



Status of Vitamin D Receptor Gene Polymorphism and 25-Hydroxy Vitamin D Deficiency with Essential Hypertension

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Abstract Essential hypertension (EH) is a multifactorial and complex disease with high rate of incidence and associated co-morbidities. Previous studies do not provide unanimous results for the risk of hypertension and association with Fok I genotype frequency and serum vitamin D levels. Hence, this study was undertaken to determine the status of Fok I vitamin D receptor (VDR) gene polymorphism along with vitamin D levels and blood pressure in patients with EH. Four hundred (200 controls and 200 cases of essential hypertension) participants from general Indian population were enrolled in this study. Peripheral blood samples were collected for genotyping Fok I-VDR gene polymorphism using PCR–RFLP method whereas 25-OH vitamin D levels in serum were quantified using high performance liquid chromatography (HPLC). Significantly reduced 25-OH vitamin D levels were observed in patients with EH (24.04 ± 8.62 vs 50.46 ± 15.46) compared to control subjects ($p = 0.0001$). Homozygous recessive genotype ‘ff’ frequency was increased by 8.06

fold (CI: 3.71–17.47, $p = 0.0001$) in patients with EH compared to dominant ‘FF’ genotype frequency. In conclusion, recessive ‘ff’ genotype frequency correlates with reduced serum vitamin D levels and results in significantly increased systolic and diastolic blood pressures leading to predisposition of EH.

Keywords Essential hypertension · Vitamin D · VDR Fok I polymorphism · SBP · DBP

Introduction

Essential hypertension (EH) is a complex, multifactorial and polygenic trait. It is also called as primary hypertension or idiopathic hypertension. In Urban population, the prevalence of EH is estimated to be 3.80–15.63% in men and 2.00–15.38% in women while in rural areas the estimated rate of prevalence has reported 1.57–6.93% in men and 2.38–8.81% in women [1]. However, yet the major cause of gender-based discrimination and prevalence of EH has not been identified.

A cross-sectional study have suggested association between low vitamin D intake (< 400 IU per day) and increase in blood pressure [2]. Vitamin D also modulates growth, hypertrophy, collagen deposition, and differentiation of cardiomyocytes, pointing towards direct role of vitamin D levels and its receptor in cardiac pathophysiology. As hypovitaminosis D has been identified an independent risk factor for total mortality in general population, high prevalence of Vitamin D insufficiency remains an important public health issue. The pandemic of hypovitaminosis D can mainly be attributed to lifestyle and environmental factors that reduce exposure to sunlight which is required for ultraviolet B radiation-induced Vitamin D

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production in skin [3]. Observational studies support the concept that vitamin D is involved in the pathogenesis of cardiovascular diseases and arterial hypertension but whether this association reflects genetic basis and causal relationship remains unclear, and needs further research.

Vitamin D-mediated triglyceride abnormalities (also referred as dyslipidemia) have been linked with two-different mechanisms [4]. Vitamin D improves serum calcium by promoting calcium synthesis in the intestine. And by reducing hepatic triglyceride production and secretion, this calcium could lower serum triglycerides. Vitamin D has a suppressive effect on serum parathormone (PTH) concentration, which is the second mechanism. Low serum PTH can reduce serum triglycerides by increasing peripheral elimination, as elevated PTH concentration reduces plasma postheparin lipolytic activity. Another potential explanation for the connection between 25-hydroxyvitamin D and triglycerides is insulin resistance: while vitamin D deficiency exists, the risk of insulin resistance increases, which is linked to an increase in very low density lipoprotein (VLDL) cholesterol and triglyceride levels. Despite the fact that this relation is becoming increasingly apparent, research on the impact of vitamin D supplementation on lowering dyslipidemia is inconsistent and ambiguous and confirmation of these results would require further research.

It has been suggested that genes regulating vitamin D levels contribute to 30 to 50% of variation in blood pressure. Thus, identifying genes that may control vitamin D levels in EH may provide a better factor to determine molecular pathogenesis of the disease. Among various genes vitamin D receptor (VDR) has been proposed as crucial regulator of vitamin D levels in our body. VDR is a nuclear receptor protein which binds to VD₃ (a form of vitamin D) with high affinity and specificity [5]. When occupied by VD₃, VDR is phosphorylated leading to a surface conformational change. Activated VDR then interacts with the retinoid X receptor (RXR) to form a heterodimer that binds to vitamin D-responsive elements in the region of the gene promoter. By recruiting complexes of either co-activators or co-repressors activated VDR/RXR regulates the transcription of genes encoding proteins that exert the traditional and non-traditional functions of VD₃ such as maintaining musculoskeletal health, calcium homeostasis, normal blood pressure and cardiovascular function. Surprisingly, liganded-1,25(OH)₂D₃ VDR can interact directly with cAMP responsive-element binding (CREB), blunting its binding to CRE. These activities seem to be carried out without the liganded-VDR heterodimerizing with RXR. As a result, unique target gene expression will be regulated, facilitating the synthesis of vitamin D-regulated proteins. In brief, lipid binding, heterodimerization with RXR, binding of the

heterodimer to vitamin D reaction elements (VDREs), and recruitment of other nuclear proteins into the transcriptional preinitiation complex are the main steps involved in the regulation of gene transcription by the VDR [6]. Therefore, the role of vitamin D in a cell, tissue, or organ depends on the local production or delivery of a sufficient amount of VD₃ and expression levels of VDR mediating vitamin D signalling. Several profound polymorphisms in the VDR gene are also very prominent, but their importance has yet to be determined, and their effects on VDR protein levels and function are uncertain. Through the years, a large number of data has been obtained on the relationship between vitamin D polymorphisms and response to different diseases. Unfortunately, the findings so far are contradictory, and the involvement of VDR polymorphisms is still uncertain in patients with EH. What seems to be apparent is that the effect of polymorphisms may not be due to changes in protein structure, but rather to variations in RNA stability and/or translation performance, or even modifications in a completely different gene.

Therefore, in this study, the role of 25-hydroxy vitamin D deficiency with dyslipidemia and association of VDR gene polymorphism in exon 2 at the 5' coding region of the gene was evaluated in newly diagnosed patients with EH. The selected Fok I polymorphism of VDR gene results in different translation initiation sites due to thymine (T) to cytosine (C) substitution in the first translation initiation codon ATG (methionine) which generates long and short variants of VDR. This is only known VDR polymorphism resulting in two different VDR protein products. Hence, determining the association of Fok I polymorphism in VDR gene along with serum vitamin D levels in EH may help in evaluating the risk of the disease pathogenesis and associated factors.

Methodology

A comparative case control study was conducted on randomly selected 200 patients with EH from the General Medicine Department Outpatient at Narayana Medical College and Hospital, Nellore, Andhra Pradesh, India. Age and gender matched 200 healthy control subjects were obtained from the general population. The major reason for randomly selecting both cases and controls was to determine the clear definition of patients with EH and healthy individuals meeting desired inclusion and exclusion criteria in a population to study more clear definition of outcome measures. In addition, multiple exposures and risk factors can be evaluated along with genetic predisposition to explore possible mechanisms based on different types of etiological factors in a larger population in comparatively lesser time. Both men and women hypertensive patients

aged between 25 and 60 years with systolic blood pressure (SBP) \geq 140 mmHg and/or diastolic blood pressure (DBP) \geq 90 mmHg were included in this study. Patients with secondary hypertension, diabetes mellitus, known cardiac abnormalities, renal, liver, nutritional disorders and pregnant and lactating women were excluded. Patients taking vitamin D supplements and drugs that could affect hypertension, lipids, and inflammatory markers were also excluded from this study. Demographic details and clinical parameters of each study participants were obtained with the help of sample collection proforma. Study was conducted after taking Institutional Ethics Committee approval (Letter No. IEC/NMCH/Date:16/11/2013) from Narayana Medical College, Nellore, Andhra Pradesh, India.

Sample Collection and Serum Separation

Peripheral blood sample (10 mL) was collected from each participant after overnight fasting and 5 mL of blood sample was transferred into a plain tube that allowed clotting adequately for 15 min at room temperature and centrifuged at 3000 rpm for 10 min for collecting the serum. 3 mL blood was transferred into a fluoride tube for collecting plasma to estimate fasting glucose and remaining 2 mL blood was collected in K3-EDTA coated vacutainer for genomic DNA extraction.

Serum Vitamin D Levels Estimation

25-OH vitamin D levels in both control and patient serum samples were quantified using High Performance Liquid Chromatography (HPLC, D-10 Hemoglobin Testing System, Bio-Rad Laboratories Inc. Hercules, CA, USA). Briefly; 500 μ L of precipitation reagent was added into a sample preparation vial following with 400 μ L of serum sample. Afterwards 400 μ L of internal standard (IS) was cooled and samples were vortexed for 30 s and finally centrifuged at 11,000 rpm for 5 min. 50 μ L of upper phase of supernatant was injected and peaks were identified. Finally, concentration of vitamin D levels was calculated using formula; Concentration of Analyte = (Area of Sample \times Concentration of analyte of calibrator)/(Area analyte of calibrator \times Recovery).

Genomic DNA Extraction

Genomic DNA was extracted from blood samples of all the enrolled subjects using phenol chloroform-based manual purification method. Briefly; red blood cells (RBCs) were lysed first using RBC lysis solution at 4 $^{\circ}$ C. Furthermore, white blood cells (WBCs) were lysed at 55 $^{\circ}$ C for 60 min using WBC lysis solution. Protein precipitation was made from the lysed WBCs using phenol and chloroform mixture

at 24:1 ratio. Finally, DNA was precipitated in aqueous phase using ethanol. Impurities from DNA was washed using 70% ethanol in addition step and pure DNA was obtained after air drying the solution at room temperature for 10 min. Finally DNA was dissolved in 100 μ L of Tris–EDTA (TE) buffer and heated at 50 $^{\circ}$ C for 10 min to dissolve the DNA pellet. Further DNA was quantified using nanodrop reader and integrity was checked using agarose gel electrophoresis. All the DNA samples were stored at -20 $^{\circ}$ C until further use.

Genotyping of Fok1 Polymorphism of VDR Gene

DNA extracted from peripheral blood samples of patients with EH and control subjects were used for single nucleotide polymorphism (SNP) genotyping of VDR gene using polymerase chain reaction followed with restriction fragment length polymorphism (PCR–RFLP) method. First, VDR gene was amplified using forward primer (5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3') and reverse primer (5'-ATGGAAA-CACCTTGCTTCTTCTCCCTC-3'). The reaction conditions were optimized for initial denaturation at 94 $^{\circ}$ C for 5 min following with 40 cycles of denaturation at 94 $^{\circ}$ C for 30 s, annealing at 58 $^{\circ}$ C for 30 s and extension at 72 $^{\circ}$ C for 60 s and one step of final extension at 72 $^{\circ}$ C for 10 min in a thermal cycler (S1000, Bio-Rad, CA, USA). The amplified PCR product of 265 bp was confirmed for the VDR gene by agarose gel electrophoresis.

20 μ L of PCR mixture of VDR gene amplicons was subjected to RFLP using 0.4 μ L of Fok 1 restriction enzyme (R0109S, NEB) in a reaction mixture of 25 μ L with 2.5 μ L reaction buffer and 2.1 μ L of molecular biology grade autoclaved water. The reaction mixture was incubated at 37 $^{\circ}$ C for 60 min in waterbath for generating restriction fragments to determine different genotypes in each sample using agarose gel electrophoresis. PCR products with an undigested 265 bp band size were genotyped as dominant 'FF' homozygote, digested bands at 169 and 96 bp were genotyped as recessive 'ff' homozygote and those with 265, 169 and 96 bp bands were genotyped as 'Ff' heterozygote.

Statistical Analysis

Results of the present study have been expressed as mean \pm SD. Sample size was estimated using creative research systems software version 1.0 (<http://hydra.usc.edu.gxe>) by taking the most probable prevalence of EH. The sample size of 400 (200 cases and 200 controls) was large enough to obtain a 90% statistical power. Hardy–Weinberg equilibrium was assessed in both cases and controls. It was primarily used for testing systematic

genotyping errors and other biases that could lead to lack of replication and genotype association of FokI polymorphism with the disease. Chi-Square test was done for risk factors distribution in different groups. Association between genotypes and essential hypertension was examined by odds ratio (OR) with 95% confidence interval (CI). Student t-test was used to calculate the statistical significance for blood pressure in patients with EH compared to control individuals. Codominant, dominant, overdominant and recessive models were used to define significance of different combinations of genotype frequencies in cases compared to controls using SNP Stats (<https://www.snpstats.net/start.htm>). These models were classified based on the presence of specific allele in each genotype such as dominant: FF vs Ff + ff, co-dominant: FF vs Ff vs ff, overdominant model: FF + ff vs Ff, and recessive: FF + Ff vs ff. A p value ≤ 0.05 was considered statistically significant for all the variables.

Results

Genotypic and allelic frequencies of Fok I polymorphism in VDR gene was identified along with vitamin D levels in 200 patients with EH and compared with age and gender matched 200 control individuals in Indian population. Furthermore, SBP and DBP levels were quantified in both patient and control samples for correlation with vitamin D levels.

Vitamin D Levels and Blood Pressure in Patients with EH

Diagnosis of EH was made for 90 mmHg DBP and 140 mmHg SBP readings. Isolated systolic hypertension was defined as SBP 140 mmHg and DBP 90 mmHg. Table 1 represents significantly increased SBP (134.53 ± 6.78 mmHg) and DBP (89.32 ± 3.00 mmHg) in patients with EH as compared to control subjects (116.65 ± 4.13 mmHg vs 76.97 ± 4.69 mmHg, ($p < 0.0001$). Further correlation between vitamin D levels and blood pressure in essential hypertension patients showed significant association of SBP, while the same turned out to be inconclusive for DBP (Fig. 1). The mean

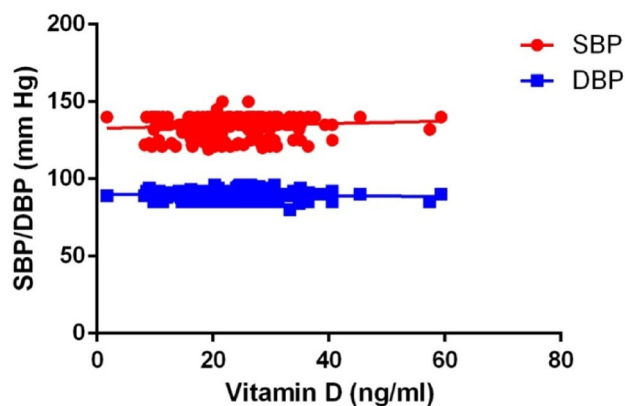


Fig. 1 Correlation between SBP and DBP in essential hypertension with respect to vitamin D levels

SBP associated with vitamin D deficiency (< 25 ng/ml) was 134.53 ± 6.78 mmHg while with vitamin D insufficiency ($25\text{--}35$ ng/ml) was 116.65 ± 4.13 mmHg. Thus, an approximate linear relation was observed for SBP and DBP levels in relation to serum vitamin D levels.

Genotype Analysis of Fok I Polymorphism in VDR Gene

Three different genotypes were observed in the study subjects represented as FF, Ff and ff. The genotype designated as FF shows absence of restriction site and yielded only one band in agarose gel electrophoresis at 265 bp region which is of same size of PCR product. The genotype designated by Ff shows presence of restriction site and yielded two bands of size 169 and 96 bp (Fig. 2). The ff genotype presented with three bands in gel electrophoresis and the size of fragments is 265, 169, and 96 bp.

Distribution of VDR Genotypic and Allelic Frequencies in Total Patients with EH

The genotypic analysis showed 66% frequency of F/F genotype in patients with EH which was almost similar to control individuals (67%). Heterozygote F/f genotype frequency was also reported almost similar in cases (30%) and controls (29%). However, the recessive f/f genotype was

Table 1 Blood pressure in essential hypertensive and control individuals showing significantly increased levels of SBP and DBP, and reduced levels of vitamin D compared to control individuals

Biochemical Parameters	Cases	Control	p Value
Vitamin D (ng/ml)	24.04 ± 8.62	50.46 ± 15.46	0.0001
SBP (mm Hg)	134.53 ± 6.78	116.65 ± 4.13	0.0001
DBP (mm Hg)	89.32 ± 3.00	76.97 ± 4.69	0.0001

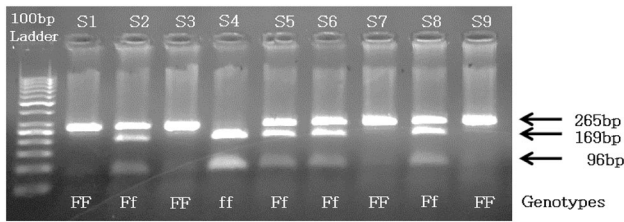


Fig. 2 Identification of different genotypes using PCR–RFLP of Fok I polymorphism in VDR gene

reported significantly increased frequency in cases (32%) compared to controls (4%) with ($p = 0.0001$) (Table 2).

The ff genotype was found to be significantly associated with 8.06 folds increased risk for EH compared to FF genotype (OR: 8.06, 95% CI: 3.71–17.47, $p = 0.0001$). Based on the dominant model, combination of Ff + ff genotypes did not show association with high risk for EH (OR: 1.01, 95% CI: 0.71–1.62, $p = 0.75$). Based on the recessive model, ff genotype showed statistical significance compared to the combination of FF + Ff genotype (OR: 11.04, 95% CI: 5.12–23.78, $p = 0.0001$). VDR allele frequency distribution analysis showed the f allele was significantly increased (33%) in cases as compared to controls (18%) with an OR = 2.16 (1.56–3.01; $p = 0.0001$).

Gender-Wise Distribution of VDR Genotypic and Allelic Frequencies in Patients with EH

Gender-wise distribution of dominant F/F genotype analysis showed 67% frequency in men and 65% frequency in women. F/f genotype showed 2% frequency in men and 3% in women and recessive f/f genotype showed 31% frequency in men and 32% in women (Table 3). The odds risk estimation of VDR gene polymorphism in men and women patients with EH showed no statistically significant values

in all the four models (codominant, dominant, recessive, and overdominant; $p > 0.05$). Different percentages of allele frequencies were observed for men and women in cases compared to control individuals. Allele frequency distribution analysis showed 68% frequency of F allele in men and 66% in women while, f allele showed 32% frequency in men and 34% in women (Table 4).

Discussion

This study explored establishment of relationship between vitamin D deficiency and genotype distribution of FokI polymorphism in VDR gene with EH. Vitamin D deficiency was significantly more prevalent in hypertensive population compared to their age and gender matched control population. Our findings are well supported with the study of Nemerovski et al. [7] wherein it was suggested that vitamin D supplementation does not improve functional capacity or quality of life in older heart failure patients with vitamin D deficiency [7]. Further, Botella-Carretero et al. [8] showed that vitamin D deficiency is associated with metabolic syndrome in morbidly obese patients [8].

In our study, we observed negative correlation of vitamin D levels with SBP and DBP. Priya et al. [9] showed vitamin D deficiency is more prevalent with EH, and low levels correlates with elevated SBP [9].

Low vitamin D levels can induce dyslipidemia, and lipid abnormalities, such as a rise in triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) levels and a decrease in high-density lipoprotein cholesterol (HDL-C) levels, have been reported as significant risk factors for atherosclerosis and cardiovascular disease in adults [10, 11]. The effects of serum vitamin D

Table 2 Odd risk estimation in VDR gene polymorphism in both essential hypertensive and healthy control individuals showing significantly increased frequency of f allele in cases compared to controls

Model	Genotype	Control (N = 200)	Cases (N = 200)	OR (95% CI)	p value
Codominant	FF	134 (67%)	131 (65.5%)	1.00	0.0001
	Ff	58 (29%)	6 (3%)	0.11 (0.04–0.25)	
	ff	8 (4%)	63 (31.5%)	8.06 (3.71–17.47)	
Dominant	FF	134 (67%)	131 (65.5%)	1.00	0.75
	Ff–ff	66 (33%)	69 (34.5%)	1.07 (0.71–1.62)	
Recessive	FF–Ff	192 (96%)	137 (68.5%)	1.00	0.0001
	Ff	8 (4%)	63 (31.5%)	11.04 (5.12–23.78)	
Overdominant	FF–ff	142 (71%)	194 (97%)	1.00	0.0001
	Ff	58 (29%)	6 (3%)	0.08 (0.03–0.18)	
Allele	F	326 (82%)	268 (67%)	OR = 2.16 (1.56–3.01) $\chi^2 = 21.99$	0.0000
	f	74 (18%)	132 (33%)		
Hardy–Weinberg equilibrium P value		0.64	< 0.0001		

Table 3 Genotypic and allelic frequencies of VDR gene polymorphism in men and women patients with essential hypertension with respect to gender matched control subjects

Model	Genotype	Control men (N = 103)	EH men (N = 84)	OR (95% CI)	p Value	Control women (N = 97)	EH women (N = 116)	OR (95% CI)	p Value
Codominant	FF	68 (66%)	56 (66.7%)	1.00	0.0001	66 (68%)	75 (64.7%)	1.00	0.0001
	Ff	32 (31.1%)	2 (2.4%)	0.08 (0.02–0.33)		26 (26.8%)	4 (3.5%)	0.14 (0.04–0.41)	
	ff	3 (2.9%)	26 (30.9%)	10.52 (3.03–36.60)		5 (5.2%)	37 (31.9%)	6.51 (2.42–17.54)	
Dominant	FF	68 (66%)	56 (66.7%)	1.00	0.93	66 (68%)	75 (64.7%)	1.00	0.6
	Ff-ff	35 (34%)	28 (33.3%)	0.97 (0.53–1.79)		31 (32%)	41 (35.3%)	1.16 (0.66–2.06)	
Recessive	FF-Ff	100 (97.1%)	58 (69%)	1.00	0.0001	92 (94.8%)	79 (68.1%)	1.00	0.0001
	ff	3 (2.9%)	26 (30.9%)	14.94 (4.33–51.54)		5 (5.2%)	37 (31.9%)	8.62 (3.23–22.99)	
Overdominant	FF-ff	71 (68.9%)	82 (97.6%)	1.00	0.0001	71 (73.2%)	112 (96.5%)	1.00	0.0001
	Ff	32 (31.1%)	2 (2.4%)	0.05 (0.01–0.23)		26 (26.8%)	4 (3.5%)	0.10 (0.03–0.29)	
Allele	F	168 (68%)	114 (66%)	OR = 0.47 (0.29–0.77)	0.002	158 (81.44)	154 (66%)	OR = 0.44 (0.28–0.70)	0.0006
	f	38 (32%)	54 (34%)	$\chi^2 = 9.36$		36 (18.55%)	78 (34%)	$\chi^2 = 12.23$	

Table 4 Genotypic and allelic frequencies of VDR gene polymorphism in men and women patients with essential hypertension

Model	Genotype	Men (N = 84)	Women (N = 116)	OR (95% CI)	p Value
Codominant	FF	56 (66.7%)	75 (64.7%)	1.00	0.89
	Ff	2 (2.4%)	4 (3.5%)	1.49 (0.26–8.44)	
	ff	26 (30.9%)	37 (31.9%)	1.06 (0.58–1.95)	
Dominant	FF	56 (66.7%)	75 (64.7%)	1.00	0.77
	Ff-ff	28 (33.3%)	41 (35.3%)	1.09 (0.60–1.98)	
Recessive	FF-Ff	58 (69%)	79 (68.1%)	1.00	0.89
	ff	26 (30.9%)	37 (31.9%)	1.04 (0.57–1.91)	
Overdominant	FF-ff	82 (97.6%)	112 (96.5%)	1.00	0.66
	Ff	2 (2.4%)	4 (3.5%)	1.46 (0.26–8.19)	
Allele	F	114 (68%)	154 (66%)	OR = 0.93 (0.61–1.42)	$\chi^2 = 0.1$
	f	54 (32%)	78 (34%)		0.82

status on lipids in Chinese adults were investigated by Wang et al., who discovered that serum 25(OH)-D levels were closely related to lipids and AIP. Furthermore, Chaudhuri et al. discovered that 25(OH)-D deficiency was linked to dyslipidemia in Indian subjects [12]. Vitamin D's roles are related to lipid levels. First, vitamin D controls calcium synthesis and raises intestinal calcium absorption, lowering fatty acid absorption in the gut [13]. As a result, lowering cholesterol levels can be achieved by reducing intestinal fat absorption. Furthermore, the calcium concentration encourages the liver to convert cholesterol to bile acids, resulting in lower cholesterol levels [14].

Further studies are desired to properly define the genetic basis of essential hypertension. Hence, in this study risk prediction for specific genotype of VDR Fok1 polymorphism causing susceptibility to EH was evaluated. VDR gene has a crucial role in regulating the renin-angiotensin system (RAS) influencing the regulation of blood pressure. Inappropriate stimulation of the RAS has been associated with hypertension [15].

In our study, significantly increased genotype frequency of homozygous 'ff' was observed compared to 'FF' genotype in cases of EH. Thus, increased 'ff' genotype frequency results in translation initiation at the first ATG

site instead of second ATG site (in case of ‘FF’ genotype) showing the reduced activity of VDR protein which is caused by the deficiency or excess of vitamin D and inactivation of its biologically active form. Whereas increased frequency of ‘FF’ genotype in cases results in formation of a truncated protein with three amino acids lesser of VDR protein (424 instead of 427) which is more biologically active form [16]. Similar to our findings, Swapna et al (2011) found an association of Fok I polymorphism of VDR gene with essential hypertension [2].

Gender-wise analysis didn’t show significant difference for genotype and allele frequencies of VDR gene polymorphisms in EH cases. However, in men with EH the genotype frequency of ‘ff’ was found significantly increased compared to control subjects. Similar findings were observed in women with EH. Collectively, our study revealed reduced activity of VDR protein due to increased ‘ff’ genotype and ‘f’ allele frequency resulting in vitamin D deficiency leading to essential hypertension. However, more data are needed to find out and validate to determine the association of VDR Fok 1 gene polymorphism with vitamin D deficiency and EH.

Conclusion

The present study demonstrated that vitamin D deficiency is more prevalent in patients with essential hypertension, and low levels correlates with elevated SBP. Further, genotype frequency distribution analysis of VDR gene polymorphism Fok 1 shows 8.06 fold increased risk of EH in presence of ‘ff’ homozygous genotype. Cumulatively ‘ff’ genotype frequency of VDR, Fok 1 gene polymorphism well correlates with reduced levels of vitamin D in patients with EH.

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Availability of data and materials Data will be made available when desired.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Study was conducted after taking Institutional Ethics Committee approval (Letter No. IEC/NMCH/Date:16/11/2013) from Narayana Medical College Nellore, Andhra Pradesh, India.

Consent to participate Written informed consent forms were collected from all the study participants using a customized proforma with defined sets of demographic and clinical information.

Consent for publication All the authors have read the manuscript and agreed to publish in Indian Journal of Clinical Biochemistry.

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