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Study of Association Between Vitamin –D-Receptor Startcodon Polymorphism with Bmd Levels in Post Menopausal Women of The South Indian District East Godavari

KEYWORDS: FOK I POLYMORPHISM, BMD, osteopenia, SCP, SOS

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ABSTRACT *Osteoporosis is one of the common chronic disorders found in post menopausal women, which is characterized by low bone mineral density (BMD). Vitamin –D Receptor (VDR) is a member of hormone receptor family. It modulates the action of genes which directly promotes intestinal calcium absorption. The aim of this study is to assess the BMD levels in post-menopausal women with respect to the FOK-I polymorphism of VDR gene.The present study includes one hundred and sixty post-menopausal women of East Godavari District of Andhra Pradesh, aged between 42 and 83. All subjects were tested for BMD using speed of sound (SOS) at calcaneum by QUS (qualitative ultrasound) method. Out of 160 subjects, 29 were normal, 63 were osteopenic and 68 were osteoporotic. PCR-RFLP analysis of start codon polymorphism (SCP) of VDR indicates that there is no significant association between VDR SCP polymorphism & Osteoporosis among South Indian East Godavari post-menopausal women*

INTRODUCTION

Osteoporosis is a multi-factorial disease which is characterized by low bone mass and micro-architectural deterioration of bone tissue with increased susceptibility to fracture. Both genetic and non genetic factors are involved in maintenance of bone mass. Genetic factors are estrogen, calcitonin, vitamin-D receptor etc and non-genetic factors include smoking, alcohol consumption, calcium uptake . Vitamin D Receptor has been suggested as one of the candidate genes for genetic control of bone mass (Kim JG et al. 2001). VDR is a member of nuclear hormone receptor super family that mediates the action of target genes which help in intestinal calcium absorption (Judith M Wishart et al. 1997). Cloning of EXON 2 of VDR gene revealed presence of 2 translation initiation codons separated apart by 3codons. Allelic variants of this polymorphism of VDR were recognized by FOK I restriction endonuclease (Young Min Choi et al. 2000). F &f alleles have more significance due to their presence at translation initiation codon. VDR coded by F is shorter than VDR f by 3 amino-acids. Thymine/Cytosine polymorphism within the first initiation codon results ACG codon (Joseph M et al. 2000). Shorter form of VDR showed greater transcriptional activation in transfection experiments. So, the aim of our present study is to assess whether there is any significant association between VDR SCP & BMD or not.

SUBJECTS & METHODS

SUBJECTS

One hundred and sixty post-menopausal women, of East Godavari District of Andhra Pradesh, India, between the ages of 42 to 83 were tested for BMD. The exclusion criteria excluded women who have undergone hysterectomy, thyroid treatment and using any drugs influencing the bone metabolism prior to the determination of BMD. Written informed consent was obtained from each subject before testing them.

DATA COLLECTION

All subjects were interviewed using a structured questionnaire. Data like height &weight were collected on the anthropometric tools.

BMD ANALYSIS

Bone mass was assessed by speed of sound (m/s) at the calcaneum using QUS device (Maarten W et al.1999).It measures bone mass in the form of T-score which is calculated by

$$\text{T - score} = \frac{\text{SOS subject - SOSpeak value for young individuals}}{\text{standard deviation peak value for young individuals}}$$

using the following equation.

GENOTYPING

Peripheral blood samples were collected from each subject & genomic DNA was isolated by LAHIRI & NURNBERGER (1991) method. EXON 2 region of VDR gene was amplified by polymerase chain reaction by using Forward Primer 5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3'Reverse Prime 5'-ATGGAAACACCTTGCTTCTTCTCCCTC-3'. PCR reaction was performed by adding 1ul of DNA to 48 μl of PCR mixture (1 μl forward primer,1 μl reverse primer, 1 U Taq polymerase , 4 μl mgcl2, 5 μl PCR buffer & 36 μl water). PCR reaction was carried out at Initial denaturation 940C /5min Followed by 35 cycles of denaturation 940C/30sec,primer annealing 610C/40sec ,extension 720C/40sec &final extension 720C/7min. 265bp PCR product was generated after amplification. The PCR products were digested with FOK I Restriction endonuclease (Fermentas) at 370C for 16hours. Then electrophoresed through 2% agarose gel. After digestion 2 types of patterns were generated. Presence of restriction site which is designated as f gave 196 bp& 69bp fragments and absence of restriction site F, resulted single fragment of 265bp

STATISTICAL ANALYSIS

The variables considered were age, height, weight, BMI and BMD. The difference between the subjects was considered significant if the P value was less than 0.05. All measurements are expressed as mean $\pm$ SD. Statistical analyses were performed using minitab ver 16.1

RESULTS

To investigate the influence of VDR polymorphism on BMD in East Godavari post menopausal women, we have selected 160 individuals aged between 42 to 83 years, with a mean age of 56.36 ± 8.86 years.

Basing on the BMD measurements at calcaneum, selected subjects were divided into normal (29) and low bone mineral density group (131). Further low BMD group were divided in to osteopenia [63 (39.375%)] and, osteoporosis [68 (42.5%)]. t-test analysis between BMD and other parameters such as age, weight, height and BMI were given in the Table 1. This data indicates that there is no statistical significant association.

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Table – 1 Statistical comparisons between normal & abnormal BMD groups

	Normal	Abnormal BMD group	T-value	P-Value
Age	53.06 ± 6.7	57 ± 9.11	2.02	0.054
Weight	58.56 ± 11.9	53.12 ± 9.9	1.71	0.104
Height	153.010 ± 4.98	151.5 ± 5.9	1.07	0.295
BMI	25.035 ± 5.12	23.13 ± 4.08	1.40	0.178

PCR RFLP products showed the respective bands at 265bp band (FF), 265,196&65bp bands (Ff) and 196&65bp bands (ff). The genotypic & allelic frequencies of FOK 1 polymorphism of all the 160 subjects were shown in Table 2. This data shows that 11.76% of FF, 9.8% of Ff and 2.9% of ff individuals in normal BMD group, in osteopenia group of 63, 16.6% of FF, 7.8% of Ff and 4.9% of ff were observed, while 31.3% of FF, 7.8% of Ff and 6.8%of ff individuals in osteoporotic group. Genotype determinants for osteoporosis, osteopenia & normal were summarized in Table 3. This data showed no significant influence of FOK 1 on calcaneal BMD as the P value is 0.223 which is determined by Chi – square analysis.

Table – 2 The genotypic & allelic frequencies of FOK 1

	FF	Ff	Ff	F	F
Normal	11.76%	9.8%	2.9%	16.6%	7.8%
Osteopenia	16.6%	7.8%	4.9%	20.5%	8.8%
Osteoporosis	31.3%	7.8%	6.8%	35.2%	10.7%

Table-3 Statistical ComparisionAmong BMD Groups& VDR Polymorphism

	Normal	Osteo-penia	Osteopo-rosis	Chi-Square	P-value
FF	11.76%	16.6%	31.3%	12.11	0.002
Ff	9.8%	7.8%	7.8%	1	0.607
Ff	2.9%	4.9%	6.8%	4.5	0.105

DISCUSSION

Osteoporosis is a multi-factorial disorder with environmental and genetic factors influencing bone density. Genetic factors are responsible for more than 70% of problems associated with BMD (Katerina Zajickova and IvanaZofkova. 2003). Literature review indicated a contradicting reports on the association of VDR polymorphism and BMD.(Cheng W. C. & Tsai K. S. (1999). The possible association of SCP of vitamin D receptor gene with differences in BMD has received great attention because, a number of studies supported that, SCP at the translation initiation site in exon 2 of the VDR gene results change in VDR structure because F allele lacks first ATG and the m-RNA translation starts from second ATG. Hence, F allele produces receptor which is shorter by 3 amino-acids than f allele (Andre .G et al .2004). However, few studies reported no or weak significant association between SCP of VDR and BMD (E. M. C. Lau et al. 2002). A previous study of FOK 1 polymorphism in Andhra Pradesh, India showed significant association with low BMD (Yasovanthi et al .2011). In contrast ,Our findings with the test group of East Godavari district, India showed no association between FOK 1 polymorphism of VDR gene & BMD as the P value is >0.05 as shown in the Table 3.

Various epidemiological studies indicate that frequency of polymorphism in these genes and their association with low bone mass varies from population to population and country to country. Studies on bone mineral density suggest that the differences in osteoporosis related phenotypes between ethnic groups may be partially the result of the different ethnic background At the same time environment and life style factors can't be ignored because ethnic differences also occurs for lifestyle factors, such as sun-light exposure, smoking, exercise and alcohol consumption. Non - genetic factors of this study has no statistically significant association (table-1) Hence, it requires further study with large sample data to evaluate the influence of genetic & non genetic markers on osteoporosis.

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