is difficulty in detecting the possible genes associated with T2DM development due to subtle changes that could occur in the candidate genes and the interaction of these changes with other genetic or non genetic factors [15]. The most

* Corresponding author at: Dyar city, middle hill, El Mokattam, Cairo 11571, Egypt.

E-mail address: noha_adly@yahoo.com (N.A. Sadik).

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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 aminotransaminase (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 length 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 $\geq$ 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 leucocytes 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 ThermoFisher. 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

FokI	Forward: 5'-AGC TGG CCCTGG CAC TGACTC GCTCT-3' Reverse: 5'- ATGGAA ACA CCT TGC TTC TTC TCC CTC-3'
BsmI	Forward: 5'- CAACCAAGACTACAAGTACCGCGTCAGTGA-3' Reverse: 5'-AACCAGCGGGAAGAGGTCAAGGG -3'
TaqI	Forward: 5'- CAG AGC ATG GACAGG GAGCAA-3' Reverse: 5'-CAC TTC GAG CAC AAG GGGCGTTAG C-3'

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 FokI 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 BsmI 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 TaqI 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 $\pm$ 8.2) years and group 2

Table 1
Demographic and laboratory data of the studied groups.

Variable	Controls n = 50		T2DM patients n = 50		P value
	Mean	Standard Deviation	Mean	Standard Deviation	
Age (years)	51	$\pm$ 8.2	52.48	$\pm$ 6.55	0.322
BMI (Kg/m ²)	30.57	$\pm$ 7.68	33.06	$\pm$ 6.07	0.075
WC (cm)	94.98	$\pm$ 16.56	110.60	$\pm$ 9.33	< 0.001*
ALT(IU/L)	23.90	$\pm$ 6.40	30.52	$\pm$ 6.45	< 0.001*
AST(IU/L)	25.08	$\pm$ 6.66	30.78	$\pm$ 6.23	< 0.001*
Creatinine (mg/dl)	0.62	$\pm$ 0.20	0.63	$\pm$ 0.16	0.784
Urea (mg/dl)	29.16	$\pm$ 4.99	32.78	$\pm$ 4.90	< 0.001*
Ca (mg/dl)	9.29	$\pm$ 0.49	8.97	$\pm$ 0.46	0.001
PO4 (mg/dl)	3.40	$\pm$ 0.42	3.48	$\pm$ 0.55	0.405
Triglycerides (mg/dl)	142.64	$\pm$ 61.19	158.9	$\pm$ 58.22	0.177
Cholesterol (mg/dl)	167.40	$\pm$ 57.47	204.10	$\pm$ 62.54	0.003
LDL-c (mg/dl)	145.02	$\pm$ 40.46	154.08	$\pm$ 45.43	0.295
HDL -c (mg/dl)	45.22	$\pm$ 10.28	45.78	$\pm$ 14.32	0.823
FBG (mg/dl)	85.94	$\pm$ 5.80	222.58	$\pm$ 85.89	< 0.001*
2hr -PPG (mg/dl)	129.90	$\pm$ 6.96	244.40	$\pm$ 87.62	< 0.001*
Hb A1c %	4.76	$\pm$ 0.54	7.18	$\pm$ 2.04	< 0.001*
25(OH) D (ng/ml)	44.65	$\pm$ 7.19	28.54	$\pm$ 10.02	< 0.001*

Values are expressed as means $\pm$ SD, *P < 0.05 is significant, WC, waist circumference, BMI, Body mass index, Ca, Serum calcium, PO4, Serum phosphorus, FBG, 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.

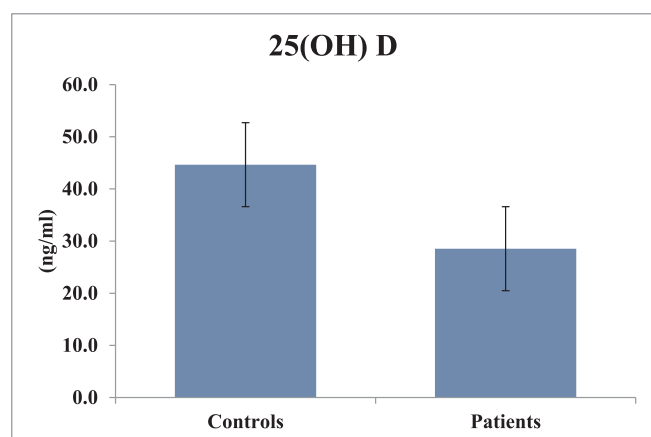


Fig. 1. Comparison of mean serum 25(OH)D between patients with T2DM and control subjects.

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 antidiabetic drugs mainly metformin, ten (20%) of them were receiving oral antidiabetic 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 I* 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 I* genotypes distribution there was no statistical significant difference between patients with T2DM and controls (Table 2, Fig. 3).

Also with *TaqI* genotypes distribution there were no significant statistical 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

Table 2
Genetic distribution of VDR polymorphisms in patients with T2DM in comparison to control subjects.

Variable	Controls (n = 50)	T2DM (n = 50)	P value	OR (95%CI)
<i>Fok I</i>				
ff	3 (6%)	8 (16%)	0.005*	5.3 (1.2–22.9) $p = 0.016$
Ff	15 (30%)	26 (52%)		3.5 (1.5–8.3) $p = 0.005$
FF	32 (64%)	16 (32%)		Reference
Allele f	21 (21%)	42 (42%)	0.001*	2.7 (1.5–5.1) $p = 0.001$
Allele F	79 (79%)	58 (58%)		Reference
<i>Bsm I</i>				
bb	5 (10%)	10 (20%)	0.261	2.8 (0.8–9.9) $p = 0.104$
Bb	24 (48%)	25 (50%)		1.5 (0.6–3.5) $p = 0.393$
BB	21 (42%)	15 (30%)		Reference
Allele b	34 (34%)	45 (45%)	0.112	1.5 (0.9–2.8) $p = 0.11$
Allele B	66 (66%)	55 (55%)		Reference
<i>TaqI</i>				
tt	7 (14%)	7 (14%)	0.689	1.2 (0.4–4.1) $p = 0.757$
Tt	20 (40%)	24 (48%)		1.5 (0.6–3.4) $p = 0.39$
TT	23 (46%)	19 (38%)		Reference
Allele t	34 (34%)	38 (38%)	0.556	1.2 (0.7–2.1) $p = 0.56$
Allele T	66 (66%)	62 (62%)		Reference

Values are expressed as numbers and percentage, * $P < 0.05$ is significant, OR: odd ratio, CI: confidence interval.

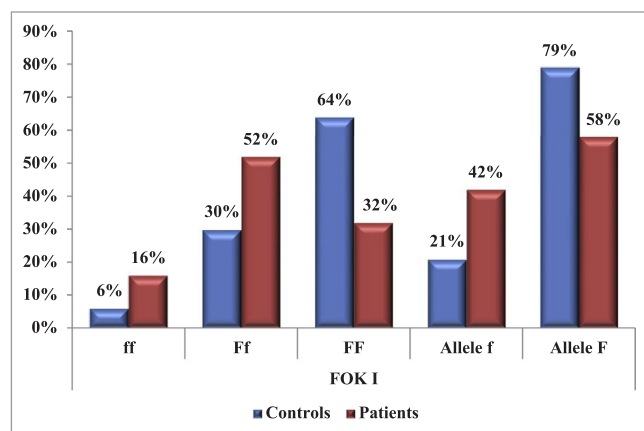


Fig. 2. Distribution of vitamin D receptor Fok 1 and alleles (F & f) genotypes in patients with T2DM in comparison to controls.

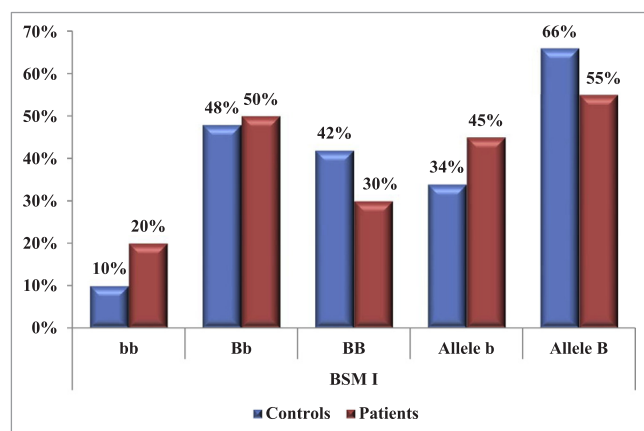


Fig. 3. Distribution of vitamin D receptor Bsm 1 and alleles (B & b) genotypes in patients with T2DM in comparison to control subjects.

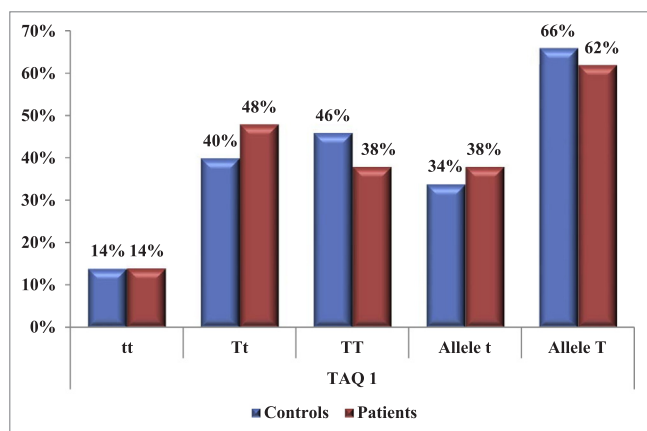


Fig. 4. Distribution of vitamin D receptor Taq 1 and alleles (t & T) genotypes in patients with T2DM in comparison to control subjects.

polymorphisms (Fok I, Taq1, Bsm I) distribution regarding BMI, lipid profile, glycemic 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 peripheral insulin resistance due to impaired insulin transduction which decreases GLUT-4 the main player in glucose metabolism which maintains glucose homeostasis. Vitamin D activates the transcription of human insulin gene and vitamin D response element found in the insulin gene promoter area which elucidates the crucial role of vitamin D with insulin [13]. Therefore vitamin D deficiency may be related to impaired insulin secretion in T2DM.

Meta analysis of 11 studies reported by Shen L et al [22] found lower vitamin D levels in T2DM patients compared to controls. In our study we found patients with T2DM had significant lower vitamin D levels associated with high prevalence 58% in comparison with controls 4% ($p < 0.001$). This result was supported by the Egyptian study done by Mackawy and Badawi [23] which revealed lower vitamin D levels among diabetic patients with more decrease in diabetic patients with metabolic syndrome. Errouagui Abdeltif et al [24] also documented high prevalence of vitamin D deficiency in T2DM (40%) compared to non diabetic controls (20%) in Moroccans population.

VDR gene polymorphisms have been identified to affect the activity of VDR protein [25]. It has supposed that genetic variations in the VDR play significant role in the pathogenesis of T2DM by altering Ca metabolism, adipocyte function, insulin release and cytokine expression [25]. We evaluated VDR (FokI – Taq1 – Bsm1) gene polymorphisms in our patients and we found significant statistical differences in comparing FokI genotypes and alleles distribution between patients with T2DM and controls with increased frequency of ff genotype in patients with T2DM. In agreement with our findings the Egyptian researchers Mackawy and Badawi [23] found the ff genotype and the f allele of the VDR FokI polymorphism significantly more frequent in diabetic patients with metabolic syndrome. Also the study done by Errouagui et al [24] on Moroccan population reported significant association regarding FokI distribution in T2DM. On the contrary other studies [26,27] showed no association between VDR FokI polymorphisms and diabetes

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].

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 FokI 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 (FokI, 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 FokI 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 FokI genotypes and alleles distribution which could be a risk factor for Egyptian patients with T2DM. Neither vitamin D nor VDR

(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

- [1] American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2005;28(1):37–42.
- [2] Roglic G, Unwin N, Bennett PH, Mathers C, Tuomilehto J, Nag S, et al. The burden of mortality attributable to diabetes: realistic estimates for the year 2000. *Diabetes Care* 2005;28:2130–5.
- [3] Unger RH, Orci L. Paracrinology of islets and the paracrinopathy of diabetes. *Proc Natl Acad Sci USA* 2010;107(37):16009–12.
- [4] Tong Y, Lin Y, Zhang Y, Yang J, Zhang Y, Liu H, et al. Association between TCF7L2 gene polymorphisms and susceptibility to type 2 diabetes mellitus: a large human genome epidemiology (HuGE) review and meta-analysis. *BMC Med Genet* 2009;10:15. <https://doi.org/10.1186/1471-2350-10-15>.
- [5] Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. *Diabetes Care* 2007;30:980–6.
- [6] Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. *Diabetes Care* 2007;30:980–6.
- [7] Pittas A, Nelson J, Mitri J, Hillmann W, Garganta C, Nathan DM, et al. Plasma 25-hydroxyvitamin D and progression to diabetes in patients at risk for diabetes: an ancillary analysis in the Diabetes Prevention Program. *Diabetes Care* 2012;35:565–73. <https://doi.org/10.2337/dc11-1795>.
- [8] Maestro B, Molero S, Bajo S, Dávila N, Calle C. Transcriptional activation of the human insulin receptor gene by 1, 25-dihydroxyvitamin D(3). *Cell Biochem Funct* 2002;20:227–32.
- [9] Dunlop TW, Vaisanen S, Frank C, Molnár F, Sinkkonen L, Carlberg C. The human peroxisome proliferator-activated receptor δ gene is a primary target of 1 α , 25-dihydroxyvitamin D3 and its nuclear receptor. *J Mol Biol* 2005;349(2):248–60.
- [10] Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, et al. The nuclear receptor superfamily: the second decade. *Cell* 1995;83:835–9.
- [11] Zhang J, Li W, Liu J, Wu W, Ouyang H, Zhang Q, et al. Polymorphisms in the vitamin D receptor gene and type 1 diabetes mellitus risk: an update by meta-analysis. *Mol Cell Endocrinol* 2012;355:135–42. <https://doi.org/10.1016/j.mce.2012.02.003>.
- [12] Haussler MR, Haussler CA, Bartik L, Whitfield GK, Hsieh JC, Slater S, et al. Vitamin D receptor: molecular signaling and actions of nutritional ligands in disease prevention. *Nutr Rev* 2008;66:S98–112. <https://doi.org/10.1111/j.1753-4887.2008.00093.x>.
- [13] Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes: a systematic review and meta-analysis (review). *J Clin Endocrinol Metab* 2007;92:2017–29.
- [14] Ortlepp JR, Lauscher J, Hoffmann R, Hanrath P, Joost HG. The VDR gene variant is associated with the prevalence of Type 2 diabetes mellitus and coronary artery disease. *Diabetes Med* 2001;18:842–5.
- [15] Xia Zheng, Yazhuo Hu, Han Zhitao, Gao Ya, Bai Jie, He Yao, et al. Association of vitamin D receptor gene polymorphisms with diabetic dyslipidemia in the elderly male population in North China. *Clin Interv Aging* 2017;12:1673–9.
- [16] ADA (American diabetes association). Classification and diagnosis of diabetes mellitus. *Diabetes Care* 2012;35(suppl 1):s64–72.
- [17] WHO (World Health Organization). expert consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004;363(9414):1077–127.
- [18] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96(7):1911–30. <https://doi.org/10.1210/jc.2011-0385>.
- [19] DNeasy * blood and tissue handbook, Qiagen. DNA extraction of vitamin D receptor polymorphism 2006.
- [20] Thermo Fisher Scientific. agarose gel electrophoresis protocols: E-Gel® EX Agarose Gel and Ultra Pure™ Agarose, available at < <https://www.thermofisher.com/...science/...gel-electrophoresis/...electr> > ; 2015.
- [21] Ozfirat Zehra, Chowdhury Tahseen A. Vitamin D deficiency and type 2 diabetes. *Postgrad Med J* 2010;86:18–25.
- [22] Shen L, Zhuang QS, Ji HF. Assessment of vitamin D levels in type 1 and type 2 diabetes patients: Results from meta-analysis. *Mol Nutr Food Res* 2016;60(5):1059–67. <https://doi.org/10.1002/mnfr.201500937>.
- [23] Mackawy AM, Badawi MEH. Association of vitamin D and vitamin D receptor gene polymorphisms with chronic inflammation, insulin resistance and metabolic syndrome components in type 2 diabetic Egyptian patients. *Meta Gene* 2014;2:540–56. <https://doi.org/10.1016/j.mgene.2014.07.002>.
- [24] Abdeltif Errouagui, Benrahma Houda, Charoute Hicham, Ghalim Nouredine, Barakat Abdel Hamid, Kandil Mostafa, Rouba Hassan. Vitamin D receptor gene polymorphisms and vitamin D status and susceptibility to type 2 diabetes mellitus in Moroccan patients. *Int J Innov Appl Stud* 2014;8(2):503–14.
- [25] Mahajan Mridula, Sharma Ritu. Current understanding of role of vitamin D in type 2 DM. *Int J Recent Sci Res* 2015;6(2):2602–7.
- [26] Bid HK, Konwar R, Aggarwal CG, Gautam S, Saxena M, Nayak VL, et al. Vitamin D receptor (FokI, BsmI and TaqI) gene polymorphisms and type 2 diabetes mellitus: a North Indian study. *Indian J Med Sci* 2009;63:187–94. <https://doi.org/10.4103/0019-5359.53164>.
- [27] Malecki MT, Frey J, Moczulski D, Klupa T, Kozek E, Sieradzki J. VDR gene polymorphisms and association with type 2 diabetes mellitus in a Polish population. *Exp Clin Endocrinol Diabetes* 2003;111:505–9.
- [28] Zhu Bo, Zhao Hui-Liu, Chao Ou, Huang Ling-Sha, Li Pei-Zhang, Lao Ming. Association of vitamin D receptor BsmI gene polymorphism with the risk of type 2 diabetes mellitus. *J Recept Signal Transduction* 2014;34:458–62. <https://doi.org/10.3109/10799893.2014.919592>.
- [29] Valdivielso JM, Fernandez E. Vitamin D receptor polymorphisms and diseases. *Clin Chim Acta* 2006;371:1–12.
- [30] Luo C, Wong J, Brown M, Hooper M, Molyneux L, Yue DK. Hypovitaminosis D in Chinese type 2 diabetes: Lack of impact on clinical metabolic status and biomarkers of cellular inflammation. *Diab Vasc Dis Res* 2009;6:194–209. <https://doi.org/10.1177/1479164109337974>.
- [31] Ogunkolade BW, Boucher BJ, Prah J, Bustin SA, Burrin JM, Noonan K, et al. Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. *Diabetes* 2002;51:2294–300.
- [32] Ortlepp JR, Metrikat J, Albrecht M, von Korff A, Hanrath P, Hoffmann R. The vitamin D receptor gene variant and physical activity predicts fasting glucose levels in healthy young men. *Diabet Med* 2003;20:451–4.
- [33] Oh JY, Barrett-Connor E. Association between vitamin D receptor polymorphism and type 2 diabetes or metabolic syndrome in community-dwelling older adults: the Rancho Bernardo Study. *Metabolism* 2002;51:356–9.
- [34] Clemente-Postigo M, Muñoz-Garach A, Serrano M, Garrido-Sánchez L, Bernal-López MR, Fernández-García D, et al. Serum 25-hydroxyvitamin D and adipose tissue vitamin D receptor gene expression: relationship with obesity and type 2 diabetes. *Clin Endocrinol Metab* 2015;100(4):E591–5. <https://doi.org/10.1210/jc.2014-3016>.
- [35] Hannemann A, Heinsbaek Thuesen B, Friedrich N, Völzke H, Steveling A, Ittermann T, et al. Adiposity measures and vitamin D concentrations in Northeast Germany and Denmark. *Nutr Metab* 2015;12:24. <https://doi.org/10.1186/s12986-015-0019-0>.
- [36] Zaki Moushira, Kamal Sanaa, Basha Walaa A, Youness Eman, Ezzat Wafaa, El-Bassyouni Hala, Amr Khaldia. Association of vitamin D receptor gene polymorphism (VDR) with vitamin D deficiency, metabolic and inflammatory markers in Egyptian obese women. *Genes Diseases* 2017;4:176–82. <https://doi.org/10.1016/j.gendis.2017.07.002>.