ABSTRACT

Background: Optimizing bone health throughout lifecycle is important as low bone mass is associated with skeletal fragility fractures and osteoporosis during later years of life. Level of peak bone mass (PBM) achieved during adolescence and early adulthood is one of the two main determinants of osteoporosis, the other being rate of bone loss during adult years. Premenarchal years are associated with rapid bone mass accrual leading to achievement of PBM during early adulthood. Due to the discrimination against girls with respect to distribution of food in the family, nutritional deficiencies have been reported to be high in adolescent Indian girls especially from low income groups. This may lead to severe consequences on bone mass accrual and future risk of osteoporosis after menopause. Further, women are at higher risk of osteoporosis as they have lower bone mass compared to men and experience rapid bone loss during the initial 5 to 10 years after menopause. Therefore, premenarchal and menopausal years are important for taking measures to achieve optimal bone health. Amongst various factors, nutrition is one of the important modifiable factors associated with bone health and thus, nutritional intervention programmes targeting premenarchal years in girls may result in improvement of future bone health.

Objectives: The study was carried out to i) To assess role of anthropometric, nutritional and other lifestyle factors in bone mineral accrual in premenarchal girls. ii) To investigate the effect of calcium along with multivitamin and zinc supplementation on bone mineral content, bone area and bone mineral density in premenarchal girls and iii) To evaluate relative importance of anthropometric, nutritional and other lifestyle factors in bone loss in post-menopausal women.

Materials and study design: To assess factors influencing bone health in premenarchal girls, a cross sectional study was carried out on school girls (8-12 yr) from two public schools in Pune city, India, catering to children from low socioeconomic strata (LSES) during December’08-January’09. In all, 214 eligible girls in classes 3rd-5th were enrolled. To obtain statistical power of 90% at 5% level of significance to detect a difference of more than 10% between the groups, a sample size of 214 was estimated based on the standard deviation in outcome parameters, i.e., total body bone mineral content (TBBMC), from previous available
data in premenarchal girls (Khadilkar et al, 2011). To compare the bone health of LSES girls with girls from higher socioeconomic stratum (HSES), 93 age-matched girls were recruited from a private school catering to high income groups. Efficacy of calcium, zinc, multivitamin and vitamin D supplementation on bone mass accrual and height velocity was evaluated in these 214 girls from LSES, who were randomly allocated to one of the three intervention groups for a period of 12 months (January’09-December,’09): Ca+MZn+D group (n=73) received calcium (500mg) along with multivitamin and zinc (15mg) 6d/wk) and vitamin D (30,000IU/3 months); Ca+D group(n=72) received calcium and vitamin D, M+D group (n=69) received multivitamin and vitamin D. In 319 women above 40 years of age, the relative importance of various factors on bone health was examined at a routine health check of a tertiary care hospital during December’07-May’08. Based on the variation in bone mineral density (BMD) as observed from a previous study in premenopausal and postmenopausal women (Salamat et al, 2008), a sample size of 300 was estimated to obtain the power of the study to be 90% at level of significance 5% to detect a difference of more than 5% between the groups. To ensure a sample size of 300, 319 women above 40 years were recruited of which 104 were identified as premenopausal and 215 as postmenopausal. Exclusion criteria for all participants were based on factors known to adversely affect bone metabolism.

Data were collected on anthropometry, blood levels of calcium, parathyroid hormone, vitamin D and zinc, diet by 2 day 24-hr recall, physical activity, sunlight exposure and sociodemographic characteristics. Bone health status was assessed using Lunar Dual energy X-ray absorptiometry (DXA). Ethical approval for both the study protocols was obtained from the ethics committee of Hirabai Cowasji Jehangir Medical Research Institute and Jehangir Clinical Development Center.

**Results and Conclusions:** The mean age of girls was 9.8±1.1 years. Underweight (Weight-for-age Z score <-2) and stunting (height-for-age Z score <-2) were observed in 39% and 24.8% of the present study population of apparently healthy premenarchal girls. Energy and protein intakes were approximately 75% of the recommended dietary allowances (RDA). Intakes of calcium, zinc, iron, thiamine, riboflavin, folic acid and ascorbic acid were below 50% of the RDA implying multiple micronutrient deficiencies. Blood status of calcium, zinc and vitamin D were also low in 71%, 55% and 34.5% girls respectively. Majority of the girls had
adequate sunlight exposure (65%) and moderate physical activity (96%) of more than 30 min/d. Z scores for total body bone mineral content (TBBMC) were low (<-2) in 15.6% of the girls while 37.5% girls were “at risk” for achieving low bone mass (Z score between -2 and -1). Prevalence of low bone mass showed an increasing trend with increasing age. Mean rate of increase in TBBMC with age (11.6%) and TBBMC with Tanner-stage (20.7%) was lower in the study population than Indian reference-database and published reference database in Caucasian and Polish population. TBBMC and total body bone area (TBBA) showed significant positive association with age, height, weight, moderate physical activity, Tanner stage, serum Vitamin D (25 OH-D) and dietary intakes of protein, calcium and zinc (p<0.05). Regression analysis revealed that low weight, 25OH-D, intakes of protein, calcium and zinc were significant factors adversely affecting TBBMC (p<0.05). Thus, findings highlight the need to improve bone health in premenarchal years through improvement in nutritional status of underprivileged girls.

Comparison of girls from LSES with well-off age-matched girls from HSES revealed that all bone parameters [(TBBMC, total body bone mineral density (TBBMD), total body bone area (TBBA), total body lean body mass (TBLBM)] and total body fat percentage (TB fat%) in LSES girls were significantly lower than that of HSES girls (p<0.05). TBBMC, TBBA and TBBMD showed significant positive associations with anthropometric parameters (age, height, weight, total body fat percentage) in girls from both LSES and HSES of the order r=0.6-0.9. After adjusting for factors such as bone area, height and TBLBM, it was observed that independent of these factors, bone mass was lower in girls from LSES compared to HSES. There was a significant effect of age and puberty on all bone parameters. The difference in TBBMC, TBBA, TBLBM and TB Fat% between the 2 socio-economic strata at Tanner stage I were not significant (p>0.1). However, by Tanner stage III, mean TBBMC of HSES girls (1258±190g) was higher than LSES girls (1008±164g) (p<0.05). From Tanner stage I to Tanner stage III, the mean rate of increase in TBBMC (16.9%) and TBBMD (4.2%) was also lower in the LSES girls than the HSES girls [TBBMC (31.8%), TBBMD (7.3%) which may result in achievement of lower peak bone mass in girls from LSES in absence of any intervention.

The supplementation trial to assess the effect of calcium, zinc+multivitamin and vitamin D on bone mass accrual of LSES premenarchal girls revealed higher
mean percent increase in TBBMC in Ca+D-group (22.3%) and Ca+MZN+D-group (20.8%) compared to M+D-group (17.6%) (p<0.05) with no significant differences observed between Ca+MZN+D and Ca+D groups (p>0.1). Improvement in bone health in terms of TBBMC-for-age Z scores showed beneficial effect in both Ca+MZN+D group (13.6%) and Ca+D group (22%) compared to the M+D-group (0.0% i.e. no improvement). This improvement was higher in the girls who were “at risk” for low bone mass (Z score between -2 and -1) than girls who already had low bone mass (Z score<-2). Also, post intervention, shift in distribution pattern of TBBMC towards the right was higher in both the calcium supplemented groups (Ca+MZN+D and Ca+D group) compared to M+D group. Thus results demonstrate the positive effect of supplementing calcium and vitamin D with or without multivitamins and zinc on bone health, especially with regards to improvement in bone related Z scores in the present study population of underprivileged premenarchal girls.

Further, supplementing zinc along with multivitamins, calcium and vitamin D showed improvement in height-for-age Z scores in the study population compared to age-matched unsupplemented controls from HSES. Change in height-for-age Z scores adjusted for Tanner stage were significantly higher in the Ca+MZN+D group (0.32±0.06) compared to its age-matched controls from HSES (C1 group) as well as Ca+D (0.18±0.06) and M+D (0.14±0.04) group (p<0.05). Twenty two percent of the girls from Ca+MZN+D group, 9.1% from Ca+D group and 16% girls from the M+D group showed improvement while majority of the girls from HSES (95.7%) showed no change in height-for-age Z scores at endline. With respect to height velocity, significantly higher height velocity was observed in the Ca+MZN+D group (6.7±0.4 cm/yr) compared to its age-matched control group, C1 (5.0±0.3 cm/yr) as well as Ca+D group (5.6±0.5 cm/yr) and M+D group (6.1±0.2 cm/yr) (p<0.05). Further, while other studies have reported peak height velocity at age 11 yrs in un-supplemented girls from LSES and HSES, the present study population showed continued increase in height velocity even at age 12 yrs indicating a possible beneficial role of zinc in improving height deficits in underprivileged girls.

Cross sectional study to assess factors affecting bone health in women above 40 years of age revealed high prevalence of osteoporosis at lumbar spine in post menopausal women (25.8%) and a high prevalence of osteopenia was observed in
premenopausal women (44.3%). Bone mineral density (BMD) was significantly lower in postmenopausal women (0.99±0.16 g/cm^2 at lumbar spine; 0.85±0.11 g/cm^2 at femoral neck and 0.90±0.13 g/cm^2 at total hip) than the premenopausal women (1.09±0.13 g/cm^2 at lumbar spine; 0.92±0.14 g/cm^2 at femoral neck and 0.97±0.12 g/cm^2 at total hip) (p<0.001). Biochemical deficiencies of calcium and vitamin D were seen in both pre and post menopausal women. Correlation analysis revealed significant positive association between BMD at all three sites (lumbar spine, total hip and femoral neck) and height, weight, waist and hip circumference (r=0.2 to 0.6) (p<0.05). Lumbar spine BMD showed negative association with age at menarche (r= -0.25) and years since menopause (r= -0.33) (p<0.05). A negative association was found between BMD at all three sites and increasing stages of menopause indicating an increase in bone loss with advancing menopausal stages. Regression analysis revealed that age, weight, height, menopause, low intakes of calcium and low 25 OH-D along with poor sunlight exposure were the major factors contributing to bone loss in women above 40 years of age. Of the total bone loss of 7-8.8% observed at the three sites, 2.1-4.5% was seen to be contributed by menopause after accounting for factors like age, height and weight.

**Contribution to the existing body of knowledge**

- The importance of premenarchal years for improvement in bone mass and height has been demonstrated especially in girls from LSES.
- Dietary inadequacies of protein, calcium and zinc, low weight and vitamin D deficiency were found to be the major factors affecting bone status of premenarchal girls.
- Positive effect of multivitamin+zinc supplementation along with calcium was observed on bone mass accrual in premenarchal girls.
- Enhancing effect of multivitamin+zinc supplementation on height velocity and height-for-age Z scores in girls indicates the potential of zinc supplementation for improving growth.
- Improvement in calcium intakes along with moderate physical activity and sunlight exposure may prove useful to minimize bone loss especially after menopause.