



A Correlative Study of Vitamin D Receptor Variants Fok1, Apa1, Bsm1, and Taq1 Polymorphism and Vitamin D Deficiency with Higher Risk Ratio of Coronary Artery Disease in Female Population

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Abstract

No studies are available on single nucleotide polymorphisms (SNPs) of the VDR gene in association with coronary artery disease (CAD) in women. The study aimed to investigate the association between SNPs in the VDR regions Fok I (rs10735810), Bsm I (rs1544410), Apa I (rs7975232), and Taq I (rs731236) and its associated risk of CAD in female patients. This prospective case-control study included 100 women diagnosed with CAD and age-matched healthy controls. The association between 1,25-dihydroxy vitamin D levels and VDR polymorph was assessed. In cases, the genotype 'ff' was reduced (14%) in co-dominant (OR, 0.13; CI, 0.03–0.65, $p=0.0018$) and recessive (OR, 0.11; CI, 0.02–0.54, $p=0.0018$) models. The 'Ff' genotype frequency in the over-dominant model was significantly higher (15%) in some cases (OR, 2.58; CI, 1.04–6.41, $p=0.031$). The frequency of genotype 'Bb' was increased (14%) in co-dominant (OR, 2.24; CI, 0.83–6.01, $p=0.029$) and over-dominant (OR, 2.65; CI, 1.01–6.94, $p=0.035$) cases. Women with CAD had a significantly lower frequency (14%) of the 'bb' genotype in both co-dominant (OR, 0.48; CI, 0.19–1.17, $p=0.029$) and recessive models (OR, 0.40; CI, 0.17–0.95, $p=0.039$). VDR genotypes such as Aa+aa, BB+Bb, Aa, Bb, FF+Ff, Tt+tt, aa, Ff, and tt were associated with an increased risk of CAD, and serum vitamin D levels were lower. Alleles 'a', 'B', and 't' increase CAD risk in women. Subjects with genotypes (BB/Bb), (Tt/tt), and (FF) have a 2-fold, 2.6-fold, and 3-fold increased risk of low 25(OH)D3 levels. We found a statistically significant association between VDR polymorphisms and 25(OH)D3 levels for Taq I and Bsm I genotypes in females with CAD.

Keywords

Coronary artery disease, vitamin D receptor, 1,25-dihydroxyvitamin D, gene polymorphism

Introduction

The distribution of coronary artery disease (CAD) risk factors differs between men and women, and failure to account for these

differences may have contributed to the belief that women are at lower risk of CAD than men. There is an urgent need to better understand the presentation of cardiac symptoms in women in order to facilitate diagnosis and improve quality of life.

Despite the importance of CAD for women, there is a persistent misconception that CAD is a man's disease. Contributing to this notion is the observation of differences in incidence rates according to age; the incidence of CAD in women is lower than men but rises steadily after the fifth decade.^[1] The clinical presentation of CAD in women ranges from asymptomatic to severe unstable angina to myocardial infarction. Women have a worse prognosis and a worse outcome than men after a myocardial infarction, percutaneous coronary intervention, and coronary artery bypass grafting.

CAD kills one in every three women, regardless of race or ethnicity.^[2] The INTERHEART study revealed that women have their first presentation of coronary heart disease approximately 10 years later than men, most commonly after menopause.^[3] Despite this delay in onset, mortality among women is increasing faster than among men. Indian studies have found a high burden of conventional risk factors such as diabetes, hypertension, and metabolic syndrome.^[4,5]

According to some studies, the heritability of CAD is estimated to be between 40% and 60%, implying that genetic factors may play a significant role in CAD susceptibility.^[6]

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The vitamin D receptor (VDR) is a crucial signal transduction molecule for vitamin D. VDR is a vital signal transduction molecule for vitamin D and has been associated with an increased risk of CAD. Van Schooten et al. observed that the rs1544410 polymorphism was linked to the severity of CAD.^[7] Ortlepp et al.^[8] followed up with a small group study confirming the earlier findings in 2001, but no association was found in a larger population study published in 2003.^[9] In the next decade, various studies were conducted not only in the rs1544410 polymorphism but also in other VDR polymorphisms among two sex groups; however, the conclusions were inconsistent.^[10] The VDR gene is located on chromosome 12q13.1 and has four common SNPs: rs2228570 (Fok I F/f in exon 2), rs1544410 (Bsm I B/b in intron 8), rs7975232 (Apa I A/a in intron 8), and rs731236 (Taq I T/t in exon 9).

There were no studies on different SNPs of the VDR gene in association with CAD in women populations.

Materials and Methods

Study design: A prospective case-control study was conducted between April 2017 and March 2019.

Study Population: A total of 100 women with CAD were included, as well as 100 age-matched individuals without CAD as controls.

Inclusion criteria: Women patients aged 25–65 years with clinically diagnosed CAD were included, along with age-matched healthy women.

Exclusion criteria: Patients with chronic disorders affecting vitamin D metabolism, patients on vitamin D/parathormone medications, pregnant or breastfeeding women, and women with chronic diseases associated with VDR gene polymorphisms were excluded.

1. Study data

All subjects were thoroughly examined in terms of clinical variables, lipid profile, high sensitivity C-reactive protein (hsCRP), serum creatinine, 25-hydroxy vitamin D levels, and genotyping using the PCR-RFLP method. The relationship between 25-hydroxy vitamin D levels and Apa I, Fok I, Bsm I, and Taq I polymorphisms was investigated.

2. Biochemical assay

Blood was drawn (12 hour fasting), centrifuged, and the serum was tested for 25-hydroxy vitamin D, lipid profile, creatinine, and CRP. The serum 25-hydroxy vitamin D levels were determined using high-performance liquid chromatography (UHPLC, Thermo Scientific, USA).

3. Nucleic acid extraction

For purposes of DNA extraction, 5 ml of blood was collected in EDTA tubes, and white blood cells were isolated using lysis solution (blood lymphocyte buffer: $\text{KHCO}_3 + \text{NH}_4\text{Cl} + \text{EDTA}$), then frozen at -80°C . In brief, genomic DNA was extracted from blood samples using a phenol chloroform-based manual purification method. At

40°C , red blood cells were extracted using RBC lysis solution. WBC lysis solution was used to lyse white blood cells for 60 minutes at 550°C . Protein precipitate was extracted from lysed WBCs using a 24:1 phenol-chloroform mixture. Impurities in DNA were removed using 70% ethanol, and pure DNA was obtained after air-drying the solution at room temperature for 10 minutes. Ice-cold ethanol was used to precipitate genomic DNA in an aqueous phase. DNA was mixed with 100 μl of Tris-EDTA (TE) buffer and heated at 50°C for 10 minutes, and quantity measured using a NanoDrop™ 2000/2000c Spectrophotometer (Thermo Scientific, USA). DNA was checked using agarose gel electrophoresis, and total DNA was stored at -20°C .

4. PCR-RFLP genotyping of VDR gene

Genotyping was done using polymerase chain reaction (PCR) followed by the restriction fragment length polymorphism (PCR-RFLP) method.

PCR was carried out in a final volume of 50 μl containing 100 ng of DNA, 0.25 mm dNTPs, 1.5 mm MgCl_2 , 100 ng of primer, 1xPCR buffer, and 0.02 U TaqDNA polymerase (Sigma-Aldrich Chemicals Private Limited, Bangalore, India). The amplification conditions for each PCR were 95°C for 5 minutes, followed by 35 cycles of 95°C for 60 seconds, 55°C for 60 seconds, and 72°C for 60 seconds, along with a final extension of 72°C for 10 minutes.

Primer 3 was used to create primers for the amplification of Bsm I, Apa I, Fok I, and Taq I SNPs, which were then tested for specificity using BLAST. The DNA sequences were obtained from the Genbank databases. The genotypes of each selected SNP were determined using different sizes of restriction fragments obtained on agarose gel after restriction digestion (at 370°C for 2–4 hours) of related amplicons using specific enzymes (Table 1).

Agarose gel electrophoresis was used to check the DNA integrity and RFLP fragments. DNA was visualized under ultraviolet (UV) light using a UV trans-illuminator and agarose gel stained with ethidium bromide. The gel documentation system was used to document the DNA bands in an agarose gel (Syngene, US).

The Hardy Weinberg genetic equilibrium principle was used to adapt genotype spreading.

For the Fok I polymorphism, PCR products with an undigested 265 bp band size were genotyped as dominant 'FF' homozygote, digested bands at 169 bp and 96 bp as recessive 'ff' homozygote, and those with 265 bp, 169 bp, and 96 bp bands as 'Ff' heterozygote.

For Bsm I polymorphism, PCR products with an undigested 800 bp band size were genotyped as dominant 'BB' homozygote, digested bands at 650 bp and 150 bp were genotyped as recessive 'bb' homozygote, and those with 800 bp, 650 bp, and 150 bp bands were genotyped as 'Bb' heterozygote.

For Taq I polymorphism, PCR products with 494 bp and 251 bp band sizes were genotyped as dominant 'TT' homozygote, digested bands with 494 bp, 293 bp, 251 bp, and 201 bp bands were genotyped as recessive 'Tt' heterozygote, and those with 293 bp and 251 bp bands were genotyped as 'tt' homozygote.

For Apa I polymorphism, PCR products with an undigested 740 bp band size were genotyped as dominant 'AA' homozygote,

Table 1 - Primers used in the study and RFLP fragment sizes for VDR SNP genotyping.

VDR gene Restriction site	Primers	RFLP fragments (bp)	Restriction enzyme
31236	F:CAGAGCATGGACAGGGAGCAAG R:GCAACTCCTCATGGCTGAGGTCTCA	494, 293, 251, 201	Taq I
1544410	F:CAACCAAGACTACAAGTACCGCGTCAGTGA R:AACCAGCGGGAAGAGGTCAAGGG	800, 650, 150	Bsm I
7975232	F:CAGAGCATGGACAGGGAGCAA R:TCATGGCTGAGGTCTCAAGGG	740, 520, 220	Apa I
10735810	F:AGCTGGCCCTGGCACTGACTCTGCTCT R:ATGGAAACACCTTGCTTCTTCTCCCTC	96, 169, 265	Fok I

digested bands at 520 bp and 220 bp were genotyped as recessive 'aa' homozygote, and those with 740 bp, 520 bp, and 220 bp bands were genotyped as 'Aa' heterozygote.

5. Statistics

The current study estimated the effect of allele contrast, the contrast of homozygotes, and the contrasts for the dominant and recessive models. The defined model for Taq I, Apa I, Bsm I, and Fok I is as follows, respectively: Taq I; dominant model (tt + Tt vs. TT), recessive model (tt vs. Tt + TT), and allelic model (t vs. T); Apa I; dominant model (aa + Aa vs. AA), recessive model (aa vs. Aa + AA), and allelic model (a vs. A); Bsm I; dominant model (bb + Bb vs. BB), recessive model (bb vs. Bb + BB), and allelic model (b vs. B); Fok I; dominant model (ff + Ff vs. FF), recessive model (ff vs. Ff + FF), and allelic model (f vs. F).

Statistical analyses were carried out using software SPSS for Windows version 20.0 (IBM, USA). To compare categorical parameters among two or more groups, the chi-square test and the Fisher Exact test were used. The Student t-test is used to compare continuous variables between two groups. The chi-square test and the odds ratio (OR) with 95% confidence intervals (CI) test were used to compare VDR genotypes between groups. Logistic regression analysis was used to investigate the relationship between VDR polymorphisms and the risk of low serum vitamin D levels. *P*-values of 0.05 were considered statistically significant (Table 2).

Results

The patient group's mean age was 51.65±17.5 years with a mean BMI of 25.2±6.8 kg/m². There was a significant difference observed between the cases and control regarding SBP, DBP, vitamin D levels, cholesterol levels, and triglyceride levels (Table 3).

Cases of women with CAD showed significantly reduced frequency (14%) of 'ff' genotype in co-dominant (OR, 0.13; CI, 0.03–0.65, *p*=0.0018) and recessive models (OR, 0.11; CI, 0.02–0.54, *p*=0.0018) (Table 3). However, 'Ff' genotype frequency in the over-dominant model showed significantly increased levels (15%) in female cases than in gender-matched control subjects (OR, 2.58; CI, 1.04–6.41, *p*=0.031). Allele frequency analysis did not show significant change in female cases than in controls (OR, 0.67; CI, 0.37–1.23; *p*=0.21).

Female cases with CAD showed significantly reduced frequency (14%) of 'bb' genotype in co-dominant (OR, 0.48; CI, 0.19–1.17,

Table 2 - Demographics, clinical, and biochemical variables in cases and control subjects.

Demographics	Controls (Mean±SD)	Cases (Mean±SD)	<i>p</i> -value
Age	49.5±16.5	51.65±17.5	>0.05
SBP	115.5±7.5	116.5±8.5	0.0001
DBP	77.8±9.5	89.5±10.9	0.0001
25-hydroxy vitamin D (ng/ml)	51.52±11.5	20.2±8.5	0.0001
Fasting blood sugar (mg/dl)	113.2±10.5	119.8±11.5	>0.05
Total cholesterol (mg/dl)	185.5±20.8	255.5±50.5	0.0001
Triglycerides (mg/dl)	99.7±19.5	240.31±52.5	0.0001
HDL-C (mg/dl)	49.6±8.5	42.18±10.5	0.0001
LDL-C (mg/dl)	58.5±12.5	131.5±45.8	0.0001
VLDL-C (mg/dl)	21.3±10.6	49.9±11.2	0.0001
hsCRP (mg/L)	0.61±1.5	6.22±2.5	>0.05
Serum creatinine (mg/dl)	0.85±0.075	0.92±0.08	>0.05

SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol; CRP: c-reactive protein.

p=0.029) and recessive models (OR, 0.40; CI, 0.17–0.95, *p*=0.039) (Table 4). However, 'Bb' genotype in co-dominant (OR, 2.24; CI, 0.83–6.01, *p*=0.029) and over-dominant (OR, 2.65; CI, 1.01–6.94, *p*=0.035) models showed significantly increased frequency (14%) in female cases than in control subjects. Allele frequency analysis did not show significant change in female cases than in controls (OR, 0.72; CI, 0.42–1.23; *p*=0.27).

Female cases with CAD did not show significant change of genotype and allele frequencies of Apa I of VDR gene compared to control subjects (Table 5).

Female cases with CAD did not show significant change of genotype and allele frequencies of Taq I of VDR gene compared to control subjects (Table 6).

Correlation analysis of genotype vs. serum 25-hydroxy vitamin D levels showed significantly upregulated levels with recessive homozygous and heterozygotes in selected polymorphisms of VDR gene. Serum 25-hydroxy vitamin D level was significantly increased in cases with genotype 'Ff' (*p*=0.004) of Fok I polymorphism. In Bsm I polymorphism, genotype 'bb' had significantly increased with 25-hydroxy vitamin D levels compared to genotype 'BB' (*p*=0.0023) and 'Bb' (*p*=0.0035). In Apa I gene polymorphism, significantly reduced levels of vitamin D in cases with genotype 'Aa' (*p*=0.004) and significantly increased levels with genotype 'aa' (*p*=0.01) compared

Table 3 - Fok I VDR gene polymorphism in cases and control subjects.

Model	Genotype	Control N=100	Cases N=100	OR (95% CI)	p-value
Co-dominant	F/F	70 (70%)	68 (68%)	1.00	0.0018
	F/f	14 (14%)	29 (29%)	2.16 (0.86–5.44)	
	f/f	16 (16%)	2 (2%)	0.13 (0.03–0.65)	
Dominant	F/F	70 (70%)	68 (68%)	1.00	0.84
	F/f-f/f	15 (30%)	31 (31%)	1.08 (0.52–2.26)	
Recessive	F/F-F/f	84 (84%)	97 (97%)	1.00	0.0018
	f/f	16 (16%)	2 (2%)	0.11 (0.02–0.54)	
Over-dominant	F/F-f/f	86 (86%)	70 (70%)	1.00	0.031
	F/f	14 (14%)	29 (29%)	2.58 (1.04–6.41)	
Allele	F	144 (77%)	166 (83%)	0.67 (0.37–1.23)	0.21
	f	46 (23%)	34 (17%)		

Table 4 - Bsm I VDR gene polymorphism in cases and control subjects.

Model	Genotype	Control N=100	Cases N=102	OR (95% CI)	p-value
Co-dominant	B/B	62 (62%)	61 (61%)	1.00	0.029
	B/b	12 (12%)	26 (26%)	2.24 (0.83–6.01)	
	b/b	26 (26%)	12 (12%)	0.48 (0.19–1.17)	
Dominant	B/B	62 (62%)	61 (61%)	1.00	0.93
	B/b-b/b	38 (38%)	38 (38%)	1.03 (0.51–2.08)	
Recessive	B/B-B/b	37 (74%)	86 (86%)	1.00	0.039
	b/b	26 (26%)	12 (12%)	0.40 (0.17–0.95)	
Over-dominant	B/B-b/b	88 (88%)	73 (73%)	1.00	0.035
	B/b	12 (12%)	26 (26%)	2.65 (1.01–6.94)	
Allele	B	136 (68%)	148 (74%)	0.72 (0.42–1.23)	0.27
	b	64 (32%)	52 (26%)		

Table 5 - Apa-I VDR gene polymorphism in cases and control subjects.

Model	Genotype	Control N=100	Cases N=100	OR (95% CI)	p-value
Co-dominant	A/A	58 (58%)	46 (46%)	1.00	0.44
	A/a	26 (26%)	31 (31%)	1.50 (0.68–3.34)	
	a/a	16 (16%)	21 (21%)	1.65 (0.65–4.23)	
Dominant	A/A	58 (58%)	46 (46%)	1.00	0.2
	A/a-a/a	42 (42%)	53 (53%)	1.56 (0.78–3.10)	
Recessive	A/A-A/a	84 (84%)	78 (78%)	1.00	0.43
	a/a	16 (16%)	21 (21%)	1.43 (0.58–3.51)	
Over-dominant	A/A-a/a	74 (74%)	68 (68%)	1.00	0.48
	A/a	26 (26%)	31 (31%)	1.32 (0.61–2.82)	
Allele	A	142 (71%)	126 (63%)	1.45 (0.86–2.44)	0.19
	a	58 (29%)	74 (37%)		

Table 6 - Taq I VDR gene polymorphism in cases and control subjects.

Model	Genotype	Control N=100	Cases N=100	OR (95% CI)	p-value
Co-dominant	T/T	27 (54%)	46 (46%)	1.00	0.64
	T/t	18 (36%)	38 (38%)	1.24 (0.59–2.58)	
	t/t	5 (10%)	14 (14%)	1.64 (0.53–5.07)	
Dominant	T/T	27 (54%)	46 (46%)	1.00	0.42
	T/t-t/t	23 (46%)	53 (53%)	1.33 (0.67–2.63)	
Recessive	T/T-T/t	45 (90%)	85 (85%)	1.00	0.45
	t/t	5 (10%)	14 (14%)	1.50 (0.51–4.43)	
Over-dominant	T/T-t/t	32 (64%)	61 (61%)	1.00	0.74
	T/t	18 (36%)	38 (38%)	1.13 (0.56–2.28)	
Allele	T	72 (72%)	132 (66%)	1.30 (0.77–2.21)	0.35
	t	28 (28%)	68 (34%)		

to genotype 'AA'; while cases with genotype 'aa' ($p=0.0042$) had significantly increased levels compared to genotype 'Aa'. In Taq I gene polymorphism, significantly increased levels of 25-hydroxy vitamin D levels in cases with genotype 'tt' compared to genotype 'TT' ($p=0.0025$) and 'Tt' ($p=0.0022$).

VDR genotype differences by 25-hydroxy vitamin D level status were noted; particularly, subjects with lower 25-hydroxy vitamin D levels were less likely to carry the recessive homozygous 'tt' genotype and 'bb' genotype.

Upon logistic regression, statistically significant associations observed between VDR polymorphisms and 25-hydroxy vitamin D levels were identified for Taq I and Bsm I genotypes. No effect was identified for Fok I and Apa I. The 't' allele genotype was associated with a 2-fold increase in the odds for lower 25-hydroxy vitamin D levels, and individuals carrying the 'Tt' genotype were at 2.5-fold increased odds when compared to TT carriers. The b allele genotype was associated with approximately half the odds of low 25-hydroxy vitamin D levels. Overall, BB/Bb, Tt/tt, and FF were considered as risk genotypes.

Discussion

Women are more likely than men to die after their first MI, and survivors face a higher risk of recurrent MI, heart failure, or death.^[11] In the Framingham Heart Study, the one-year mortality rate after a MI was 44% in women and 27% in men.^[12] The overall short-term and long-term CAD mortality following an MI are about 40% higher in women after adjustment for age and other risk factors. Women's higher hospital CAD mortality almost compensates for their lower pre-hospital mortality. Despite their increased risk, women are only half as likely as men to be prescribed aspirin, beta-blockers, or thrombolytic therapy, or to be referred for a revascularization procedure.

According to a study, mortality from MI in women 50 years of age is double that of men, and excess mortality in women is limited to 60 years of age.^[13] Hypertension, diabetes, low levels of high-density

lipoprotein, and high levels of total cholesterol, triglycerides, low-density lipoprotein, and Lp (a) are all linked to CAD in Indian women.^[14]

Many factors influence the pathogenesis of CAD, including age, ethnicity, and geographic location. The prevalence of CAD is also affected by genetic factors. SNPs in the vitamin D receptor (VDR) have been identified as a possible CAD risk factor, possibly linked to low vitamin D levels in CAD patients, but the exact mechanism underlying the effect of VDR polymorphisms on the pathogenesis of CAD remains unknown.

While genetic factors play a significant role in the heterogeneity of circulating levels of 25(OH)D3, with heritability estimates ranging from 30% to 40%, whether Bsm1, Apa1, Taq1 polymorphisms play a functional role in raising CAD risk indirectly through vitamin D deficiency requires further investigation. The structure specialization of the VDR mRNA may affect vitamin D deficiency.

Hence, in the current study, the association between four lead SNPs (Fok I, Bsm I, Apa I, and Taq I) of the VDR gene was determined in patients with CAD and compared to control subjects. The genotypes of these SNPs were correlated with their serum vitamin D levels.

In our study, recessive homozygote 'ff' of the Fok I polymorphism showed significantly lower frequency in CAD cases compared to controls, and 'f' allele frequency represented protective nature in cases compared to controls. In CAD patients, the 'Ff' genotype had significantly higher serum vitamin D levels than the 'FF' genotype.

A recent study found an association between VDR Bsm I polymorphisms and autoimmune and inflammatory disorders in several populations around the world,^[15] making Bsm I an appealing candidate marker to study in Indian populations to determine its true significance. Because the majority of genetic studies are currently being conducted in diabetic patients, its role in Indian patients with CAD requires further investigation.

In our study, the frequency of recessive homozygote 'bb' of Bsm I polymorphism was significantly lower in cases compared to controls. Heterozygote 'Bb' had a significantly higher frequency in the over-dominant model of cases vs. control. Allele frequency analysis

revealed no significant differences between cases and controls. The correlation analysis revealed significantly higher serum vitamin D levels in patients with genotype 'bb' compared to 'BB' and 'Bb' ($p=0.0001$).

Sarma et al. investigated the relationship between VDR Bsm I polymorphisms and type 2 diabetes mellitus patients in northeastern India. Bsm I was found to be strongly related to HbA1C. Vitamin D deficiency was found to be slightly higher in cases compared to controls. The prevalence of the heterozygous genotype of the Bsm I polymorphism was slightly higher in type 2 diabetics than in controls.^[16] Apa I had a significantly higher frequency of recessive homozygote 'aa' in cases compared to controls. Furthermore, the frequency of heterozygote 'Aa' in cases was significantly higher than in controls. We found significantly lower levels of vitamin D in 'Aa' genotype cases compared to 'AA' ($p=0.001$) and significantly higher levels in 'aa' genotype cases compared to 'AA' ($p=0.01$).

Franczek et al.^[17] investigated whether the polymorphisms rs731236, rs7975232, and rs2228570 in the VDR gene are associated with an increased risk of CAD events in stable Polish people with a family history of P-CAD (FH of P-CAD). They genotyped 845 participants from a cohort of 386 healthy volunteers with a recorded P-CAD event in a first-degree relative and 459 healthy volunteers without a P-CAD incident in their family. VDR polymorphisms in Taq I, Apa I, and Fok I were genotyped. While the Taq I and Apa I genotype frequencies were not statistically significant, the AA genotype of the Fok I polymorphism was significantly more common in the research group than in the control group (24.61% vs. 16.99%). Under the recessive paradigm, there was a strong correlation between Fok I polymorphism and FH of P-CAD in healthy people (OR, 1.26; 95% CI, 1.07–1.49); however, the frequency of VDR haplotypes did not differ significantly between the control and sample populations. They concluded that the Fok I polymorphism is related to P-CAD FH. The Fok I polymorphism may predispose healthy people to developing P-CAD in the coming years.

Correlation analysis of genotype and vitamin D levels revealed significantly higher levels with recessive homozygotes and heterozygotes in selected VDR gene polymorphisms.

Early prevention based on genetic polymorphism can reduce the incidence of CAD.

In our study, four single nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) gene (rs2228570, rs1544410, rs731236, and rs7975232) were thoroughly investigated in women with CAD.

According to our findings, subjects carrying either the B or T allele appeared to have a twofold increased risk of developing vitamin D deficiency compared to the reference allele, as the 'b' or 'T' allele appeared to be associated with higher serum 25(OH) D3 levels. The combined effect of the risk genotypes (BB/Bb, Tt/tt, and FF) increased the risk of low 25-hydroxy vitamin D levels. Subsequent studies looked into whether VDR polymorphisms affect serum 25-hydroxy vitamin D levels in vitamin D-deficiency diseases. A study in Bangladesh discovered that VDR polymorphisms had no effect on serum 25-hydroxy vitamin D concentration.^[18] Apa I, Fok

I, and Taq I AA, FF, and TT showed significantly higher vitamin D values in Egyptian obese women.^[19]

There were no studies available in the literature to compare with our study findings, because all available literature studies included both male and female CAD candidates. In contrast, in our study, we only included women with CAD to examine the relationship between serum 25-hydroxy vitamin D levels and vitamin D genotype. Hence, it is the first study of this in India. Further, we would like to continue this study by enrolling higher numbers of patients to generalize the results.

Conclusion

The study analysis supports the role of SNPs within the VDR gene as a risk factor for CAD in the female population; it was found to be associated with an increased risk of CAD. The study found a significant association between low 25-hydroxy vitamin D levels and the presence of 'B' and 't' alleles of Bsm I and Taq I region in the VDR gene. Further research into the genetic involvement of VDR gene polymorphisms in the regulation of vitamin D metabolite concentrations may have important implications for the use of the genetic profile to identify individuals who may be at risk for vitamin D deficiency and recommend daily preventive vitamin D supplementation. Hence, assessing the VDR genetic profile in addition to measuring 25-hydroxy vitamin D concentrations could be useful in prevention of vitamin D deficiency diseases in the female population.

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Ethics approvals and consent to participate

The study protocol was approved by the Ethics Committee of Narayana Medical College (NMC/IEC/2017).

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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