

## 5.

## MENOPAUSE

As a part of the aging process, women above 45 years enter into the phase of menopause that leads to silent physical change including osteoporosis and genitor-urinary problems, which are preventable to some extent. The Institute's research programmes have focused on prevention and management of postmenopausal osteoporosis; genito-urinary dysfunction and to educate women about lifestyle changes and medications that may help lead a better quality of life after menopause.

### 5.1 **Assessment of Prevalence of Osteoporosis in Adult Population in India** (ICMR multicentre study)

Principal Investigator: **Rashmi Shah**

Project Associates: Lalita Savardekar, M. I. Khatkhatay,  
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Pramodini Phatak, Suchita Utekar, Reshma  
Sathe, Harvinder Kaur Sudan, Madhu Singh,  
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Duration: 2002 -2006

Osteoporosis, a major public health problem is associated with substantial morbidity and socio-economic burden. It is a condition that can be prevented and treated if diagnosed early and accurately. Measuring bone density is the most important tool in the diagnosis of osteoporosis. The gold standard for measuring bone density is the dual energy x-ray absorptiometry (DEXA), a useful tool for both the axial and appendicular skeleton. The bone mineral density (BMD) values measured by the Hologic DEXA machine are based on Caucasian data. There are studies that document inter ethnic variation in BMD measurements and no norms are available for the Indian population.

The objectives of this study are (a) to establish peak bone mineral density reference values for Indian men and women and (b) to assess the prevalence of osteopenia and osteoporosis in Indian population.

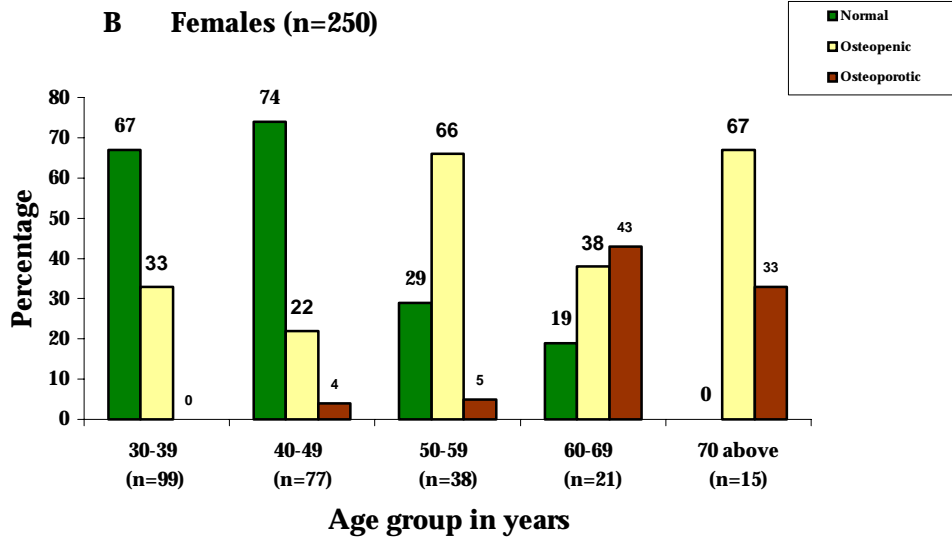
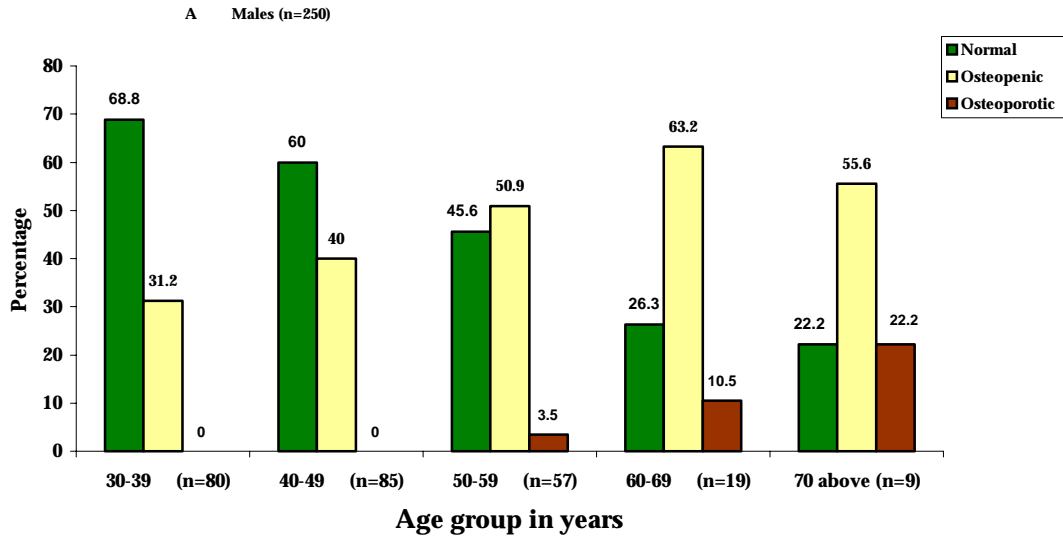
The staff attended a training workshop at NIN, Hyderabad during February 2003 and at AIIMS, New Delhi in April 2003. To establish BMD reference values, 100 healthy men and women each between 20 to 30 years of age belonging to the higher socioeconomic group will be enrolled. This study will generate reference values for the Indian population. To assess the prevalence of osteopenia and osteoporosis, 1500 subjects (750 men & 750 women) in the age group 30 to 70+ years from the three socio-economic groups i.e. higher, middle and lower will be enrolled. Each socio-economic group will have 500 subjects (250 men and 250 women) enrolled. Osteopenia and osteoporosis will be identified using the values generated in the first part of the study.

Biochemical tests which include haemoglobin, serum calcium, alkaline phosphatase, albumin, creatinine, vitamin D, intact PTH and urinary fluoride will be estimated in all subjects of the reference group and 20 percent of the randomly selected subjects of the prevalence group.

Survey of households from middle-income group was carried out from February 2003 to July 2003. Selection of volunteers for the study was done as per random selection of household. The enrollment of 500 volunteers (250 males and 250 females) and blood collection in 100 of the randomly selected volunteers has been completed. Biochemical tests are being carried out in the collected samples. Data verification has been completed. Data entry of 500 subjects has been completed and sent to New Delhi for further data analysis.

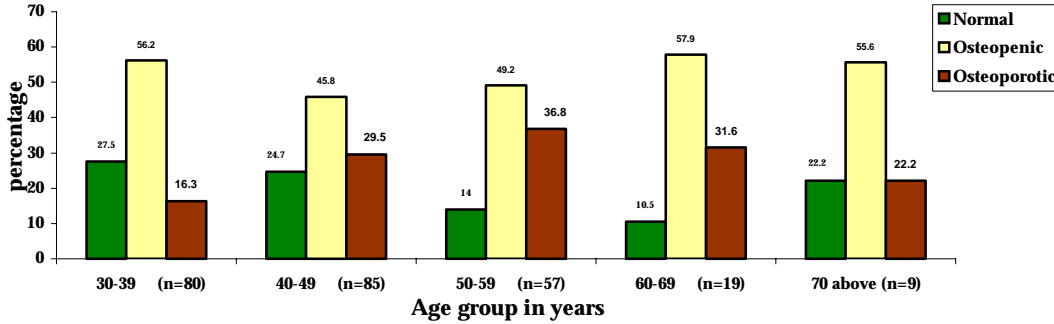
The diagnosis of osteopenia and osteoporosis using the Caucasian norms reflects that after 50 years of age, increasing number of women develop osteopenia and osteoporosis as seen in the hip and spine than men (Fig. 97). Osteoporosis of the spine appears earlier than at the hip and is more common in women as compared to men (Fig. 98).

Household survey of volunteers from lower & higher income group has been initiated from March 2004. The study is ongoing.

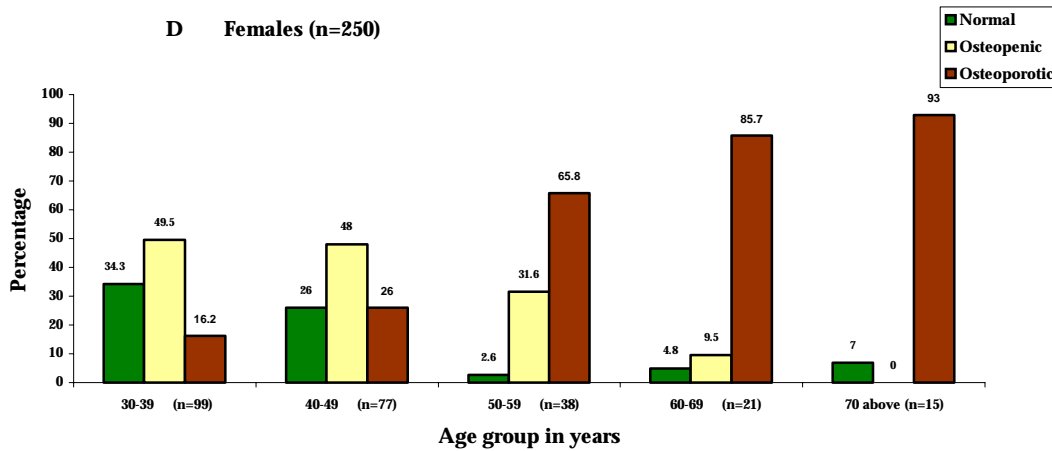


**Fig. 97: Age-wise BMD estimation of the Hip in males (A), females (B) with respect to Hologic standard**

**C Males (n=250)**



**D Females (n=250)**



**Fig. 98: Age-wise BMD estimation of the Spine in males (C) and females (D) with respect to Hologic standard**

**5.2 Biochemical Markers for Early Diagnosis of Osteoporosis** *(Funded by the Department of Science and Technology, Government of India)*

Principal Investigator: **Meena Desai**

Project Associates: M.I. Khatkhatay, U.M. Donde, A.H.Bandivdekar, K. Bhanu Prakash, Rashmi S. Shah and Lalita Savardekar

Collaborators: K.E.M. Hospital, BYL Nair Hospital, Mumbai ,S.L. Raheja Hospital, Mumbai

Duration: 2003-2007

Osteoporosis is a condition in which the absolute bone mass is below normal and is so reduced that there is an increased risk of fracture in absence of significant trauma. Bone status is generally assessed using bone mineral density

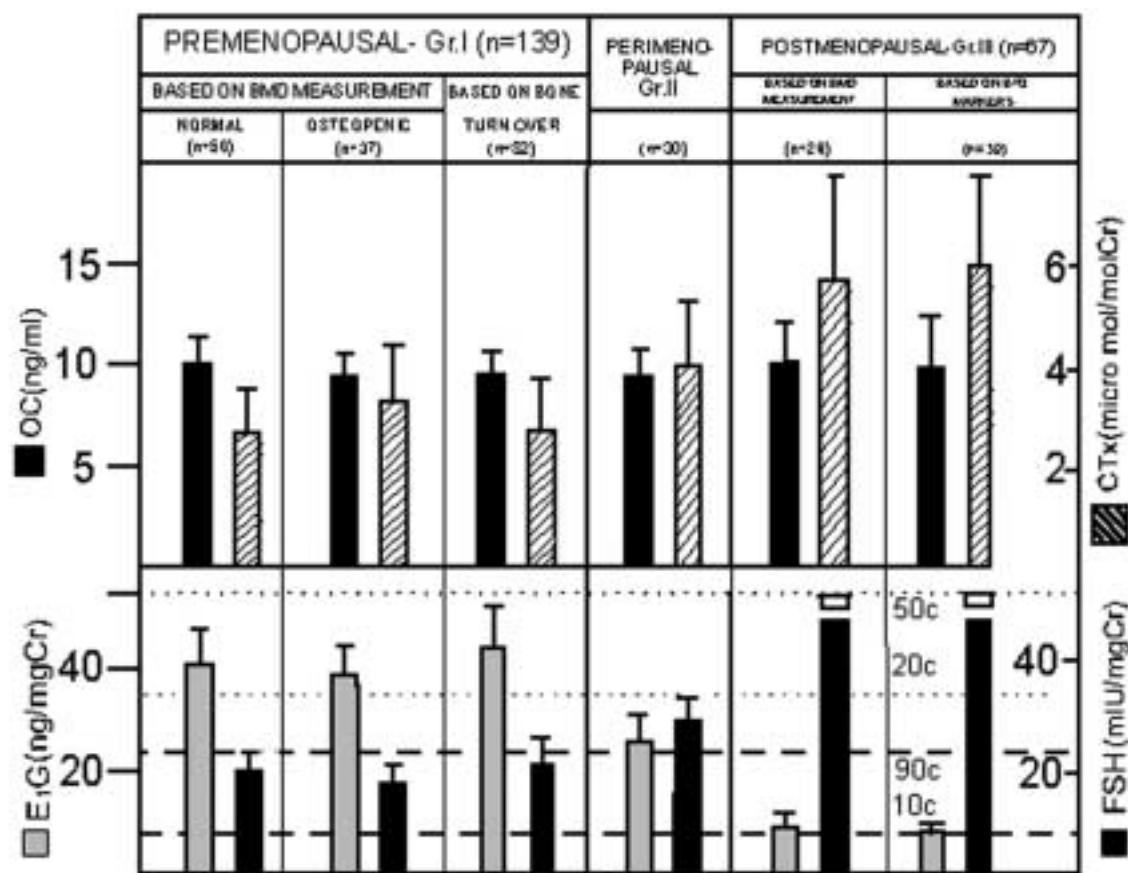
measurement and assays of biochemical markers of bone turnover. The main objectives of the study are to (1) develop simple ELISAs for osteocalcin (OC), bone specific alkaline phosphatase (BSAP), c-terminal telopeptides (CTx) and collagen-cross links (Pd/Dpd); (2) establish base line data of these markers in Indian subjects and correlate them with hormonal profiles, and to identify women at risk of developing osteoporosis.

*Purification of OC and development of an ELISA:*

Approximately 600 $\mu$ g OC with 98 percent purity was isolated from 90 g of bovine bones and used for generating polyclonal antibodies, resulting in relatively high titre antiserum. These were used to develop an ELISA. The assay so developed was validated and fulfilled the general criteria defined for labeled immunoassays.

*Age related changes in bone turnover markers and ovarian hormones:*

The age related changes occurring in bone turnover markers and ovarian hormones during pre and post menopause were studied. Data obtained from 236 women from neighboring hospitals, was analyzed for their relation between the hormonal and bone turnover markers. The women were categorized into premenopausal (Group I, n=139), perimenopausal (Group II, n=30) and postmenopausal (Group III, n=67). Fifty women in Group I were with normal bone mineral density (BMD) measurements and normal estrone glucuronide (E<sub>1</sub>G) and FSH levels (Fig. 99). In 37 women of Group I with osteopenia, the mean levels of E<sub>1</sub>G though normal were below 20<sup>th</sup> centile of reference norm and normal urinary FSH levels with moderately elevated OC and CTx levels. In 30 perimenopausal women (Group II) with irregular cycles, the mean E<sub>1</sub>G levels were significantly low with elevated FSH and CTx levels reflecting perimenopausal changes as compared to the premenopausal women. These observations indicated that women with irregular cycles, low E<sub>1</sub>G and elevated FSH levels should be screened for osteoporosis.



**Fig. 99: Relation between E<sub>1</sub>G, FSH, OC and CTx levels in premenopausal, perimenopausal and postmenopausal women.**

*Establishment of reference values for bone turnover markers.*

Two hundred and fifteen women were enrolled from the Elderly Woman’s Clinic, Naigaum during the year. BMD were measured in these women by DEXA and biochemical markers of bone turnover are being estimated in serum and urine samples including OC, BSAP, CTx and Dpd. This data will establish reference norms for biochemical markers of bone turnover in Indian women. In-house assays will be developed for bone specific alkaline phosphatase and Dpd.

**5.3 Relevance of Changes in Bone Related Proteins and their Utility for Diagnosis of Osteoporosis in Indian Women.** *(Funded by Ministry of Health and Family Welfare, Government of India)*

Principal Investigator: **M.I. Khatkhatay**  
 Project Associates: Meena Desai, U.M. Donde, Vrinda V. Khole  
 Duration: 2002-2005

During last few years' progress has been made towards understanding mechanism of regulation of bone remodeling especially in relation to postmenopausal osteoporosis. Several investigations have suggested that estrogen prevents bone loss by modulating the secretion or release of cytokines that are known to influence bone remodeling. Recent studies also suggest that T cells are capable of modulating bone resorptive cytokines and thus have focused attention on the immune system playing an important role in the control of bone resorption.

Therefore, the objectives of the study were to (1) establish profiles of IL1, IL 6 and TNF $\alpha$  in healthy and osteoporotic women. (2) study the T-lymphocyte subsets (CD4, CD8) and correlate with bone mineral density measurements and bone turnover markers.

ELISA's for interleukin 1 (IL1), interleukin (IL6) and tumor necrosis factor (TNF $\alpha$ ) the well-known bone resorptive cytokines, were standardized and preliminary observations indicate that IL6 plays a dominant role in bone loss. Standardization RT-PCR for quantification of IL6 is under way and will be correlated with serum concentrations. Flow cytometric analysis of T subset population (CD4, CD8) and distribution pattern will be studied in peri and post-menopausal women.

#### **5.4 Genetic Factors Contributing To Osteoporosis: Study of Gene Poly Morphism in Vitamin D Receptor Gene and Estrogen Receptor Gene in Indian Population. (Partly funded by WHO)**

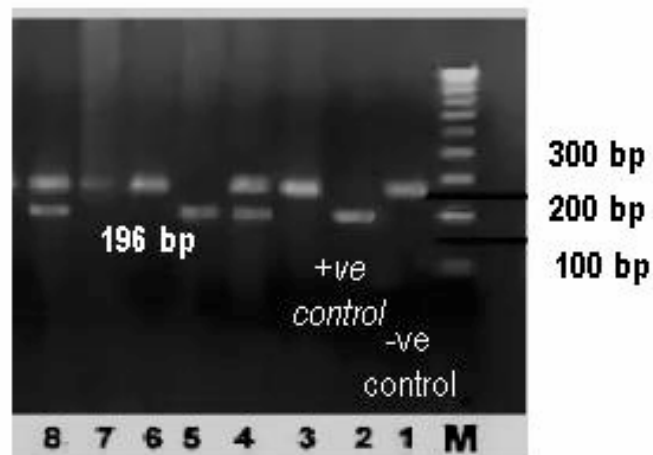
Principal Investigator:	<b>M.I. Khatkhatay</b>
Project Associates:	Meena Desai, Sumegha Mitra,
Project Collaborators:	Mahatma Gandhi Hospital, Mumbai Parsee General Hospital, Mumbai
Duration:	2002-2004

Earlier familial studies in twins suggest that bone mass and age related bone loss has a strong genetic determinant. Vitamin D receptor (VDR) and estrogen receptor (ER) genes have been implicated as important genetic factors affecting bone mass. Polymorphisms in these genes have been strongly correlated with low bone mass. It has also been reported that the frequency of polymorphisms in these genes varies from population to population. However, similar data has yet not been established for Indian women. Therefore, objectives of the study are to (1) study molecular epidemiological studies of polymorphisms in VDR and ER genes in Indian women; (2) correlate the frequency distribution of these polymorphic alleles in women with low bone mass; (3) identify individuals with mutation in genes resulting in increased risk of developing osteoporosis.

During the year methods for studying restriction fragment length polymorphism for vitamin D receptor gene and estrogen receptor gene were standardized.

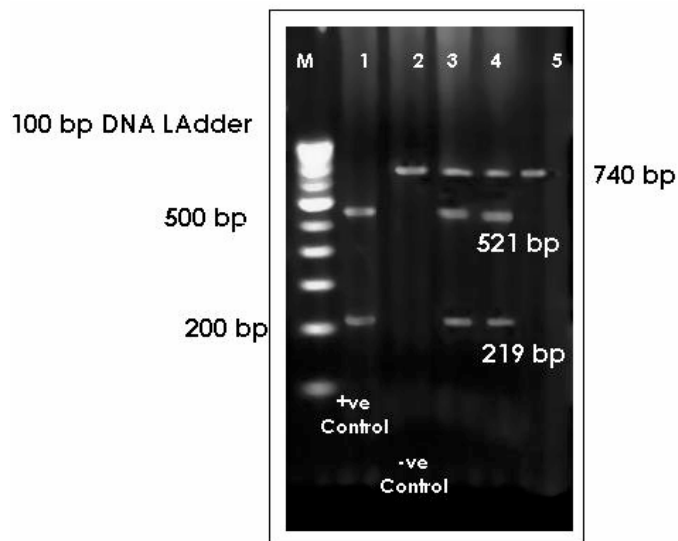
#### *Vitamin D receptor gene polymorphoism*

The Fok I polymorphic region of exon 2 was amplified using specific primers and the 265 bp PCR product was subjected to digestion with Fok I restriction enzyme. The presence of the Fok I restriction site on both the alleles (defined as ff) generated 196 and 69 bp fragments, whereas the absence (FF) yielded one undigested 265 bp fragment. Heterozygous Ff exhibited fragments of 265, 196 and 69 bp (Fig. 100).



**Fig. 100: Restriction Fragment length Polymorphism Analysis of Fok I Polymorphism. Lane 1-8 Restriction Digestion of osteoporotic 8 sample**

A single amplification was performed using specific primers for both Apa I and Taq I Restriction Fragment Length Polymorphism detections. In the case of Apa I, following restriction digestion the presence of restriction site on both the alleles designated as aa generated 521 and 219bp fragments, whereas absence yielded one undigested 740 bp fragment designated as AA and heterozygous Aa yielded 740, 521,219 bp fragments (Fig. 101).



**Fig. 101: Restriction Fragment length Polymorphism Analysis of Apa I Polymorphism. Lane 1-5 PCR Restriction Digestion of osteoporotic sample.**

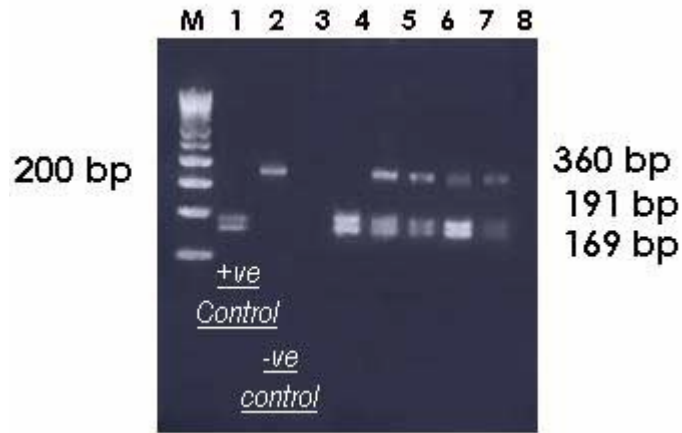
For Taq I genotypes PCR products were restriction digested with Taq I enzyme and it revealed homozygous TT (absence of Taq I restriction site) yielding bands of 245 bp and 495 bp. The homozygous tt exhibited 205, 245, 290 bp and the heterozygous Tt showed 495, 205, 245, 290 bp fragments (Fig. 102).



**Fig. 102: Restriction Fragment length Polymorphism Analysis of Taq I Polymorphism. Lane 1-8 Restriction digestion of osteoporotic sample.**

PCR amplification of the polymorphic Bsm I site was performed with specific primers resulting in a 360 bp fragment, which following restriction

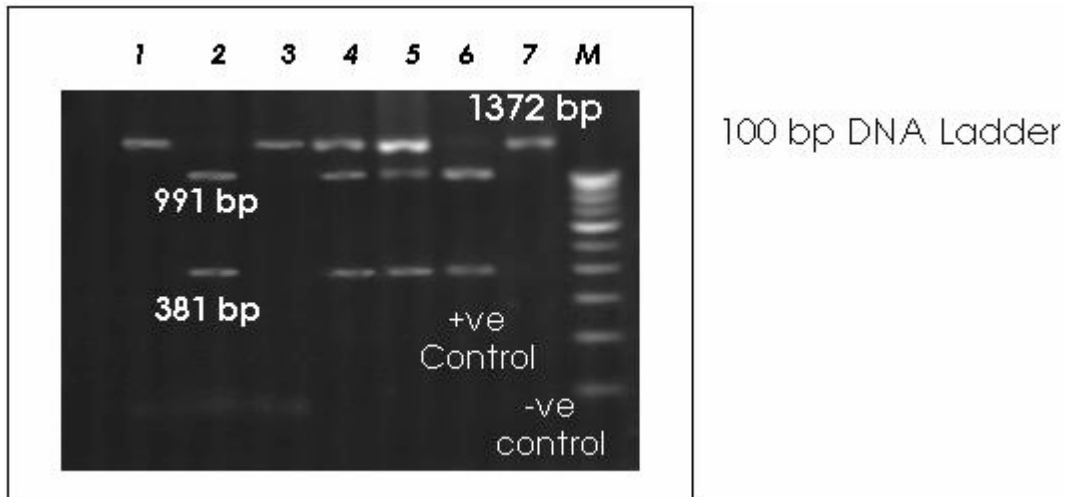
digestion separated into genotype BB (360bp), bb (191, 169 bp) and Bb (360, 191,169 bp) (Fig. 103).



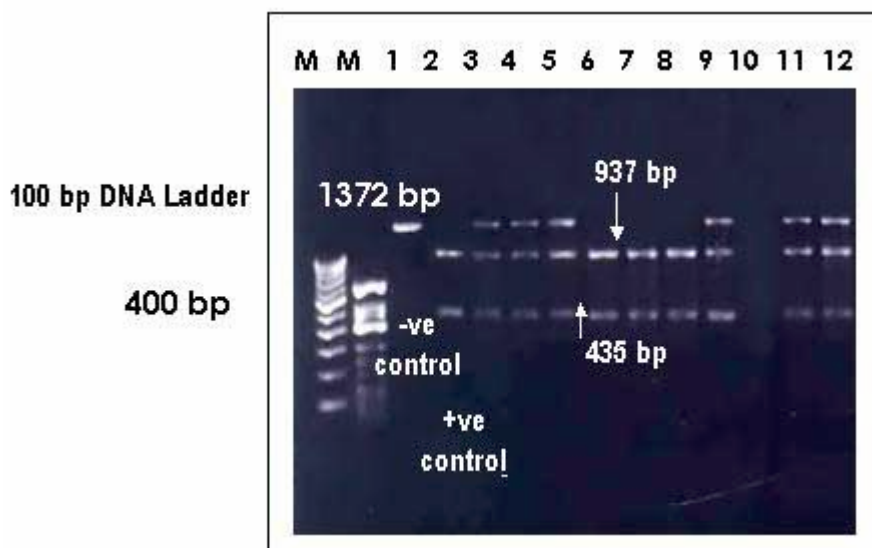
**Fig. 103: Restriction Fragment length Polymorphism Analysis of Bsm I Polymorphism. Lane 1-8 amplification of sample.**

*Estrogen receptor gene:*

The ER polymorphism was studied using specific primers amplifying 1.37 kbp PCR product. The PCR product was digested with Xba I and Pvu II at 65<sup>o</sup>C and 37<sup>o</sup>C respectively. The fragments following digestion with Xba I were found to be 991 and 381bp for presence of restriction site on both the alleles designated as xx and whereas 1372 bp for absence of restriction site designated as XX (Fig. 104). The fragments following digestion with Pvu II were found to be 937 and 435 bp for presence of restriction site on both the alleles designated as pp (Fig. 105).



**Fig. 104: Restriction Fragment Length Polymorphism Analysis of estrogen receptor Xba I Polymorphism. Lane 1-7 PCR Restriction Digestion of Osteoporotic samples.**



**Fig. 105: Restriction Fragment Length Polymorphism Analysis of estrogen receptor Pvu II Polymorphism. Lane 1-12: PCR Restriction Digestion of Osteoporotic samples.**

These observations corroborate well with those reported in literature. The impact of genetic factor is likely to be more significant amongst Parsees due to their intra-community marriages. Hence we plan to study VDR and ER gene polymorphisms in members of Parsee community.