4 MENOPAUSE AND OSTEOPOROSIS

As part of the aging process, women above 45 years of age enter into the phase of menopause that leads to silent physical changes including osteoporosis and genito-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.

The studies being pursued include: (i) estimation of peak bone mineral density reference values for Indian men and women and to assess the prevalence of osteopenia and osteoporosis in Indian women; (ii) establishment of base-line data for biochemical markers of bone turnover; (iii) assessment of the molecular and cellular mechanisms regulating osteoporosis; (iv) study of genetic factors contributing to osteoporosis with special reference to polymorphism in vitamin D receptor, estrogen receptor and collagenase I receptor genes.

4.1 Assessment of Prevalence of Osteoporosis in Adult Population in India (*ICMR multicentric study*)

Principal Investigator:	**Rashmi Shah**
Project Associates:	Lalita Savardekar, M.I. Khatkhatay, Hemali Dalvi, Reshma Sathe, S. Chawan, Harvinder Kaur Gill, Prerna Gade, Kirti Bodke, K. Chavan, Shalini Baji, Pramodini Phatak, Bhagyashree Kanje
Duration:	2002-2006

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

To establish BMD reference values, 100 healthy men and women each, in the age group of 20 to 30 years belonging to the higher socioeconomic group will be recruited. To determine the prevalence of osteopenia and osteoporosis, BMD measurements will be taken in 1500 subjects (750 men & 750 women) ranging from 30 to 70+ years and from three socio economic groups i.e. higher income group (HIG), middle income group (MIG) and lower income group (LIG). Osteopenia & 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.

The enrollment of 500 volunteers (250 males and 250 females) and blood collection in 100 of the randomly selected volunteers from MIG has been completed. Biochemical tests are being carried out in the collected samples. Data verification and data entry of 500 subjects (except blood report columns) has been completed & sent to New Delhi in June 2004 for further data analysis.

Household survey of volunteers from LIG was carried out from April 2004 to February 2005. 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. The data entry of these 500 subjects (except blood report columns) has also been completed and mailed to Headquarters in February 2005.

Standardization of Block Phantom of DEXA machine for quality control had been carried out under supervision of the BMD technician from NIN, Hyderabad. Urine samples of 100 subjects each of MIG & LIG groups have been sent to Lucknow for fluoride estimation. Thirty quality control samples for estimation of serum calcium, alkaline phosphatase and phosphorus were received from AIIMS, which have been analyzed and results sent back to AIIMS.

Preliminary data on BMD examination showed that osteoporosis at the hip was more in females as compared to males (8% vs 3%) both in MIG and LIG groups (Fig. 90). Similar findings were seen at the spine (Fig. 91). However, at the forearm, men had a slightly higher incidence of osteoporosis as compared to females (Fig. 92). As regards dietary calcium intake only 2-3 percent of subjects, both males and females, had an intake of 600-800 mg/day in both MIG and LIG groups. An intake of 400-600 mg/day was seen in 63 percent females in MIG as compared to 40 percent in LIG and in 53 percent males in MIG as compared to 33 percent in LIG (Fig. 93). As for sunlight exposure, almost 75 percent of females had exposure between 0-1 hr. Exposure to sunlight in males showed a wide range from 1 hour to more than 7 hours. Most of the males and females in both MIG and LIG groups had a mild to moderate degree of physical activity ranging from 77-92 percent.

Household survey of volunteers from HIG group and reference group has been initiated. The study is ongoing.

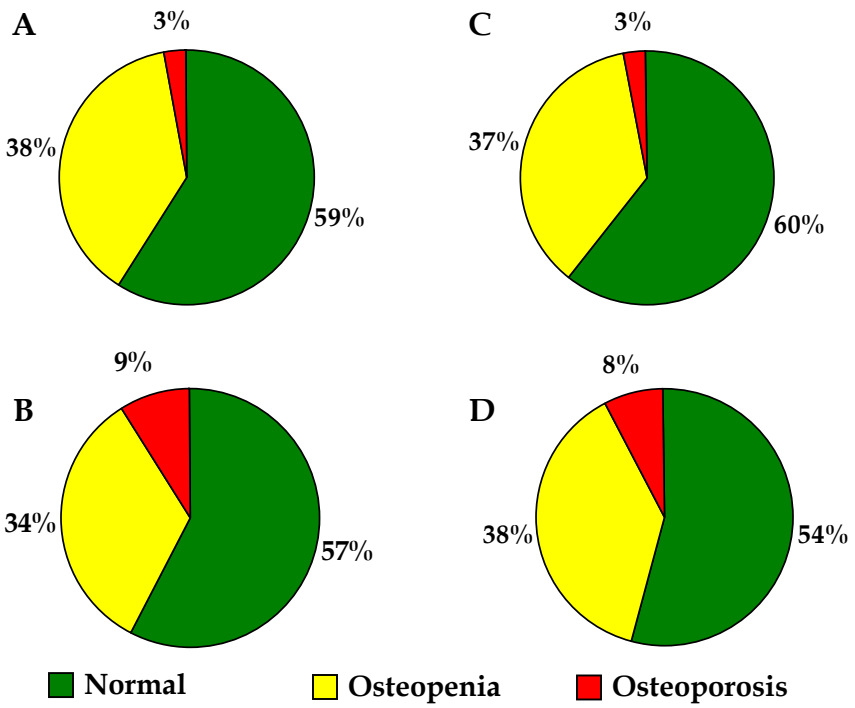

Fig. 90: BMD estimation of the hip in males (A) and females (B) in MIG and in males (C) and females (D) in LIG groups with respect to Hologic standard.

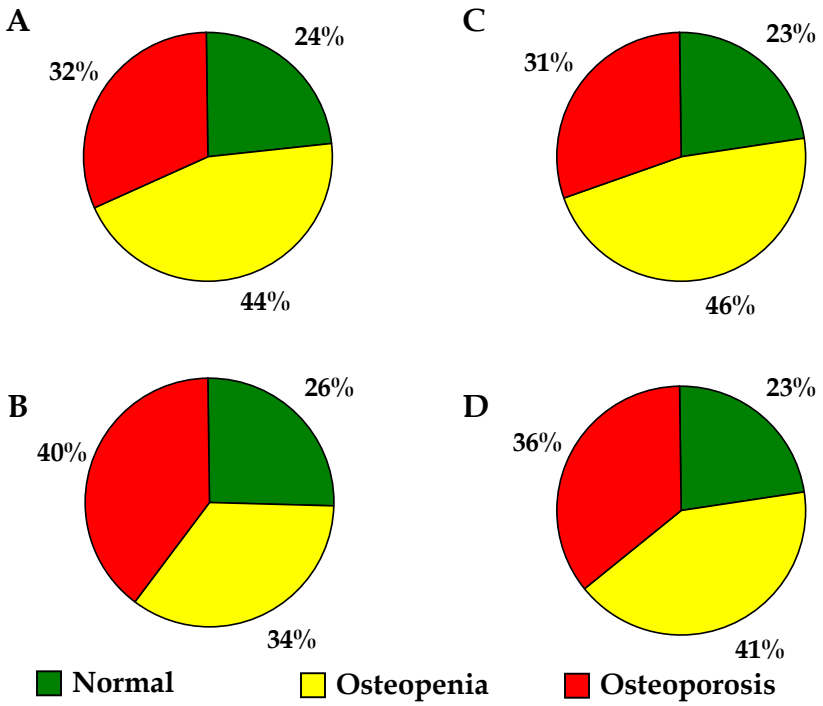

Fig. 91: BMD estimation of the spine in males (A) and females (B) in MIG and in males (C) and females (D) in LIG groups with respect to Hologic standard.

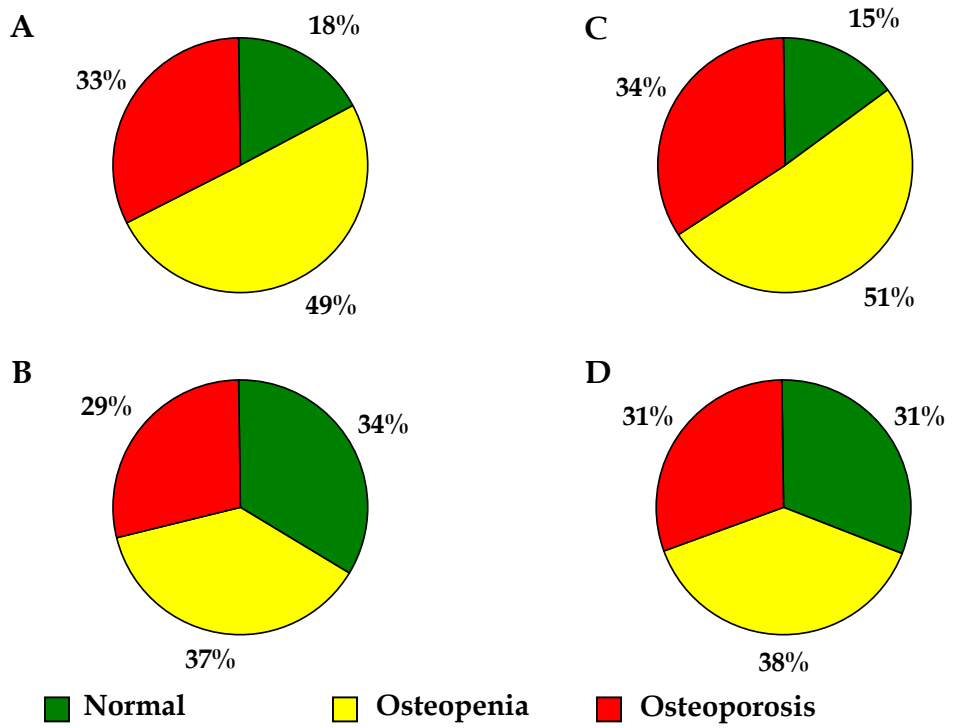

Fig. 92: BMD estimation of the forearm in males (A) and females (B) in MIG and in males (C) and females (D) in LIG groups with respect to Hologic standard.

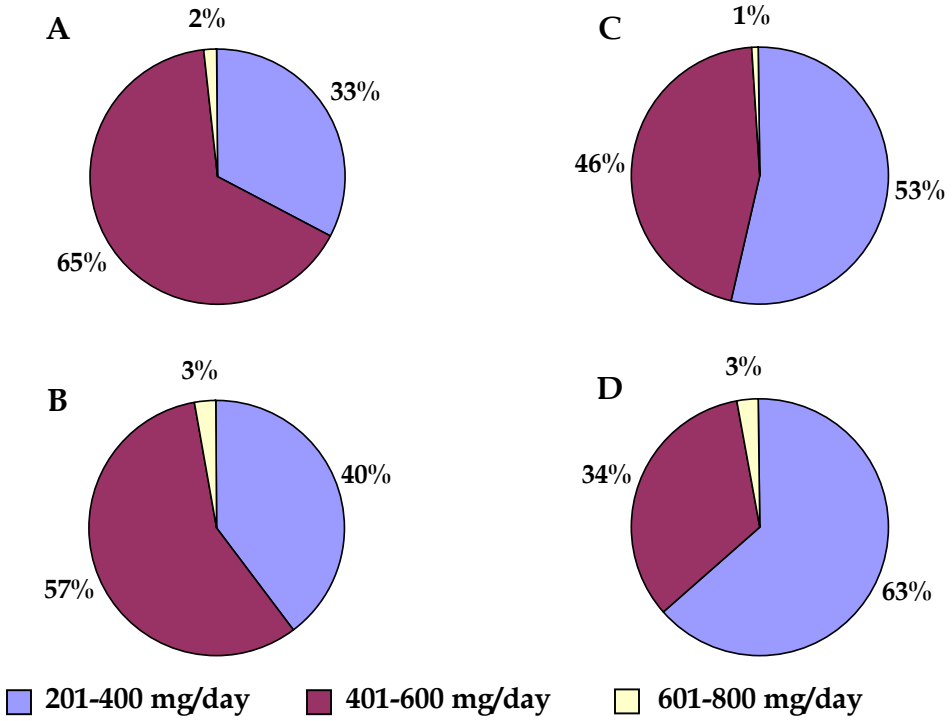

Fig. 93: Dietary calcium intake in males (A) and females (B) of MIG & in males (C) and females (D) of LIG groups.

4.2 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, K. Bhanu Prakash, Rashmi S. Shah and Lalita Savardekar

Duration: 2004-2007

Osteoporosis is one of the most common consequences of menopause with increased incidence of morbidity and mortality. Bones are constantly remodeled with old bone being replaced by new bone. There is gradual decline in bone mass in both men in women. However, women experience rapid bone loss after the age of 50 due to decline in estrogens during menopause. It is therefore, essential to identify these women at risk of developing osteoporosis. Bone status can be assessed by bone mineral density (BMD) measurements by DEXA and assays of biochemical markers of bone turn over. The project aims at developing simple immunoassays for selective markers of bone formation and bone resorption and establishment of reference values in Indian women for these markers.

Establishment of reference values for the bone turn over markers:

Three hundred and two women were enrolled from the Elderly Clinic during the year. Bone mineral bone density (BMD) was measured in these women by DEXA and biochemical markers of bone turn over namely Osteocalcin (OC), bone specific alkaline phosphatase (BSAP), c-terminal telo-peptide (CTx) and deoxypyridinoline (Dpd) were estimated in the serum and urine samples by ELISA. In addition, urinary hormones estrone glucuronide (E_1G) and FSH were estimated by indigenously developed ELISA while calcium, phosphorous and creatinine were estimated by standard methods. Data obtained from 302 women in the defined age group was segregated into women with normal bone mass, osteopenic and osteoporotic bone mass by DEXA. The levels of calcium, phosphorous and PTH were within the normal range indicating normal bone homeostasis in the subjects enrolled. The data was also classified into 5- yearly age band to depict the age related changes in levels of bone turn over markers reflecting the age related bone loss (Fig. 94).

The levels of bone formation and resorption markers were static up to the age-band 31-35 years. Once the peak bone mass is achieved, the bone resorption exceeds bone formation. The bone resorption, to certain extent, is compensated by increase in bone formation. However, there is a significant rise in the bone resorption due to the decline in the estrogen levels during menopause. The

markers of bone resorption namely CTx and Dpd are relatively more sensitive indicators of increased bone remodeling as seen by the higher percentage change in these markers (Fig. 95). Moreover, levels of bone resorption markers correlate well with fall in BMD and E_1G levels. A significant correlation coefficient was observed between the bone-turn over markers and BMD (Table 10-13). The purification of CTx and BSAP to develop in house assay is in progress.

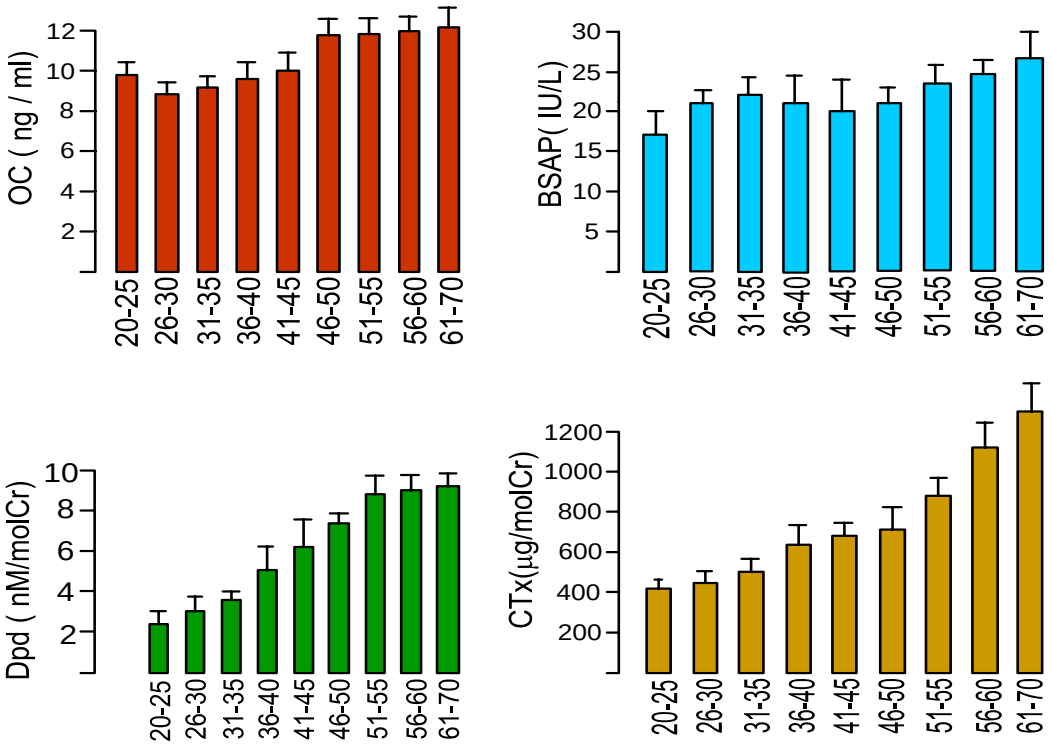

Fig. 94: Mean levels of bone turnover markers across the age bands (n= 220). Results are expressed in mean ± SD.

Table 10: Correlation coefficient between osteocalcin and BMD.

OC v/s Spine BMD				
Age	**r**	**A**	**B**	**p**
20-60	-0.954	1.759	-0.0701	< 0.001
46-60	-0.849	2.051	-0.0939	

OC v/s Hip BMD				
Age	**r**	**A**	**B**	**p**
20-60	-0.921	1.395	-0.0499	< 0.5
46-60	-0.897	1.815	-0.0840	

(A=Intercept; B=Slope)

Table 11: Correlation coefficient between bone specific alkaline phosphatase and BMD.

BSAP v/s Spine BMD				
Age	**r**	**A**	**B**	**p**
20-60	-0.877	1.827	-0.0372	
46-60	-0.999	1.850	-0.0394	< 0.05

BSAP v/s Hip BMD				
Age	**r**	**A**	**B**	**p**
20-60	-0.9138	1.465	-0.0276	
46-60	-0.991	1.583	-0.0331	< 0.5

(A=Intercept; B=Slope)

Table 12: Correlation coefficient between C-terminal telopeptide and BMD.

CTx v/s Spine BMD				
Age	**r**	**A**	**B**	**p**
20-60	-0.982	1.280	-0.0003	< 0.001
46-60	-0.992	1.218	-0.0030	

CTx v/s Hip BMD				
Age	**r**	**A**	**B**	**p**
20-60	-0.984	1.052	-0.0002	< 0.5
46-60	-0.992	1.054	-0.0002	

(A=Intercept; B=Slope)

Table 13: Correlation coefficient between deoxypyridinoline and BMD.

Dpd v/s Spine BMD				
Age	**r**	**A**	**B**	**p**
20-60	-0.902	1.257	-0.041	< 0.001
46-60	-0.842	1.703	-0.0945	

Dpd v/s Hip BMD				
Age	**r**	**A**	**B**	**p**
20-60	-0.857	1.026	-0.027	< 0.5
46-60	-0.801	1.433	-0.076	

(A=Intercept; B=Slope)

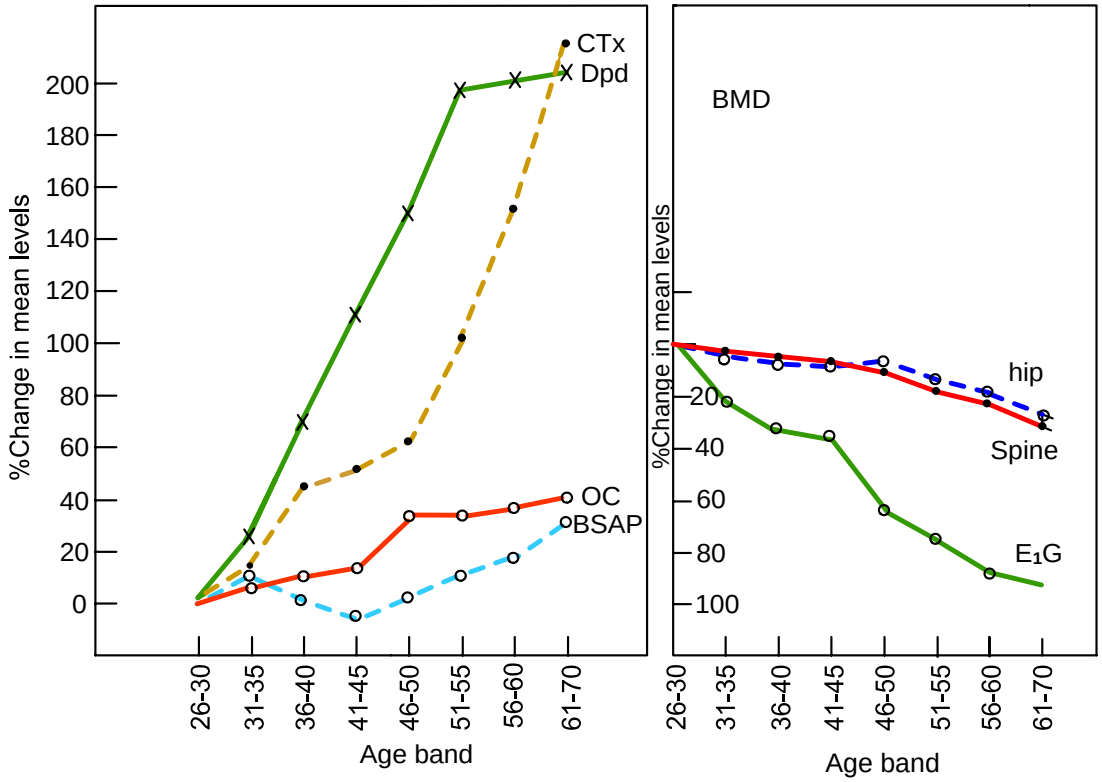

Fig. 95: Percent change in the levels of bone turnover markers and BMD across the age bands (n=220).

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

Principal Investigator: **M.I. Khatkhatay**

Project Associates: Meena Desai, U.M. Donde, Vrinda V. Khole,

Collaborator: V.R.Tasker, Streehitkarini, Mumbai

Duration: 2002-2005

During the last few years progress has been made towards understanding mechanism of regulation of bone remodeling specially in relation to post-menopausal osteoporosis. Several studies have suggested that the bone loss in women during postmenopausal period in response to estrogen deficiency is mediated by increased production of cytokines such as interleukin 1 (IL 1), interleukin 6 (IL 6) and tumor necrosis factor (TNF α). With availability of these evidences, it is interesting to know profiles of peripheral secretion of cytokines in healthy and osteoporotic women and correlate with the levels of bone turnover

markers and the measurements of bone mineral density at spine and femoral neck. It may then enable us to explore the possibility of these cytokines as important indicators of impending osteoporosis. The broad objectives of the study are to: (1) assess the profiles of cytokines IL 1, IL 6 and TNF α in Indian women (2) correlate the cytokine profiles with the changes in biochemical markers of bone turnover in Indian women (3) assess the apoptotic bone cell pattern in various types of osteoporosis.

The study involves estimation of cytokines in about 500 samples. The ready to use kits for cytokines available are expensive. We therefore decided to standardize ELISAs for cytokines by procuring individual components of the assays. Antisera to IL 1, IL 6, TNF α and enzyme conjugates were procured from BD Pharmingen, USA. Microtitre polystyrene plates were obtained from NUNC Laboratories, Denmark. We have successfully standardized in house non-competitive "Sandwich ELISAs" for IL 1, IL 6 and TNF-alpha. The developed assays have the desired sensitivity.

4.4 Genetic Factors Contributing to Osteoporosis: Study of Gene Polymorphism in Vitamin D Receptor Gene and Estrogen Receptor Gene in Indian Population *(Partly funded by WHO)*

Principal Investigator:	**M.I. Khatkhatay**
Project Associates:	Meena Desai and Sumegha Mitra
Project Collaborators:	M. Ahuja, Mahatma Gandhi Hospital, Mumbai
Duration:	2002-2004

Osteoporosis is a multi-factorial disorder with environmental and genetic factors influencing bone density. In fact up to 80 percent variance in bone mineral density (BMD) values can be explained by genetic factors. The two most strongly related candidate genes with bone mass are vitamin D receptor (VDR) gene and estrogen receptor (ER) gene. The polymorphisms in this gene have been strongly correlated with low bone mass. 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. Epidemiology of variants of candidate genes for osteoporosis has yet not been reported from any of the South Asian countries including India. We, therefore, initiated a study, objectives of which 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, the relationships between intron 1 polymorphism of ER alpha gene and intragenic polymorphism of VDR gene and BMD were investigated in 192 postmenopausal Indian women. These polymorphisms were studied as restriction fragment polymorphism using restiction endonucleases Xba 1 and Pvu II in case of ER gene; and Apa 1, Bsm I, Fok I, and Taq I in case of VDR gene. BMD was measured at lumber spine and at the femoral neck by Dual Energy X-ray Absorptiometry (DEXA).

The frequency of genotype XX (absence of polymorphism for Xba I) and PP (absence of polymorphism for Pvu II) were found to be more in normal group (XX= 35 %, PP=43%) than osteoporotic (XX= 15 %, P=23%; chi square= 13.9, p<0.001) (Table 14). On an average subjects with the XX genotype demonstrated 14.5 percent higher BMD at spine (p<0.001, ANOVA) and 15.1 percent higher BMD at the hip (p<0.001, ANOVA) than those with xx (presence of Xba I restriction site) genotype (Table 15). Similarly average BMD was 5.6 percent higher at spine (p<0.001, ANOVA) and 4.8 percent higher at hip (p<0.001, ANOVA) in subjects with PP genotypes than with pp (presence of Pvu II restriction site).

In case of VDR gene, women with genotype aa (presence of restriction site Apa l) and bb (presence of restriction site) had average BMD 13.5 percent and 18 percent higher at spine and 11.6 percent and 13.8 percent higher at hip respectively (p<0.001, ANOVA) than AA (absence of restriction site for Apa1) and BB (absence of restriction site for Bsm1) (Table 16). Similarly, women with genotype FF (absence of restriction site for Fok 1) and TT (absence of restriction site for Taq 1) had mean BMD 15.6 percent and 18.5 percent higher at spine and 10.9 percent and 13.2 percent higher at hip respectively (p<0.001, ANOVA) than ff (presence of restriction site for Fok 1) and tt (presence of restriction site for Taq 1) genotypes (Table 17). The frequency of genotype bb was significantly higher in normal group than osteoporotic group (chi square= 13.85, p<0.001) (Table 18).

Table 14: Frequency of estrogen receptor genotypes (Xba I and Pvu II) in normal and osteoporotic subjects.

Genotype/ Allele	Osteoporotic	Normal	Chi-square significance
XX	15	35	
Xx	49	51	
xx	36	14	p<0.01
PP	23	43	
Pp	29	39	
pp	48	18	p<0.001

Table 15: Estrogen receptor genotypes (Xba 1 and Pvu II) and BMD measurements at lumber spine and femoral neck.

BMD (g/cm²)	XX (n=41)	Xx (n=103)	xx (n=53)
Lumbar spine (L1-L4)	0.937 ± 0.084	0.869 ± 0.077	0.818 ± 0.064
Femoral neck	0.862 ± 0.071	0.798 ± 0.062	0.749 ± 0.053

BMD (g/cm²)	PP (n=41)	Pp (n=103)	pp (n=53)
Lumbar spine (L1-L4)	0.894 ± 0.076	0.878 ± 0.084	0.846 ± 0.074
Femoral neck	0.820 ± 0.062	0.803 ± 0.065	0.782 ± 0.062

P < 0.001; XX vs xx, XX vs Xx and Xx vs xx at spine and hip.

P < 0.001; PP vs pp. No level of significance seen between PP vs Pp and Pp vs pp.

Values are in mean ± SD.

Table 16: Vitamin D receptor genotypes (Apa 1 and Bsm 1) and BM measurements at lumber spine and femoral neck.

Apa 1	AA	Aa	aa	p
BMD (g/cm²)				
Lumbar spine (L1-L4)	0.831 ± 0.073	0.882 ± 0.011	0.944 ± 0.014	P<0.001
Femoral neck	0.761 ± 0.056	0.806 ± 0.061	0.849 ± 0.067	P<0.001

Bsm 1	BB	Bb	bb	p
BMD (g/cm²)				
Lumbar spine (L1-L4)	0.811 ± 0.066	0.862 ± 0.079	0.957 ± 0.086	P<0.001
Femoral neck	0.748 ± 0.052	0.791 ± 0.062	0.851 ± 0.065	P<0.001

Values are in mean ± SD.

Table 17: Vitamin D receptor genotypes (Fok 1 and Taq 1) and BMD measurements at lumber spine and femoral neck.

Fok 1	FF	Ff	ff	p
BMD (g/cm²)				
Lumbar spine (L1-L4)	0.911 ± 0.078	0.888 ± 0.077	0.788 ± 0.073	P<0.001
Femoral neck	0.821 ± 0.065	0.810 ± 0.060	0.740 ± 0.057	P<0.001

Taq 1	TT	Tt	tt	p
BMD (g/cm²)				
Lumbar spine (L1-L4)	0.943 ± 0.073	0.861 ± 0.077	0.796 ± 0.073	P<0.001
Femoral neck	0.844 ± 0.062	0.792 ± 0.064	0.745 ± 0.054	P<0.001

Values are in mean ± SD.

Table 18: Frequency of Vitamin D receptor genotypes (Xba I and Pvu II) in normal and osteoporotic subjects.

Genotype/ Allele	Osteoporotic (%)	Normal (%)	Chi-square significance
AA	56.4	30.2	
Aa	28.7	33.8	
aa	14.8	35.9	p<0.01
BB	42.5	19.7	
Bb	38.6	39.4	
bb	18.8	40.8	p<0.001
FF	31.6	47.9	
Ff	35.6	33.8	
ff	32.6	18.3	P<0.02
TT	28.7	45.0	
Tt	35.6	32.3	
tt	35.6	22.5	p<0.05

Values are in mean ± SD.

4.5 Osteoporosis in Men: To Study Impact of Genetic and Environmental Factors on Bone Health in Indian Men

Principal Investigator:	**M.I. Khatkhatay**
Project Associates:	Meena Desai and Kalpana Venkat
Collaborators:	Lt Col Parduman Singh and Lt Col MM Arora Armed Forces Medical College, Pune
Duration:	2003-2006

Osteoporosis is no more considered as health problem that affects elderly women. Recent epidemiological studies point out that osteoporotic fractures are not uncommon in men and it can lead to same life threatening consequences as seen in women. For unknown reason, the mortality associated with hip fractures as well as vertebral and other major fractures is higher in men than in women. Peak bone mass is influenced by genetic factors, age at puberty, exercise and calcium intake during childhood whereas causes of bone loss include declining sex steroids concentrations, physical inactivity, smoking, alcohol consumption and vitamin D deficiency. Therefore, it is necessary to study impact of genetic and environmental factors on bone health of Indian men. Thus the objectives of the study are to (1) study changes in levels of sex steroid, biochemical markers of bone turn over and their correlation with bone loss in men (2) study polymorphism in COLIA1 gene as genetic marker for osteoporosis (3) effect of interaction between environmental and genetic factors leading to osteoporosis.

During the year, about 200 cadets/officers from AFMC were enrolled following well-defined inclusion criteria. A detailed clinical history including life style factors such as habits of smoking and alcohol consumption was obtained from each subject enrolled for the study. Their bone mineral density (BMD) at lumber spine and femoral neck was estimated by DEXA at K.E.M Hospital and Research Centre, Pune. The data obtained was broadly classified into alcoholics and non-alcoholics. The alcoholic group was further sub-grouped into light, moderate and severe alcoholics depending upon average grams of alcohol intake during the last six months. The data was also classified into non-smokers, light and severe smokers on the basis of average number of cigarettes smoked per 6 months.

The preliminary results indicate that mean BMD at spine and femoral neck of non-alcoholic (1.075 ± 0.09 and 1.033 ± 0.122 g/cm^2) was not significantly different from alcoholic group (1.113 ± 0.132 and 1.107 ± 0.106 g/cm^2) indicating that alcohol up to 720 g/6 months has no effect on BMD. However, a significant decrease in mean femoral BMD ($P<0.05$) was observed in subjects who smoke (0.934 ± 0.078 g/cm^2) more than 7 cigarettes per day compared to non-smokers (1.063 ± 0.1342 g/cm^2).