A Study of VDR Foki Polymorphism in Osteoporosis Susceptibility in Central India

Arpana Mishra[1], Alpana Mishra[2], Udita Singh[3], Santosh Agnihotry[4]
[1, 2, 3] Centre for Biotechnology Studies, APS University Rewa M.P
[4]Govt. Science College Rewa (M.P.)

Abstract- Background: *Osteoporosis, or porous bone, is a disease characterized by low bone mass density (BMD) and structural deterioration of bone tissue, leading to bone fragility and increased risk of hip, spine, and wrist fractures. There are numerous risk factors for osteoporosis. While many of these factors are non-genetic in nature, there is a definite genetic component responsible for this condition. The main aim of this study was to evaluate the association between VDR (Vitamin D receptor gene) polymorphisms (Fok1) A>G (rs2228570) osteoporosis in an Indian population.*

Methods: *The study participants comprised of 108 Indian patients as well as 120 controls recruited from the city of Rewa and satna. All samples were genotyped for VDR genes (Fok1) polymorphism with polymerase chain reaction, using a predesigned PCR-RFLP method*

Results: *There was no significant association between Fok1 polymorphism and osteoporosis*
Chi square (P value)= 2.266, (0.3383)

Conclusion: *Fok 1 polymorphism of VDR gene is not involve in pathogenecity of osteoporosis in vindhyan region.*

Keywords- osteoporosis, VDR Fok 1 Polymorphism, PCR-RFLP Method.

I. INTRODUCTION

Osteoporosis is a skeletal disorder characterized by compromised bone strength, predisposing to an increased risk of fracture. The composition of the mineral and matrix, the fine structure of the trabecular bone, the porosity of the cortical bone, and the presence of micro-fractures and other forms of damage in bone are all important in determining bone strength. The concept of osteoporosis was spawned already in 1824 by the English surgeon Sir Astley Cooper, who noted a relation between reduced bone mass and hip fractures in elderly . Today, an osteoporotic fracture is considered to be the most adverse health event in older adult women and is strongly associated with age and a previous bone fracture. In women, the loss of BMD accelerates perimenopause or menopause transition, leads a condition of menopause, and ultimately develops postmenopause. It is crucial to examine the polymorphism of VDR gene in osteoporosis among women age 50 and older to reaching natural menopause. Despite previous studies that have supported menopause is associated with osteoporosis in postmenopausal women (Kim, Lee, Chung, & Park, 2011). There are many association studies that confirm the functional significance of VDR genes polymorphisms and its potential effects on disease susceptibility. No such studies are available from India. Therefore there is a need for further data to determine whether these polymorphisms can act as possible genetic markers of ostioprotic postmenopausal women susceptibility in our country.

II. MATERIALS AND METHODS

Sample collection

osteoporosis patients were recruited from Sanjay Gandhi hospital, Rewa, Madhya Pradesh, Government. Ayurveda Medical College Rewa, Birla Hospital, Satna during the year 2004–2017. One hundred and eight patients were enrolled in the study and 120 respective controls having similar ethnicity and socioeconomic status were recruited, to achieve more than 80% power in additive and 91% in multiplicative model (CaTs Model) for present investigation.

DNA isolation and quantification

Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller et al.6 The isolated genomic DNA samples were then tested for purity by measuring their absorbance values at 230 nm, 260 nm, 280 nm, and 300 nm using an ultraviolet visible spectrophotometer (Systronic (India) Ltd, Bhopal, India). Gel electrophoresis of the genomic DNA was carried out for qualitative estimation of samples prepared. Horizontal agarose slab gel electrophoresis apparatus (Bangalore Genei Pvt, Ltd, Bangalore, India) was used.

VDR polymorphism screening

The VDR gene is ≈75 kb long, and is made up of eleven exons together with intervening introns. The gene encoding the VDR is on chromosome 12q, and has several known allelic variants including a FokI restriction fragment length polymorphism in intron 2.

Primers

The oligonucleotide sequences (primers) used were those described by Bid and Mittal. Sequences are as follows:

Forward primer – 5′ AGCTGGCCCTGGCACTGACTCTGCTCT 3′.

Reverse primer – 5′ ATGGAAACACCTTGCTTCTTCTCCCTC 3′.

PCR mix

The PCR reaction was carried out in a total volume of 25 μL containing 100 ng of genomic DNA, 10 pM of each primer, 2 mM MgCl2, 0.2 mM dNTPs, 1× buffer and 2 U of Taq polymerase. PCR amplifications were performed on each sample in a 25 μL reaction volume consisting of 10 μL PCR buffer (Perkin Elmer, Boston, MA, USA), 0.5 mM of each primer, 0.1 mM of each dNTPs (Bangalore Genei Pvt, Ltd), 1.25 U of Taq Gold DNA polymerase (Perkin Elmer Inc.), 3 mM MgCl2 and 50 ng of genomic DNA, diluted to the final volume with H2O.

Thermal profile

The PCR cycles included initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 61°C for 30 seconds, and amplification at 72°C for 1 minute, and one final cycle of extension at 72°C for 7 minutes.8 The PCR products were separated by 9% Page stained with ethidium bromide using a 100 bp molecular weight marker to confirm the PCR product size (Figure 1).

Restriction digestion by FokI and polymorphism study

The PCR product was digested with 1.0 U of FokI restriction enzyme, and the reaction buffer, incubated at 37°C for 4 hours. Digestion of the amplified 265 bp PCR product gave two fragments of 169 bp and 96 bp respectively if the product was digested by FokI. Depending on the digestion pattern, the FF genotype (homozygote of common allele) lacked a FokI site and showed only one band of 265 bp. The ff genotype (homozygote of infrequent allele) generated two fragments of 169 and 96 bp. The heterozygote had three fragments of 265, 169, and 96 bp, designated as Ff.

III. RESULTS

VDR FokI genotypes, alleles and carriage rates with susceptibility to disease (postmenopausal ostoporosis) cases compared to HC population in Central India.

VDR GENOTYPE	CASE N= 108		CONTROL N=120		χ^2 VALUE (*P* VALUE)
	N	%	N	%	
FF	55	50.9	51	42.5	2.266, (0.3383)
Ff	49	45.4	61	50.8	
Ff	4	3.7	8	6.6	
ALLELES					
F	159	73.6	163	67.9	1.773, (0.1318)
F	57	26.4	77	32.1	
CARRIGE RATE					
F	104	96.4	112	93.6	0.7447, (0.3882)
F	53	48.2	69	56.8	

N - Number of individuals carrying particular genotype in a study group

% - Genotype frequency, allele frequency and carriage rates in percentage;

*- significant values

χ^2 (*P* Value) - indicates χ^2 *P* Value when HC is compared to Osteoporosis

Detection of VDR FokI gene polymorphism

Overall genotype pattern of VDR gene was not significantly different between the case and control groups ($\chi 2$ =2.266, df =2, P-value =0.3221). The osteoporosis group showed an increase in the "FF" genotype as compared to the control group (50.9% versus 42.5%), but was not significantly different. The Genotype "ff" was not significantly higher in the HC group as compared to the osteoporosis group (6.6% vs 3.7%). The overall allele distribution was also not found to be significant, but less common "f" allele was found in higher frequency in the controls as compared to the osteoporosis patients (32.1% versus 26.4%), and the F allele was found at a higher frequency in the cases as compared to the control group (73.6% versus 67.9%), but the difference was not statistically significant ($\chi 2$=1.773, P=0.1712). Carriage rate of the F allele was equivalent to the HC group and the osteoporosis group. Whereas carriage rate of allele f was higher in the control group (56.8% versus 48.2%), but not significantly different between cases and controls ($\chi 2$=0.7447, P=0.3882). The pattern of genotype and allele distribution in the disease and control groups suggested a lack of association of VDR FokI

(rs10735810) in osteoporosis susceptibility, as shown in Table 1 and Figure 1.

Association of VDR *FokI* genotypes, alleles and carriage rates with susceptibility to disease in postmenopausal ostoporosis cases compared to controls using Fisher exact test.

VDR GENOTYPE	CASE N=108	%	CONTROL N=120	%	P VALUE	ODDS RATIO & CI
FF	55	50.9	51	42.5	0.1952	1.412, 0.8458-2.358
Ff	49	45.4	61	50.8	0.4347	0.7937, 0.4758-1.324
Ff	4	3.7	8	6.6	0.3898	0.5580, 0.1635-1.904
ALLELES						
F	159	73.6	163	67.9	0.1876	1.322, 0.8857-1.974
F	57	26.4	77	32.1		0.7563, 0.5066-1.129
CARRIGE RATE						
F	104	96.4	112	93.6	0.4339	1.214, 0.7815-1.885
F	53	48.2	69	56.8		0.8239, 0.5306-1.280

N - Number of individuals carrying particular genotype in a study group

% - Genotype frequency, allele frequency and carriage rates in percentage;

*- significant values χ^2 (*P* Value) - indicates χ^2 *P* Value when HC is compared to Osteoporosis

IV. DISCUSSION

Osteoporosis is a metabolic bone disease, characterized by low bone mass and bone tissue deterioration, which leads to osteoporotic fracture risk (2,3). It is a disease caused by the interaction of genetic and environmental factors. According to many studies, the contribution of genetic and environmental factors is about 70% and 30%, respectively. The environmental factors; however, can control gene expression and the process of the disease (4). Different genes are reported to be linked with osteoporosis, the most important of which is vitamin D receptor gene (VDR) (5). The most common genetic markers that have been investigated in genetic association studies of BMD or osteoporosis are the single nucleotide polymorphisms (SNPs) in the VDR gene (6). In osteoporosis, bone density decreases due to the high activity of osteoclasts (7). The prevalence of osteoporosis is different in various ethnicities and thus it is different in multiethnic countries (8,9). Several studies have evaluated the association between polymorphisms of vitamin D receptor gene (Fok1, Bsm1, Taq1, Apa1) and low bone mass density or osteoporosis (10.11). Overall genotype pattern of VDR gene was not significantly different between the case and control groups ($\chi 2$ =2.266, df =2, P-value =0.3221). The osteoporosis group showed an increase in the "FF" genotype as compared to the control group (50.9% versus 42.5%), but was not significantly different. A systematic review published in 2014 revealed a significant association between the two polymorphisms (Fok1 and Bsm1) and osteoporosis in more than 50% of the available studies. Based on the articles, 60.0% of these studies reported a significant correlation between Fok1 and higher osteoporosis risk (12,17). They concluded that ethnicity and race, like gender, can influence the risk of osteoporosis and BMD As for the Indian population, FF was seen in 59%, Ff in 36%, and ff in 5% of the studied population (13). In the Lampedusa Italian population, the observed proportions of Fok1 genotypes were 33.2% for FF, 32.8% for Ff, and 34% for ff (14,16). In the Spanish population, the prevalence of these genotypes was 40.4, 48.0 and 11.6% for FF, Ff and ff, correspondingly (15).

REFERENCE

[1] Kim HJ, Lee CH, Shin S, Lee JH, Kim YW, Chung HS, et al. The impact of nutritional deficit on mortality of in-patients with pulmonary tuberculosis. Int J Tuberc Lung Dis. 2010; 14(1): 79-85.

[2] Kelly P, Eisman J, Sambrook P. Interaction of genetic and environmental influences on peak bone density. Osteoporosis international. 1990;1:56–60. [PubMed]

[3] Pollitzer WS, Anderson JJ. Ethnic and genetic differences in bone mass: a review with a hereditary vs environmental perspective. The American journal of clinical nutrition. 1989;50:1244–59. [PubMed]

[4] Ferrari S. Osteoporosis: a complex disorder of aging with multiple genetic and environmental determinants. World review of nutrition and dietetics. 2005;95:35. [PubMed]

[5] Hustmyer FG, Peacock M, Hui S, Johnston C, Christian J. Bone mineral density in relation to polymorphism at the vitamin D receptor gene locus. Journal of Clinical Investigation. 1994;94:2130.[PMC free article] [PubMed]

[6] Uitterlinden AG, Pols HA, Burger H, Huang Q, van Daele PL, van Duijn CM. et al. A large-scale population-based study of the association of vitamin D receptor gene polymorphisms with bone mineral density. Journal of Bone and Mineral Research. 1996;11:1241–8. [PubMed]

[7] Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis 1. Endocrine reviews. 2000;21:115–37. [PubMed]

[8] Barrett-Connor E, Siris ES, Wehren LE, Miller PD, Abbott TA, Berger ML. et al. Osteoporosis and fracture risk in women of different ethnic groups. Journal of Bone and Mineral Research. 2005;20:185–94.[PubMed]

[9] Looker AC, Orwoll ES, Johnston CC, Lindsay RL, Wahner HW, Dunn WL. et al. Prevalence of low femoral bone density in older US adults from NHANES III. Journal of Bone and Mineral Research. 1997;12:1761–8. [PubMed]
[10] 10.Eisman JA. Vitamin D receptor gene alleles and osteoporosis: an affirmative view. Journal of Bone and Mineral Research. 1995;10:1289–93. [PubMed]
[11] Shrikant Kol et al. Identification of microbes related to crude oil components consumption and optimization of their growth parameters: International Journal of Development Research, Vol. 4, Issue, 11, pp. 2437-2441, November, 2014
[12] Abbasi M, Hasani S, Sheikholeslami H, Alizadeh S, Rashvand Z, Yazdi Z. et al. Association between Vitamin D Receptor APA1 AND TAQ1 genes polymorphisms and osteoporosis in postmenopuasal wemen. The Journal of Qazvin University of Medical Sciences. 2012:4–10.
[13] Mohammadi Z, Fayyazbakhsh F, Ebrahimi M, Amoli MM, Khashayar P, Dini M. et al. Association between vitamin D receptor gene polymorphisms (Fok1 and Bsm1) and osteoporosis: a systematic review. Journal of Diabetes & Metabolic Disorders. 2014;13:98. [PMC free article] [PubMed]
[14] Bhanushali AA, Lajpal N, Kulkarni SS, Chavan SS, Bagadi SS, Das BR. Frequency of fokI and taqI polymorphism of vitamin D receptor gene in Indian population and its association with 25-hydroxyvitamin D levels. Indian J Hum Genet. 2009 Sep-Dec;15(3):108–113. [PMC free article] [PubMed]
[15] Falchetti A, Sferrazza C, Cepollaro C, Gozzini A, Del Monte F, Masi L. et al. FokI polymorphism of the vitamin D receptor gene correlates with parameters of bone mass and turnover in a female population of the Italian island of Lampedusa. Calcif Tissue Int. 2007;80:15–20. [PubMed]
[16] Saket, Rishabh Dev, et al. "Compliment receptor type-1 (CD35) gene polymorphism and Plasmodium falciperum Maleria." International Journal of Medical Research & Health Sciences 4.2 (2015): 422-431.
[17] Quesada JM1, Casado A, Díaz C, Barrios L, Cuenca-Acevedo R, Dorado G. Allele-frequency determination of BsmI and FokI polymorphisms of the VDR gene by quantitative real-time PCR (QRT-PCR) in pooled genomic DNA samples. J Steroid Biochem Mol Biol. 2004 May;89-90(1-5):209–14.[PubMed]

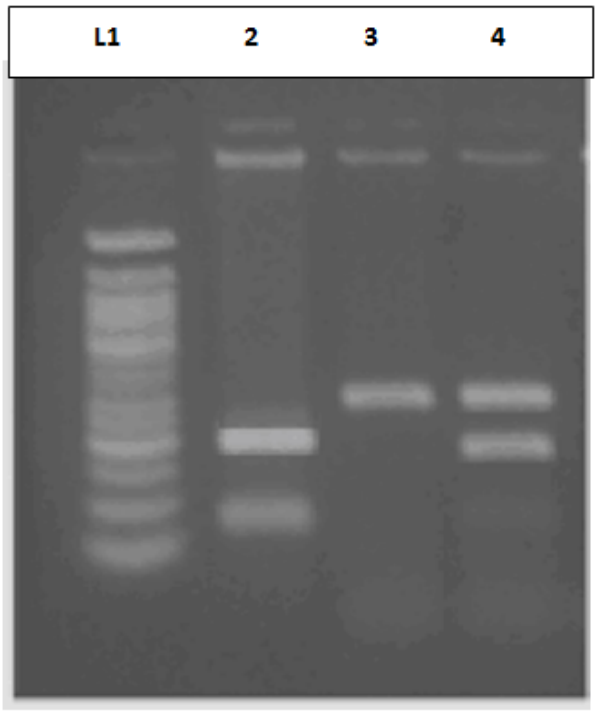

Figure 13: Represents PCR-based analysis of VDR gene Fok1 polymorphism

M =marker (50 bp ladder), Lane 1 represents excisable fragment (169 bp and 96 bp),
Lane 2 unexcisable fragment of (265 bp), Lane 3 shows heterozygosity (265;169 and 96 bp).