Vitamin D receptor gene polymorphisms (BsmI and FokI) and serum levels of 25-hydroxyvitamin D in Egyptian patients with type 2 diabetes and their association with atherosclerosis

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Background/aim
A large number of individuals with type 2 diabetes mellitus (T2DM) die owing to atherosclerotic cardiovascular diseases (CVD). The role of vitamin D deficiency and vitamin D receptor (VDR) gene polymorphisms in the development of T2DM-related CVD has yet to be established. We aimed to determine the association of 25(OH) vitamin D and VDR gene (BsmI and FokI) polymorphisms with T2DM and its accompanying atherosclerosis.

Patients and methods
This study enrolled 45 male patients with T2DM and manifested CVD who were admitted to the Department of Internal Medicine of Medical Research Institute Hospital, Alexandria University, Egypt, in addition to 45 healthy male volunteers. 25 (OH) vitamin D was measured in all cases. Carotid intima-medial thickness was measured, in addition to analysis of VDR gene (BsmI and FokI) polymorphisms using PCR and restriction fragment length polymorphism for all studied participants.

Results
Significantly lower vitamin D levels were observed in the diabetic atherosclerotic group than controls. The minor allele f of the FokI polymorphism and the minor allele b of BsmI polymorphism were associated with a higher risk of coronary artery disease in patients with T2DM, with an odds ratio of 12.750 (P=0.002) and 6.122 (1.202–30.078), respectively. FF genotype had significantly lower levels of total cholesterol and low-density lipoprotein cholesterol than Ff and ff genotypes (P=0.01 and 0.04, respectively).

Conclusion
Presence of the f allele of FokI as well as the b allele of BsmI polymorphisms of the VDR gene could increase the risk of atherosclerosis in Egyptian patients with T2DM, through influencing lipid metabolism. Vitamin D deficiency might contribute to increased risk of atherosclerosis in T2DM independent of the variants of VDR gene.

Keywords:
atherosclerosis, type 2 diabetes mellitus, 25(OH) vitamin D, vitamin D receptor polymorphisms

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Introduction

Diabetes mellitus (DM) is a huge and rising health problem all over the world. DM is one of the main noncommunicable chronic diseases reaching about 422 million by 2019, with type 2 diabetes mellitus (T2DM), representing more than 90% of the cases of diabetes [1]. Egypt was ranked among the top 10 countries with diabetes with a prevalence rate of ~15.56% among adults between 20 and 79 years of age. This prevalence is fast rising and expected to increase by about 13.1 million by 2034 [2].

Diabetic complications have become a major cause of morbidity and mortality worldwide [3]. Increased incidence of atherosclerosis is found in diabetic patients when accompanied by other major cardiovascular risk factors such as hypertension, dyslipidemia, and smoking. However, atherosclerosis process in diabetes is indistinguishable from that of the nondiabetic population, but it begins earlier and is often more extensive and more severe [3]. Several diabetes-induced abnormalities may add to the atherosclerosis process, for example, lipid abnormalities, increased lipoprotein oxidation, increased lipoprotein glycosylation, accentuated platelets aggregation and adhesion, as well as endothelial cell dysfunction [4].

Many studies have found a strong association between vitamin D deficiency and T2DM development [5,6]. Moreover, the involvement of vitamin D in the components of metabolic syndrome has been suggested [7]. Metabolic syndrome is defined as a group of disorders that when together lead to occurrence of increased risk of both cardiovascular disease (CVD) and T2DM. Some studies have supported the role of vitamin D in vascular protection, especially against the effects of advanced glycation end products, which is the main damaging diabetic effect on cardiovascular system [6,8].

Vitamin D receptor (VDR) regulates the transcriptional functions of 1, 25$(OH)_2$ vitamin D-responsive genes by forming a complex with a vitamin D-response element located in the promoter region of the target genes. VDR mediates the effects of vitamin D on bone metabolism, oxidative damage, chronic diseases, and inflammation as it is present in more than 38 tissues [9]. The VDR gene is a nuclear receptor located on chromosome 12q13.1 [10]. It has four mostly studied single nucleotide polymorphisms (SNPs) that are known to alter VDR protein activity: BsmI (rs1544410), ApaI (rs7975232), TaqI (T>C; rs731236), and FokI (rs10735810) [10]. Some of these polymorphisms were found to have an association with T2DM, insulin secretion, in addition to metabolic changes related to obesity [6,11].

The FokI polymorphism occurs at the VDR gene starting codon [12]. It has two alleles, the allele F and the infrequent f allele, where the allele F codes for shorter (424 amino acid) but more active VDR isoform than the infrequent f allele [12]. The *BsmI* polymorphism occurs at intron 8 of VDR gene near the 3'end, where it has a strong linkage disequilibrium with a polyadenosine (poly (A)) microsatellite repeat in the 3'untranslated region; thus, it may affect the translational activity of VDR [13].

Accumulating evidence suggests that low serum levels of vitamin D are associated with an increased risk of atherosclerosis, endothelial dysfunction, and CVD [12,13]. Moreover, in the last few years, the association between vitamin D deficiency and DM-related complications especially vascular ones has drawn great attention [8]. However, few studies have examined the role of VDR gene polymorphisms as a potential candidate for occurrence of atherosclerosis-related cardiovascular complications accompanying T2DM. Therefore, this study was conducted to assess the association of 25 (OH) vitamin D and VDR gene (BsmI and FokI) polymorphisms with T2DM and its associated atherosclerosis.

Patients and methods

Patients

The present study was conducted on 45 male patients who were admitted to the Department of Internal Medicine of Medical Research Institute Hospital, Alexandria University, from May 2019 to December 2020. The inclusion criteria were male patients definitely diagnosed with T2DM (according to the American Diabetes Association) above 18 years of age, with manifested cardiovascular complications in the form of ischemic heart disease (stable angina) with or without hypertension, as the diabetic atherosclerotic group.

Patients with acute illness, advanced chronic liver and renal disease (other than diabetic nephropathy), type 1 diabetes mellitus, and who were taking medications known to alter vitamin D metabolism such as calcium and vitamin D supplements were excluded from the study. Females were not included in our study to avoid the effect of menopause on hormonal level and its consecutive effect on vitamin D and calcium metabolism.

Study design

The present study included 90 participants divided into two groups: 45 male patients as the diabetic atherosclerotic group, in addition to 45 healthy male volunteers of comparable age and socioeconomic status as a control group. All data were collected using a predesigned questionnaire that involved sociodemographic data and clinical data including history of other diseases, treatment received, duration of diabetes as well as history of CVD.

Ethical approval

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. This study has been approved by the local Ethics Committee of Medical Research Institute – Alexandria University with approval number E/C S/M R3/2019 at June 2019. All participants provided written consent before enrollment in the study.

Methods

(1) Full clinical examination included complete physical examination with stress on cardiovascular examination, and clinical investigations included 12-lead ECG and

ultrasound 'Doppler' examination using a β-mode ultrasound of the carotid artery for measuring carotid intima-medial thickness (CIMT) to assess the degree of atherosclerosis.

(2) Biochemical and laboratory investigations
Five milliliters of venous blood was withdrawn from each participant after 12 h of fasting and divided into two tubes: plain tube for serum separation and EDTA tube for glycated hemoglobin. The plain tube was used for determination of routine biochemical tests related to atherosclerosis and glycemic control of diabetes, including fasting blood glucose, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), total and ionized calcium, phosphorus, as well as alkaline phosphatase activity. Postprandial blood glucose was measured in blood samples withdrawn from all participants 2 h following intake of a meal. All biochemical tests were performed on an Olympus AU400 automated clinical chemistry analyzer (Olympus Europa SE & CO. KG, Hamburg, Germany). Moreover, 25(OH) vitamin D was measured quantitatively in serum by the use of enzyme-immuno-assay, (ImmunoDiagonstic), 25-OH vitamin D enzyme-immuno-assay kit [14]. Vitamin D status was classified according to the practical guidelines as vitamin D deficiency if its serum level was less than or equal to a cutoff of 50 nmol/l or 20 ng/dl [15]. Moreover, the tube with EDTA was used for glycated hemoglobin (measured on Olympus AU400 automated clinical chemistry analyzer) as well as for molecular analysis.

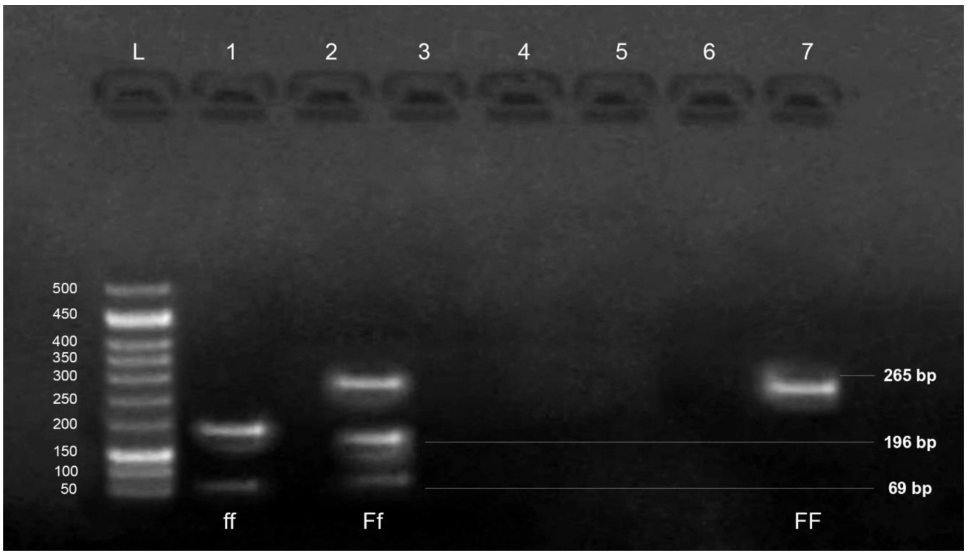
(3) DNA extraction
DNA extraction was done from the EDTA tube, and VDR gene (BsmI and FokI) polymorphism detection was done by PCR-restriction fragment length polymorphism analysis as follows:

(a) DNA was extracted from the buffy coat using QIAamp DNA blood Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions [16].
(b) The extracted genomic DNA was kept at −20°C until time of PCR. The purity and concentration of the stored extracted genomic DNA was checked using the Thermo Scientific NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA), just before performing PCR.
(c) For FokI polymorphism, the following primers were used: forward: 5-AGCTGG CCC TGG CAC TGA CTA TGC TCT-3, and reverse: 5-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3 [17]. The thermal cycler was adjusted as follows: initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 45 s, 58°C for 45 s, followed by 72°C for 45 s, and a final extension at 72°C for 7 min (Applied Biosystems, Waltham, Massachusetts, USA), and then the amplified PCR product of 265 bp was then restricted using the restriction endonuclease Fok1 (New England Biolabs (UK) Ltd, Hitchin, Hertfordshire) at 37°C for 4 h. Digested restriction fragments were separated on 3% agarose gels with ethidium bromide staining and then visualized by ultraviolet transillumination. The FF genotype (homozygote of common allele) showed only one band of 265 bp. The ff genotype (homozygote of infrequent allele) showed two bands of 196 and 69 bp. The heterozygote displayed three fragments of 265, 196, and 69 bp, designated as Ff, as shown in Fig. 1.
(d) For VDR polymorphism BsmI, the following primers were used: forward 5-CAA CC AA GA CTACAA GTA CCG CGT CAG TGA-3, and reverse: 5-AAC CAG CGC GAA GAG GTC AAG GG-3 [17]. The thermal cycler was adjusted as follows: 94°C for 5 min for denaturation followed by 35 cycles at 94°C for 1 min, 64°C for 1 min, and 72°C for 30 s, and a terminal extension at 72°C for 10 min. The amplified PCR product 825 bp was digested with restriction endonuclease BsmI enzyme after incubation at 37°C overnight and electrophoresed in a 3% agarose gel containing ethidium bromide and then visualized by ultraviolet transillumination. Homozygotes for the BsmI restriction site are designated bb, which showed bands at 650 and 175 bp, whereas homozygotes for the absence of the site are designated BB and give one band at 825 bp. The heterozygote type gives three bands at 825, 650, and 175 bp, as shown in Fig. 2.

Statistical analysis

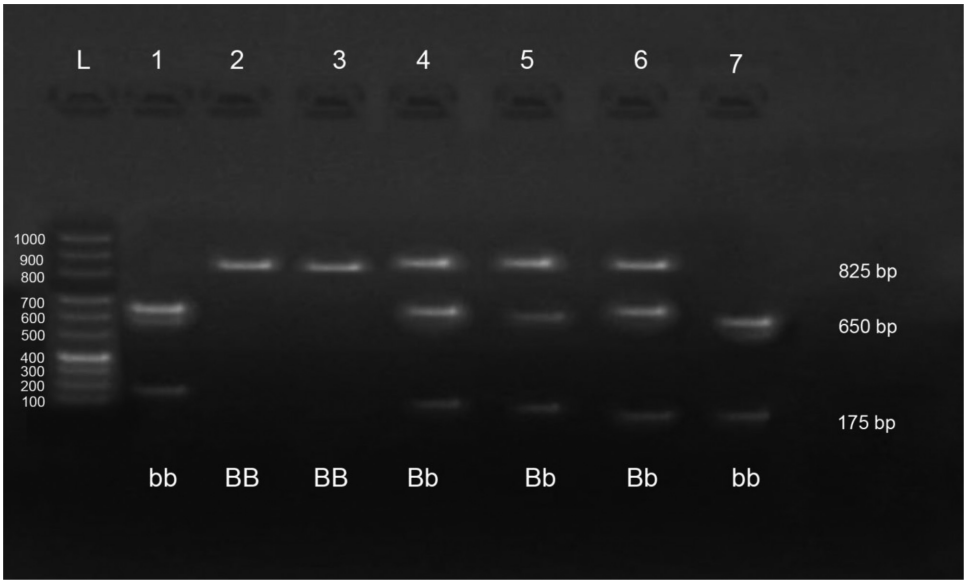
Data were analyzed using the Statistical Package for the Social Sciences Program (SPSS, IBM, USA), version 20.0. In this case–control study, the sample size was calculated using Kolmogorov–Smirnov test. A minimum sample size of 45 is required for cases and 45 for the control to achieve 80% power with a significance level of 0.05. The qualitative variables were summarized by frequency and percentage. The comparison between control and diabetic groups was done using Student t test. χ^2 test was used to test the

Figure 1



Amplified FokI genotype on agarose gel electrophoresis (3%) by PCR-RFLP technique showing: L DNA size marker 50–500 bp. Lane 1: homozygote genotype ff showing two bands at 196 and 69 bp. Lane 2: heterozygote genotype Ff showing three bands at 265, 196, and 69 bp. Lane 7: homozygote FF showing one band at 265 bp. RFLP, restriction fragment length polymorphism.

Figure 2



Amplified BsmI genotypes on agarose gel electrophoresis (3%) by PCR-RFLP technique showing the following: L DNA size marker 100–1000 bp. Lanes 1 and 7: homozygote genotype bb showing two bands at 650 and 175 bp. Lanes 2 and 3: homozygote (BB) showing one band at 825. Lanes 4, 5, and 6: heterozygote genotype (Bb) showing three bands at 825, 650, and 175 bp, respectively. RFLP, restriction fragment length polymorphism.

statistical comparison of qualitative variables between the studied groups (diabetic and controls). Quantitative variables were expressed as median and range and statistically tested using Mann–Whitney test for comparison between the two groups. Kruskal–Wallis test was used for comparing between more than two groups, and Spearman's correlation was done to correlate the quantitative variables with each other. *P* value of less than or equal to 0.05 was considered significant for all comparisons.

Genotype-specific odds ratios (ORs) were computed using logistic regression analysis under codominant, dominant, and recessive genetic models. The estimated

risk associated with these models were adjusted by the most common risk factors of atherosclerosis studied in our patients, namely, BMI, CIMT, and TC level.

Results

A total of 90 male participants were included in our study. The mean age of the diabetic atherosclerotic group (diabetic group; n=45) was 56.0±10.1 years, whereas of the control group (n=45) was 55.0±7.1 years, without significant difference between the two groups regarding age or sex. The diabetic group included 31 (68.9%) patients with hypertension and 14 (31.1%) patients without. The mean value of CIMT was significantly higher in the diabetic atherosclerotic group compared with the controls (P=0.000). Regarding findings by Doppler ultrasound, 36 (75%) patients with diabetes had plaques and calcification, whereas nine (25%) patients did not show plaques or calcification (Table 1).

The data presented in Tables 2 and 3 show significantly higher mean values of glycemic markers in the diabetic atherosclerotic group when compared with controls (P=0.000). In addition, it shows significantly higher mean levels of TC and LDL-C in the diabetic atherosclerotic group than controls (P=0.03 and 0.01, respectively). Moreover, significantly lower mean levels of ionized calcium and higher serum alkaline phosphatase were detected in the diabetic atherosclerotic group compared with controls (P=0.000 and 0.001, respectively). Meanwhile, a significantly lower mean level of 25(OH) vitamin D was observed in the diabetic atherosclerotic group than controls (P=0.000). Vitamin D deficiency was observed in most diabetic patients [24 (53.3%)], whereas only four (8.9%) controls were deficient by definition, with a statistically significant difference (P<0.01).

On comparing the genotypes and allele frequency of FolkI and *BsmI genes* between the studied groups, it was found that the genotype distribution pattern of FolkI gene among the diabetic atherosclerotic group was statistically significantly different from that of controls, where the diabetic atherosclerotic group had a higher frequency of both the homo-infrequent genotype 'ff' (P=0.000) and a lower frequency of the homozygous genotype 'FF' than the control group (P<0.001). However, there were no statistical significant differences regarding the frequency of BsmI genotypes (BB, Bb, and bb) (P=0.756) as well as allele frequency (B and b) (P=0.551) between diabetic atherosclerotic group and controls (Table 4). The genotype distribution patterns of FolkI and *BsmI genes* in the present study were in agreement with Hardy–Weinberg equilibrium (P=0.848 for FolkI and P=0.632 for BsmI).

There was a significantly higher level of LDL-C as well as TC in ff genotype compared with FF genotype (P=0.01 and 0.04, respectively), as shown in Table 5. Phosphorus showed a statistically significant difference among the three FolkI genotypes (P=0.001) with higher levels in FF genotype than ff genotype (P=0.03) as well as Ff genotype (P=0.005) (Table 5).

Table 1 Comparison of clinical parameters of the studied groups

Items	Diabetic group (N=45) [n (%)]		Control group (N=45) [n (%)]
Age (years)			
Mean±SD	56±10.1		55.7±7.8
P value		0.542	
Hypertension			
Yes	31 (68.9)		0
No	14 (31.1)		45 (100)
P value		0.004**	
CIMT (mm)			
Mean±SD	1.01±0.4		0.55±0.15
P value		0.000*	
BMI (kg/m^2)			
Mean±SD	23.36±1.39		24.38±0.75
P value		0.254	
Plaques and calcification			
Yes	36 (75)		0
No	9 (25)		45 (100)
P value		0.013**	

CIMT, carotid intima-medial thickness. *Significant differences between two groups at P value less than 0.05 using Student t test.
**Significant differences between two groups at P value less than 0.05 using χ^2 test

Regarding BsmI genotypes, there were statistically significant differences in ionized calcium and phosphorus among the three genotypes (BB, Bb, and bb) (P=0.048 and 0.001, respectively), being the lowest in bb genotype compared with the other genotypes (Table 6).

In the present study, there were significant correlations regarding the biochemical markers and different genotypes and alleles of FolkI and BsmI genes in the whole studied group as well as in the diabetic atherosclerotic group, as illustrated in Tables 7 and 8, respectively, where no significant difference was found in serum vitamin D level among the different genotypes of the two studied VDR genes. Furthermore, we did not find any significant correlation between vitamin D level and the different VDR genotypes neither with the studied biochemical markers in the diabetic atherosclerotic group except for total and ionized calcium (P=0.01 for both).

The data represented in Table 9 demonstrate the association of VDR genetic variants (FolkI and

Table 2 Statistical significance of laboratory markers of glycemic control of the studied groups

Items	Diabetic group (N=45)		Control group (N=45)
Fasting serum glucose (mg/dl)			
Mean±SD	203.53±72.13		87.35±9.4
P value		0.000*	
Postprandial serum glucose (mg/dl)			
Mean±SD	190.025±76.33		108.15±15.39
P		0.000*	
HbA1c (%)			
Mean±SD	9.6±2.49		4.63±0.37
P value		0.000*	

HbA1c, glycated hemoglobin. *Significant differences between two groups at P value less than 0.05 using Student t test.

Table 3 Statistical analysis regarding some biochemical markers between diabetic atherosclerotic and controls

Variables	Diabetic group	Control group	Mann–Whitney (U) (P values)
Total calcium (mg/dl)			
Median (minimum–maximum)	9.3 (8.1–10)	9.4 (8.9–9.9)	0.620
Ionized calcium (mg/dl)			
Median (minimum–maximum)	1.17 (0.8–1.3)	1.2 (1.1–1.3)	0.000*
Vitamin D (ng/ml)			
Median (minimum–maximum)	18 (0.09–80)	52 (19–90)	0.000*
LDL-C (mg/dl)			
Median (minimum–maximum)	126 (10.2–311)	109 (65.8–131.4)	0.010*
SAP (U/l)			
Median (minimum–maximum)	109 (40–150)	90 (70–113)	0.001*
Phosphorus (mg/dl)			
Median (minimum–maximum)	3.4 (1.9–4.7)	3.5 (2.5–4.4)	0.480
Cholesterol (mg/dl)			
Median (minimum–maximum)	200 (96–419)	188 (142–200)	0.030*

CIMT, carotid intima-medial thickness; LDL-C, low-density lipoprotein cholesterol; SAP, serum alkaline phosphatase. *Significant differences between two groups at P value less than 0.05 using Mann–Whitney nonparametric test (U).

Table 4 Genotype distribution and allele frequency of the vitamin D receptor gene variants (FolkI and BsmI) in the studied groups

	Diabetics (N=45) [n (%)]	Control (N=45) [n (%)]	χ^2	P values
FolkI				
FF	18 (40)	27 (60)	15.027*	0.001*
Ff	10 (22.2)	16 (35.6)		
ff	17 (37.8)	2 (4.4)		
F allele	46 (51.1)	70 (77.8)	13.966*	<0.001*
f allele	44 (48.9)	20 (22.2)		
Bsm1				
BB	11 (24.4)	12 (26.7)		
Bb	22 (48.9)	24 (53.3)	0.559	0.756
bb	12 (26.7)	9 (20)		
B allele	44 (48.9)	48 (53.3)	0.356	0.551
b allele	46 (51.1)	42 (46.7)		

*Statistically significant at P value less than or equal to 0.05 using Pearson χ^2.

Table 5 Statistical analysis of the variables affecting atherosclerosis among the three different (FokI) genotypes (ff, Ff, and FF)

Variables	ff	Ff	FF	Kruskal–Wallis test (*P* values)
Total calcium (mg/dl)				
Median (minimum–maximum)	9 (8.5–9.9)	9.2 (8.5–9.8)	9.4 (8.1–10)	0.09
Ionized calcium (mg/dl)				
Median (minimum–maximum)	1.2 (0.8–1.24)	1.2 (0.9–1.27)	1.2 (1.1–1.3)	0.28
Vitamin D (ng/ml)				
Median (minimum–maximum)	36 (5.4–70)	30 (0.09–90)	35 (0.09–90)	0.927
CIMT(mm)				
Median (minimum–maximum)	1 (0.49–1.4)	0.5 (0.35–1.2)	0.7 (0.2–2.1)	0.079
HbA1c (%)				
Median (minimum–maximum)	9.4 (5.2–11.4)	5.8(5.1–12.4)	5.9 (4.8–16.2)	0.197
LDL-C (mg/dl)				
Median (minimum–maximum)	155 (116–226)	110 (65.8–311)	108.6 (10.2–220)	(0.011) * FF Vs ff (0.01)**
SAP (U/l)				
Median (minimum–maximum)	113 (88–120)	90 (40–125)	93.5 (68–150)	0.132
Phosphorus (mg/dl)				
Median (minimum–maximum)	3 (2.7–3.6)	3.4 (1.9–4)	3.6 (2.5–4.7)	0.001* FF vs. ff (0.03)** FF vs. Ff (0.005)**
Cholesterol (mg/dl)				
Median (minimum–maximum)	224 (185–310)	194 (142–419)	187.5 (96–339)	(0.028)* FF vs. ff (0.04)**

CIMT, carotid intima-medial thickness; HbA1c, glycated hemoglobin; LDL, low-density lipoprotein cholesterol; SAP, serum alkaline phosphatase. *Significant difference at *P* values less than 0.05, using Kruskal–Wallis test. **P* values less than 0.05 were considered statistically significant for pairwise comparison.

Table 6 Statistical analysis of the variables affecting atherosclerosis among the three different (BsmI) genotypes (bb, Bb, and BB)

Variables	Bb	Bb	BB	Kruskal–Wallis test (*P* values)
Total calcium (mg/dl)				
Median (minimum–maximum)	9.3 (8.1–10)	9.3 (8.5–10)	9.4 (8.5–9.9)	0.90
Ionized calcium (mg/dl)				
Median (minimum–maximum)	1.17 (0.9–1.26)	1.2 (1.06–1.3)	1.21 (0.8–1.31)	0.048*
Vitamin D (ng/ml)				
Median (minimum–maximum)	03 (2.4–90)	35 (0.09–80)	36 (0.09–90)	0.908
CIMT(mm)				
Median (minimum–maximum)	0.54 (0.27–2.1)	0.8 (0.35–1.6)	0.8 (0.35–1.5)	0.359
HbA1c (%)				
Median (minimum–maximum)	8 (4–12.4)	5.75 (4.8–16.6)	6 (4.8–11.8)	0.73
LDL-C (mg/dl)				
Median (minimum–maximum)	108 (18.5–208)	117 (10.2–311)	116 (64–226)	0.863
SAP (U/l)				
Median (minimum–maximum)	97 (71–122)	90 (40–125)	95 (70–150)	0.26
Phosphorus (mg/dl)				
Median (minimum–maximum)	3 (1.9–3.8)	3.6 (1.9–4.4)	3.5 (2.7–4.7)	0.001*
Cholesterol (mg/dl)				
Median (minimum–maximum)	187 (107–318)	190 (96–419)	194 (128–310)	0.774

CIMT, carotid intima-medial thickness; HbA1c, glycated hemoglobin; LDL, low-density lipoprotein cholesterol; SAP, serum alkaline phosphatase. *Significant difference at *P* values less than 0.05, using Kruskal–Wallis test.

BsmI) with atherosclerosis risk in DM using logistic regression analysis. After adjusting for the confounding factors, including BMI, CMT, and TC, we found that the heterozygous (Ff) genotype and the f allele were associated with atherosclerosis risk in DM (P=0.002, P<0.001), with a crude OR=12.750 (2.621–62.020) and 3.348 (1.754–6.390), respectively. Moreover, the homozygous (bb) genotype and the b allele were associated with atherosclerosis in DM (P=0.04 and 0.01), with an adjusted OR=6.122 (1.202–30.078) and 4.712 (1.457–15.45), respectively.

Discussion

The incidence of coronary artery diseases (CAD) is believed to increase along with age; however, it occurs in younger age patients with diabetes [18]. Moreover, it was found that a large proportion of diabetic patients

Table 7 Correlation between vitamin D receptor gene variants (FokI and BsmI) genotypes, alleles, and biochemical markers among the whole study sample

Items	Whole study sample	
	Correlation coefficient	*P* value
Genotype FF and cholesterol	–0.246	0.020*
Genotype FF and phosphorus	–0.422	0.000*
Allele F and cholesterol	–0.234	0.027*
Allele F and phosphorus	0.210	0.047*
Allele f and cholesterol	0.215	0.042*
Allele f and phosphorus	-0.415	0.000*
Allele B and ionized calcium	0.246	0.019*
Allele f and phosphorus	0.340	0.001*

*Significant correlation at *P* less than 0.05 using Spearman's correlation.

Table 8 Correlation between vitamin D receptor gene variants (FokI and BsmI) genotypes, alleles, and biochemical markers among the diabetic atherosclerotic group

Items	Diabetic atherosclerotic group	
	Correlation coefficient	*P* value
Genotype FF and cholesterol	–0.357	0.016*
Genotype F and LDL	–0.415	0.005*
Genotype FF and phosphorus	–0.434	0.003*
Allele F and FBS	0.327	0.028
Allele F and calcium	0.336	0.024*
Allele f and cholesterol	0.347	0.020*
Allele f and LDL	0.385	0.009*
Allele f and phosphorus	-0.436	0.003*
Allele B and ionized calcium	0.338	0.023*
Allele B and phosphorus	0.369	0.013*

LDL, low-density lipoprotein. *Significant correlation at *P* value less than 0.05 using Spearman's correlation.

Table 9 Association of vitamin D receptor gene variants (FokI and BsmI) with atherosclerosis risk in patients with type 2 diabetes mellitus adjusted by the most common risk factors of atherosclerosis studied; BMI, carotid intimal thickness, and total cholesterol level

	Diabetics (*N*=45) [*n* (%)]	Control (*N*=45) [*n* (%)]	Crude OR		Adjusted OR	
			OR (95% CI: LL–UL)	*P*	OR (95% CI: LL–UL)	*P*
Folk						
FF®	18 (40)	27 (60)	–	–	–	–
Ff	10 (22.2)	16 (35.6)	0.938 (0.38–2.52)	0.898	4.252 (0.49–36.96)	0.190
ff	17 (37.8)	2 (4.4)	12.750 (2.62–62.02)	0.002*	8.378 (0.57–124.12)	0.122
Ff+ff	27 (60)	18 (40)	2.250 (0.97–5.23)	0.060	2.531 (0.52–12.23)	0.248
F allele®	46 (51.1)	70 (77.8)	–	–	–	–
f allele	44 (48.9)	20 (22.2)	3.348 (1.75–6.39)	<0.001*	2.526 (0.77–8.27)	0.126
Bsm1						
BB®	11 (24.4)	12 (26.7)	–	–	–	–
Bb	22 (48.9)	24 (53.3)	1.000 (0.367–2.724)	1.000	1.397 (0.20–9.54)	0.733
bb	12 (26.7)	9 (20)	1.455 (0.443–4.782)	0.537	6.012 (1.20–30.08)	0.040*
Bb+bb	34 (75.6)	33 (73.3)	1.124 (0.436–2.901)	0.809	4.987 (0.78–31.92)	0.090
B allele®	44 (48.9)	48 (53.3)	–	–	–	–
b allele	46 (51.1)	42 (46.7)	1.195 (0.666–2.145)	0.551	4.712 (1.46–15.45)	0.010*

CI, confidence interval; .L, lower limit; OR, odds ratio; ®, reference; UL, upper limit. *Statistically significant at *P* value less than or equal to 0.05, using univariate and multivariate logistic regression analysis test.

die owing to CVD, mainly caused by advanced atherosclerosis [19].

Studies on the association between SNPs of *VDR* gene and T2DM and CVD have been inconsistent, and actual differences have been observed between SNPs and the ethnic population analyzed [20–23]. However, studies on their association with CAD among diabetics are lacking. The aim of this study was, therefore, to examine the association of the two common variants of VDR gene and T2DM with associated CAD in a cohort of Egyptian patients.

In the present study, we found that the atherosclerotic T2DM had significant lower vitamin D levels compared with controls, suggesting the possible role of the low vitamin D in causing CAD in patients with T2DM. Similarly, Ma *et al.* [24] found that patients with T2DM had a lower level of 25(OH) vitamin D when compared with healthy controls. Moreover, they found that vitamin D levels were the lowest in patients with T2DM with CAD. This was also in line with other earlier studies [25–27].

Although the exact mechanism by which vitamin D deficiency contributes to the development of cardiovascular complications in patients with T2DM is unclear; a direct link between vitamin D deficiency and cardiovascular events has been documented in many studies [28–30].

Vitamin D involvement in atherosclerosis was suggested to occur mainly through two different

pathways based on both human and experimental studies, where human studies showed its inverse correlation to coronary artery calcification [31].

Although experimental studies revealed the role of vitamin D deficiency in enhancing chronic inflammation in the arterial wall [32,33], the role of vitamin D in patients with T2DM with CAD remains conflicting [24].

As vitamin D is known to exert its actions through VDR, variations in the VDR gene could alter vitamin D signaling process that could be associated with a wide variety of diseases.

Various studies have been conducted in different populations to assess the possible association of VDR gene polymorphisms with T2DM [34,35] and with the CAD [36–38]. In the present study, we selected FokI and BsmI polymorphisms for combined analysis as they were observed from literature to be independently associated with T2DM.

In the current study, there was no statistically significant difference regarding genotype and allele distribution of *BsmI gene* between T2D patients with CAD and controls. However, carriers of the minor b allele were found to be significantly associated with higher atherosclerosis risk, with adjusted OR=6.012 (P=0.040). Other previous studies [6,39] did not find any significant differences regarding VDR BsmI genotypes among controls and patients with T2DM only. In addition, a recent study of Egyptian population by Gendy *et al.* [40] showed a similar nonsignificant difference in BsmI genotype distribution between T2DM and controls. However, in contrast to our results, Ferrarezi *et al.* [41], who conducted a prospective cohort study on 3137 patients with type 2 diabetes, demonstrated an increased risk of CAD among carriers of the B allele. The variation in the results from different studies including ours could be explained by different ethnicities of the studied populations, different sample sizes, possible exposure to environmental factors, as well as different methods used for polymorphism analysis.

Notably, a meta-analysis done by Alizadeh and colleagues did not find any associations between the four common FokI, TaqI, BsmI, and ApaI polymorphisms of the VDR gene and the risk of CAD in Caucasians, and in Asians, however, they recommended that their results should be interpreted with caution owing to the small number of their included studies, in addition to the relatively small sample size of some of these studies [42].

In the present study, regarding FokI polymorphism (rs2228570, formerly known as rs10735810). We found statistically significant higher ff genotype and f allele frequency of *FokI gene* in patients with T2DM with CAD compared with controls. Moreover, we found that the minor allele (f) is associated with a higher risk of CAD in patients with T2D, with an OR=12.750 (P=0.002). A study done by Maia *et al.* [12] found no association between FokI polymorphism and T2DM among postmenopausal women. However, they suggested an association between the recessive model of FokI polymorphism and CAD in postmenopausal women with T2DM as a protective factor.

Moreover, another Chinese study carried out by Ma and colleagues on VDR polymorphism association with CAD in type 2 diabetics revealed that the prevalence of heterozygous infrequent (Ff) and minor allele (f) for FokI polymorphism was higher in patients with T2D with or without CAD compared with healthy controls (Ff: OR=4.27, f: OR=2.49, and Ff: OR=3.38, f: OR=2.41, respectively) [24]. Although some in vitro studies showed that the FokI polymorphism causes a frameshift mutation in the VDR protein, with the genetic variant (f) having longer and less effective VDR protein variant than the shorter VDR protein encoded by the F allele, the functional activity of this SNP is still not fully understood [12].

Furthermore, in our study, there was no significant association observed between neither VDR FokI nor BsmI polymorphism and serum level of 25(OH) vitamin D among the different VDR variants in T2DM with CAD. In contrast to our results, Ma *et al.* [24] observed a significant association of VDR FokI and TaqI polymorphism with serum vitamin D level and susceptibility to T2DM development. This could be explained by measurement of different forms of vitamin D, as we measured 25(OH) vitamin D whereas they measured $1,25(OH)_2$ vitamin D.Lastly, previous studies have shown an association between the FokI polymorphism and serum lipid parameters as cardiovascular risk factors [6,43]. One of them was an Egyptian study that investigated this association in a cohort of patients with T2DM with and without metabolic syndrome, and they revealed higher plasma TC, TG, and LDL-C with lower HDL-C in TT (ff) genotype than CC (FF) genotype carriers of the VDR 2228570 C>T polymorphism [6]. Our

results go in line with these previous studies as it demonstrated that carriers of the homozygous CC (FF) genotype of the FokI polymorphism have significantly lower levels of TC and LDL-C compared with the homozygous infrequent genotype (ff) (P=0.04 and 0.01, respectively) (Table 5). Moreover, we found an additional significant positive correlation between both allele (f) with serum levels of TC and LDL-C in the diabetic atherosclerotic patient group (P=0.02 and 0.009, respectively) (Table 8). This association could be explained by the fact that vitamin D-activated VDR may regulate several hundreds of genes by binding to vitamin D-responsive elements, thus affecting several biological activities. Interestingly, it was found that VDR activation increases the expression of one of the core enzymes for cholesterol metabolism, namely, cholesterol 7α-hydroxylase in hepatocytes, which in turn may lead to a reduction in cholesterol levels. Thus, this could be one of the possible mechanisms by which VDR influences lipid profile and in turn predispose to atherosclerosis risk in diabetic patients [44].

Although the current study was limited by the sample size, it shed light on FokI polymorphism and its potential association with cardiovascular risk in patients with T2DM, in addition to the added risk of low vitamin D levels in such patients, a finding to be further validated by future studied on a larger sample of Egyptian population, including female participants and more diabetic atherosclerotic participants and other participants without atherosclerosis.

Conclusion

The presence of the f allele of FokI polymorphism of the VDR gene could increase the atherosclerosis risk in Egyptian patients with T2DM, not through vitamin D deficiency but through influencing lipid profile and metabolism. Our results found that b allele of BsmI VDR gene may have a role in the development of atherosclerosis in Egyptian patients with T2DM. Moreover, vitamin D deficiency may be another contributing factor that increases the atherosclerosis risk in T2DM independent of the variants of VDR gene. However, it is recommended that further investigation is necessary to evaluate the role of vitamin D deficiency in CVD among type 2 diabetic adults and to determine possible mechanisms of CVD prevention in T2DM by vitamin D supplementation.

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Conflicts of interest

There are no conflicts of interest.

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