Research Article **Mishra & Agnihotri, 9(3): March, 2018:5757-5761]**
CODEN (USA): IJPLCP ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

VDR Taq1 gene polymorphism in osteoporosis: A study from central India

Arpnna Mishra[1] and Santosh Agnihotri[2]
1, Centre for Biotechnology Studies, A.P.S. University, Rewa, (M.P.) - India
2, Department of Botany, Govt. Science College, Rewa, (M.P.) - India

Abstract

Menopause is associated with an imbalance in bone metabolism, and the first five to ten years after menopause is the period of higher bone turnover and bone loss Approximately 35% of postmenopausal women lose significant amounts of bone mineral during this period and are at a higher risk for osteoporosis and fragility fractures later in life Introduction. Osteoporosis is a polygenic disorder that is determined by the effects of several genes, each with relatively modest effects on bone mass and other determinants of fracture risk. In this study, we investigated the association between the Taq 1 polymorphism of vitamin D receptor (VDR) gene and BMD in a population of vindhayan region. Taq 1 polymorphism was analyazed by PCR –RFLP METHOD. PCR Amplification products of 293bp, 340bp and 47bp were obtained. product which was digested with taq-1 enzyme . TT homozygous for the absence of TaqI site 340 bp only; tt homozygous for the presence of TaqI site 293 bp and 47 bp, in case of heterozygosity Tt, all three bands (340 bp, 293 bp and 47 bp) were exhibited. The pattern of genotype and allele distribution in disease and HC group suggested strong association of VDR Taq1 polymorphism in postmenopausal osteoporosis susceptibility.

Key words: Osteoporosis, VDR, Taq1 polymorphism

Introduction

Osteoporosis is a systemic skeletal disease, characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture" (Bonnick and Lewis *et al*., 2006). Before this definition, many people believed that this was an age-related disease meaning that age was the only factor in acquiring this disorder and that there was no definite way to prevent it. 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. it's clinical consequences occur late and are detected late. It happens silently and suddenly and it's signs and symptoms are seen after a fracture has already occurred so that it is also known as silent thief. There are a number of different ways in which osteoporosis can develop, with the skeleton becoming more fragile and the risk of fracture increasing (Raisz and Rodan *et al*., 2003).
with post menopausal risk of osteoporosis.

*** Corresponding Author**
E.mail: arpnna10mishra@gmail.com

Many people have relatively weak bones even as young adults because of their genes or because of suboptimal nutrition and lifestyle. The menopause occurs as a natural part of a woman's ageing process and marks the end of the fertile phase of life. Osteoporosis is a polygenic disorder that is determined by the effects of several genes, each with relatively modest effects on bone mass and other determinants of fracture risk. Population-based studies and case-control studies have similarly identified polymorphisms in several candidate genes that have been associated with bone mass or osteoporotic fracture, including the vitamin D receptor (VDR) (Morrison et al., 1994). Vitamin D, through its principal bioactive form 1,25-dihydroxyvitamin D3 (1,25-(OH)2D3), plays a crucial role in bone metabolism.

The action of 1,25-(OH)2D3 is mediated through a specific hormone receptor (Ralston *et al*., 2006). Mutations in the VDR gene cause the syndrome of vitamin D-resistant rickets, which is a recessive condition characterized by alopecia, hypocalcaemia, hypophosphatemia, and severe rickets and is resistant to treatment with vitamin D and its active metabolites (Ralston ; de Crombrugghe *et al*., 2006). VDR was the first candidate gene to be studied in relation to

osteoporosis (Morrison *et al*., 1994; corrected in 1997), and most attention has focused on polymorphisms of VDR that are recognized by the restriction enzymes BsmI, FokI and TaqI (Ralston *et al*.,2006). In present investigation VDR gene Taq1 polymorphism is investigated for its possible association

Material and Methods

Patient recruitment

Postmenopausal osteoporotic patients were recruited from Sanjay Gandhi hospital, District hospital , Ayurvedic hospital Rewa, (M.P.) during the year 2016-2017, 112 patients were enrolled in the study. All the patients were of Vindhyan Indian origin. The diagnosis of Postmenopausal osteoporotic patients women was based on the case history of patients and clinical tests (i.e. DEXA ,Postmenopausal osteoporotic Factor). The study population consisted of 120 unrelated subjects as case and 200 samples of healthy controls . the age of case and control is above then 45 years. Approximately 5 ml. of blood sample was collected in 0.5 M EDTA tubes from each as well as from healthy controls. These samples were stored frozen at -80˚C until DNA was extracted from them.

Method for DNA isolation

Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller and coworkers (Miller *et al*., 1988).

Detection of Vitamin- D Receptor TaqI (VDR Taq1) SNP via PCR-RFLP method

The PCR was carried out in a final volume of 25 µl, containing 100 ng of genomic DNA(4-5 µl), 2.5 µl of 10X Taq polymerase buffer (10 mMTrisHCl pH 8.8, 50 mMKCl, 1.5 mM MgCl2, 0.01% gelatin, 0.005% Tween-20, 0.005% NP-40; final concentration 1X; Genetix Biotech Asia Pvt. Ltd.,India), 1 µl of 10 mMdNTPs (BangloreGenei, Bangalore, India), 1 µl of 25 pmol/µl of forward and reverse primers specific for and 1 µl of unit of 1U/ µl Red Taq DNA polymerase (Merck biosciences).

Thermal Profile

The PCR conditions were - initial denaturation at 94°C for six minutes followed by 35 cycle of 94°C for 45 seconds, 63°C for 60 seconds, followed by 72°C for 75 seconds, and a final extension at 72°C for seven minutes.

Restriction Digestion by Taq1

Following amplification the site on VDR gene was detected by RFLP (Restriction Fragment Length Polymorphism) using the restriction endonuclease Taq1 (GENEI, Banglore, INDIA) at 65°C for four hours. Digested restriction fragments were separated on 2.5% (w/v) agarose (Sigma) gels. Bands were visualized on an UV Transilluminator Imaging system for further analysis of genotype.

Restriction Digestion by Taq1

Following amplification the site on VDR gene was detected by RFLP (Restriction Fragment Length Polymorphism) using the restriction endonuclease Taq1 (GENEI, Banglore, INDIA) at 65°C for four hours. Digested restriction fragments were separated on 2.5% (w/v) agarose (Sigma) gels. Bands were visualized on an UV Transilluminator Imaging system for further analysis of genotype.

Genotype

Taq 1 polymorphism was analyzed by PCR –RFLP METHOD. PCR Amplification products of 293bp, 340bp and 47bp were obtained. product which was digested with taq-1 enzyme . TT homozygous for the absence of TaqI site 340 bp only; tt homozygous for the presence of TaqI site 293 bp and 47 bp, in case of heterozygosity Tt, all three bands (340 bp, 293 bp and 47 bp) were exhibited.

Results and Discussion

Biochemical and clinical findings

Table 5: Anthropometric and Biochemical profile of osteoporotic patients and healthy controls

Characteristic	Osteoporotic patients (108)	Healthy control (120)	*P* value
Age(yr)	55.40 ±4.79	56.13 ±5.13	0.2630
Weight	61.3±9.5	68.8±7.9	0.001
BMI(Kg/m2)	27.76±2.25	28±1.71	0.01
Hip BMD(gr/cm2)	0.97±0.1	0.92±0.1	0.002
Spine BMD(gr/cm2)	1.15±0.14	1.1±0.21	0.05
Serum Vitamin D(nmol/L)	26.05 ± 8.22	29.02 ± 6.17	0.004

*denotes level of significant change between case and control

Biochemical test performed in the blood sample for following clinical parameters and the findings were tabulated. Statistical analysis was done by using student's t test and p value obtained suggest the level of significant changes here. The descriptive data and comparison of biochemical parameters of osteoporotic patients versus healthy controls are presented in Table 5. As expected the osteoporotic patients had markedly higher levels of weight (P=0.001), BMI(P= 0.01), serum Vitamin D

(P<0.004) compared to that of healthy control subject.

The TaqI polymorphism, located in VDR exon 9, is in the same PCR fragment as the ApaI polymorphism. Therefore, the same PCR product for ApaI was used to assess TaqI genotypes. The fragment of exon 9, loaded on an agarose gel and submitted to electrophoresis, also presents a fragment of Genotypes were assigned as follows: TT homozygous for the absence of TaqI site 340 bp only; tt homozygous for the presence of TaqI site 293 bp and 47 bp, in case of heterozygosity Tt, all three bands (340 bp, 293 bp and 47 bp) were exhibited.

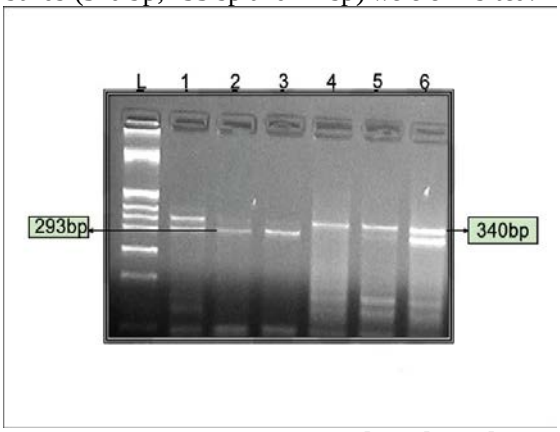

Figure 10: Represents PCR-based analysis of VDR gene Taq1 polymorphism

The distribution of the polymorphisms of VDR Taq-I polymorphism (rs731236) was consistent with Hardy- Weinberg equilibrium (HWE) in healthy controls. Overall genotype pattern of VDR gene was significantly different between case and control ($\chi 2$ =6.495, P value= 0.0389). osteoporotic postmenopausal women group showed increase in 'AA' genotype as compared to healthy control group (20.5% vs 12.8%). Genotype 'GG' was higher in HC group as compared to osteoporotic postmenopausal group (50.4% vs. 39.3%). An odds ratio of 0.6368 for 'GG' genotype indicated a protective effect of this common genotype in our population. An odds ratio of 1.761 of common 'AA' genotype group respectively was consistent with disease causing effect of this genotype in postmenopausal osteoporosis susceptibility. The heterozygous genotype 'AG' was higher in HC group as compared to postmenopausal osteoporotic group (50.4% vs 39.3%). An odds ratio of 0.153 of 'AG' showed weak or no association in postmenopausal osteoporosis susceptibility. Overall allele distribution was also non-significant but less common 'G' allele was found in higher frequency in control as compare to postmenopausal osteoporosis patients (68.8% vs 59.4%) and A allele was found at higher frequency in case as compare to control (40.6 vs 31.2%) and the difference was significant ($\chi 2$ =4.574 P= 0.0325). Carriage rate of allele 'G' was higher in HC group as compare to postmenopausal osteoporosis group. Whereas carriage rate of allele 'A' was higher in disease group as compare to healthy control group (60.7% vs. 49.6%) but not significantly different between case and healthy control ($\chi 2$=1.703, P=0.1919). odds ratio of minor allele 'A' which clearly indicates its little or moderate protective effect of minor allele "A" in our population meanwhile odds ratio of 1.509 (1.034 to 2.201) .

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

Fisher exact test value

Genotype	CASE N=112	CONTROL 125	P Value	odds ratio (CI)
AA AG GG	23 20.5 45 40.2 44 39.3	16 12.8 46 36.8 63 50.4	 0.1176 0.5960 0.0911	1.761 (0.8769 to3.535) 1.153 (0.6828 to 1.949) 0.6368 (0.3799 to 1.067)
Allele frequency A G	 91 40.6 133 59.4	 78 31.2 172 68.8	 0.0349*	 1.509 (1.034 to 2.201)
Carriage rate A G	 68 60.7 89 79.4	 62 49.6 109 87.2	 0.2144	 1.343 (0.8618 to 2.094)

Discussion

Environmental factors that influence bone density include dietary factors such as calcium, alcohol, and caffeine intake and lifestyle factors such as exercise and smoking. Ethnic differences in the susceptibility

to non-traumatic bone fracture suggest that genetic variations such as gene polymorphisms play an important role in this matter. Some examples of genetic markers that have been investigated in association studies of bone mineral density (BMD) or osteoporosis are as follows: the single nucleotide repeats (SNPs) in the vitamin D receptor (VDR) gene using Bsm1, Taq1, Apa1, Tru91, and Fok1 restriction enzymes; the estrogen receptor gene using Xba1 and Pvu1 restriction enzymes; and nucleotide repeat polymorphism in the Sp1 binding site in the collagen type 1 alpha 1 gene promoter. The nuclear receptors represent a group of important transcription factors, where the 48 members of this superfamily belong to an identified group of mammalian genes involved in transcriptional regulation (Carlberg C *et al*.,2009). By screening with different restriction enzymes, only some restricted areas of VDR gene could be analysed to verify DNA sequence variations (Kostner K *et al*.,2009). The most commonly investigated VDR polymorphisms are FokI (rs10735810 C>T), located in exon 2 of VDR, BsmI (rs1544410 G>A), located in intron 8, and TaqI (rs731236 T>C), located in exon 9 of VDR (Lemos MC *et al*.,2008) . These are single nucleotide polymorphisms (SNPs), where FokI is located at the 5' end of VDR gene and the other three SNPs are at the 3' end of the gene (Buttigliero C *et al*.,2011; Lemos MC *et al*.,2008). Most efforts to identify functional sequence variations in the VDR gene have been focussed on the 3' regulatory region. While the TaqI RFLP is located near the 3' end of the gene, the LD extends into the 3' regulatory region containing the UTR [S Mitra *et al*., 2006]. A.G. Uitterlinden et al. 2004 saw that the 3'UTR of the VDR gene contains many polymorphisms and thus, through the strong LD, these might explain associations observed with TaqI RFLP. The 3'UTR of genes is known to be involved in regulation of expression, especially through regulation of mRNA stability, including for steroid receptors which contain extensive 3'UTRs. For the latter, receptor polymorphisms in the 3'UTR have been described in the so-called AUUUA-motifs which influence the mRNA stability[AG Uitterlinden *et al*., 2004].

Overall genotype pattern of VDR gene was significantly different between case and control ($\chi 2$ =6.495, P value= 0.0389). osteoporotic postmenopausal women group showed increase in 'AA' genotype as compared to healthy control group (20.5% vs 12.8%). Genotype 'GG' was higher in HC group as compared to osteoporotic postmenopausal group (50.4% vs. 39.3%). An odds ratio of 0.6368 for 'GG' genotype indicated a protective effect of this common genotype in our population. An odds ratio of 1.761 of common 'AA' genotype group respectively was consistent with disease causing effect of this genotype in postmenopausal osteoporosis susceptibility. The heterozygous genotype 'AG' was higher in HC group as compared to postmenopausal osteoporotic group (50.4% vs 39.3%). An odds ratio of 0.153 of 'AG' showed weak or no association in postmenopausal osteoporosis susceptibility.

Overall allele distribution was also non-significant but less common 'G' allele was found in higher frequency in control as compare to postmenopausal osteoporosis patients (68.8% vs 59.4%) and A allele was found at higher frequency in case as compare to control (40.6 vs 31.2%) and the difference was significant ($\chi 2$ =4.574 P= 0.0325). Carriage rate of allele 'G' was higher in HC group as compare to postmenopausal osteoporosis group. Whereas carriage rate of allele 'A' was higher in disease group as compare to healthy control group (60.7% vs. 49.6%) but not significantly different between case and healthy control ($\chi 2$=1.703, P=0.1919). odds ratio of minor allele 'A' which clearly indicates its little or moderate protective effect of minor allele "A" in our population meanwhile odds ratio of 1.509 (1.034 to 2.201) .For the TaqI (rs731236) polymorphism Gursoy et al., 2008, Marozik et al., 2013 and Mercado et al., 2013 showed no association to osteoporosis, while Douroudis et al., 2003 showed an association of the TT genotype with osteoporosis. Mitra et al., 2006 and Duman et al., 2004 showed the association of tt genotype with osteoporosis. It has been suggested some of these contrasting results may have been due to insufficient sample size or because of possible genetic effects were masked by different gene-gene and gene-environment interaction. The inconsistent findings between our study and the data reported in literature are likely related to both the ethnic differences among the study populations and to the different inclusion criteria, as well as to the lack of a standardized approach to define pathological phenotypes .

References

1. Bonnick, S.L. and Lewis, L.A. (2006). Bone Densitometry for Technologists. Toronto, Canada: Humana Press. pp 8-9, 221-44, 258-265, 289-312.
2. Raisz LG, Rodan GA. Pathogenesis of osteoporosis. Endocrinol Metab Clin North Am. 2003 Mar;32(1):15-24.
3. Ralston SH, Uitterlinden AG, Brandi ML et al (2006) Large-scale evidence for the effect of the COLIA1 Sp1 polymorphism on

osteoporosis outcomes: the GENOMOS study. PLoS Med 3:e90
4. S A Miller, D D Dykes, and H F Polesky A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988 Feb 11; 16(3): 1215.
5. Carlberg C, Seuter S. A genomic perspective on vitamin D signaling. Anticancer Res. 2009;29(9):3485-93. 46
6. Kostner K, Denzer N, Muller CS, Klein R, Tilgen W, Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. Anticancer Res. 2009;29(9):3511-36.
7. Buttigliero C, Monagheddu C, Petroni P, Saini A, Dogliotti L, Ciccone G, et al. Prognostic role of vitamin d status and efficacy of vitamin D supplementation in cancer patients: a systematic review. Oncologist. 2011;16(9):1215-27.
8. Lemos MC, Fagulha A, Coutinho E, Gomes L, Bastos M, Barros L, et al. Lack of association of vitamin D receptor gene polymorphisms with susceptibility to type 1 diabetes mellitus in the Portuguese population. Hum Immunol. 2008;69(2):134-8.
9. Mitra S, Desai M, Ikram M. Vitamin D receptor gene polymorphisms and bone mineral density in postmenopausal Indian women. Maturitas 2006.
10. Uitterlinden AG, Fang Y, van Meurs JB, van Leeuwen H & Pols HA. Vitamin D receptor gene polymorphisms in relation to Vitamin D related disease states. J Steroid Biochem Mol Biol 2004 89-90 187-193.
11. Marozik P[1], Mosse I, Alekna V, Rudenko E, Tamulaitienė M, Ramanau H, Strazdienė V, Samokhovec V, Ameliyanovich M, Byshnev N, Gonchar A, Kundas L, Zhur K.Association Between Polymorphisms of VDR, COL1A1, and LCT genes and bone mineral density in Belarusian women with severe postmenopausal osteoporosis. Medicina (Kaunas). 2013;49(4):177-84.
12. Douroudis K[1], Tarassi K, Ioannidis G, Giannakopoulos F, Moutsatsou P, Thalassinos N, Papasteriades C. Maturitas.Association of vitamin D receptor gene polymorphisms with bone mineral density in postmenopausal women of Hellenic origin.Maturitas. 2003 Jul 25;45(3):191-
13. GURSOY S ,Emin ERDAL, Belgin ALASEHIRL , Ali AYDENIZ,Nuran ERDAL TaqI Polymorphism of the Vitamin-D Receptor Gene and Quality of Life in Postmenopausal Turkish Women 2008; 38 (1): 21-26
14. Duman, Refik Tanako, Nevin Erensoy, Melek uzturk, Selma Yılmazer Vitamin D Receptor Alleles, Bone Mineral Density and Turnover in Postmenopausal Osteoporotic and Healthy Women Med Princ Pract 2004;13:260–266
15. Mercado A , Sanchez-Lopez JY , Ibarra B Risk factors for osteoporosis in postmenopausal women from Guadalajara, Jalisco 2013 Dec;55(6):627-30.

How to cite this article
Mishra A. and Agnihotri S. (2018). VDR Taq1 gene polymorphism in osteoporosis: A study from central India. *Int. J. Pharm. Life Sci.*, 9(3):5757-5761.
Source of Support: Nil; Conflict of Interest: None declared

Received: 09.02.18; Revised: 23.02.18; Accepted: 16.03.18