Original Article

A Comparative Study of Bone-Health and Associated Factors in Healthy Indian Adolescents and Young Women

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Abstract

Introduction: Adolescence is a critical period for the accrual of bone mass. However, few studies have assessed the bone geometry in slum-dwelling girls/young women. We thus conducted this study: (1) to assess bone health in Indian adolescent girls and young women residing in slum vs nonslum (NS) areas and (2) to identify factors associated with poor bone health. **Methods:** This cross-sectional case–control study was performed on 110 apparently healthy unmarried, nonpregnant, nonlactating, postmenarchal adolescent girls and young women aged 11 to 24 years residing in urban slums with the same number of age-matched controls from NS areas. Anthropometric, dietary, physical-activity and bone-health parameters (using dual-energy X-ray absorptiometry and peripheral quantitative computed tomography—DXA and pQCT—respectively) were evaluated using standard protocols. A P value of < 0.05 was considered statistically significant. **Results:** Slum girls were significantly shorter and lighter and had lower dietary intakes of calcium and protein after adjusting for total daily calorie intake than NS girls ($P < 0.05$). Areal bone-mineral density (BMD) at lumber spine (0.940 ± 0.13 vs1.042 ± 0.15 g/cm^2), femur and total body (less-head), bone-mineral apparent density adjusted for volume at the lumbar spine (0.295 ± 0.04 vs 0.319 ± 0.04 g/cm^3) and height-adjusted bone density at femur (0.862 ± 0.011 vs 0.905 ± 0.011 g/cm^2) were significantly lower in slum dwelling participants as compared to their NS counterparts ($P < 0.05$). After adjusting for confounders like sunlight exposure, anthropometric parameters and dietary calcium intake, area of dwelling was still a significant factor for the difference in BMD. **Conclusions:** The potential determinants of poor bone density and geometry in girls and young women residing in slums include residential location, dietary habits, and physical activity levels. Despite adjustments for confounding factors, differences in bone health between those in slum and nonslum areas suggest adaptations developed over generations of deprivation in these individuals, necessitating urgent intervention.

Keywords: Adolescent girls, age at menstruation, calcium intake, DXA, pQCT, slum

Introduction

As per the World Health Organisation (WHO), adolescence is the phase of life between childhood and adulthood. There is rapid physical, cognitive and psychosocial growth during this period that lays a foundation for future good health.[1] Adolescent years are particularly important for peak bone mass acquisition, which is a major predictor and modifiable determinant of bone health. It is reported that every 10% increase in bone mass during adolescence potentially reduces fracture risk by 50% among postmenopausal women.[2] Nearly 40% of adult bone mass is accrued during adolescence, within two years of attaining peak height velocity. Thus, approximately 95% of peak bone mass is accrued by the age of 19 years, of this, more than half is acquired during the adolescent years.[3,4] During adolescence, bone formation exceeds bone resorption; low bone mass during adolescence

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may be due to bone loss or failure to accrue bone. As lifelong bone health is dependent on maximizing bone mass during the critical periods of growth and maturation, optimizing bone accrual during adolescence is essential.

During puberty and early adult life, endosteal apposition and trabecular thickening provide maximum skeletal mass influenced by locally and systemically produced factors and mechanical forces that control the function of osteoclasts and osteoblasts. A complex interaction of environmental, behavioural and genetic factors determines the bone mass of which genetic factors account for the maximum variability (60–80%).[5] These genetic polymorphisms mainly affect bone health prior to puberty in childhood. However, in adolescents, polymorphisms of oestrogen receptors, aromatase, interleukin-6, low-density lipoprotein receptor-related protein 5 **(**LRP5), and osteocalcin-related genes are independent predictors of bone size, bone-mineral content (BMC) and bone-mineral density (BMD).[6] Modifiable factors (environmental and behavioural) that account for 20–40% of the variability in bone mass mainly include nutritional status, physical activity, weight-bearing exercise and body mass.[6] Sex steroids (mainly oestrogen in women) also play an essential role in optimizing bone mass through their direct effect on bone as well as by increasing growth hormone and insulin-like growth factor-1 (IGF-1) concentrations which are potent mediators of bone formation, thereby reducing bone resorption and increasing bone accrual.[7]

India is home to the world's largest population living in slums with the highest concentration being in Maharashtra, Western India.[8] Various studies have highlighted the disparity in urban slum and nonslum population health indicators, including infant and child undernutrition. Our previous study which compared girls from lower socioeconomic class (LSEC) with age-matched Caucasian girls revealed low bone mass for bone area (dual-energy X-ray absorptiometry, DXA-derived parameters) among these underprivileged girls from Pune.[9] Also, when compared with upper socioeconomic class (USEC) girls, these LSEC girls showed lower bone health parameters.[10]

The effect of physical activity on the improvement of bone geometry has been studied previously using peripheral quantitative computed tomography (pQCT).[11] However, to the best of our knowledge, no study has compared bone health including geometry as assessed by pQCT of Indian adolescent postmenarchal girls belonging to urban slum vs nonslum populations.[12] We hypothesize that area of residence would affect bone health and bone geometry in adolescent girls due to differences in environmental and behavioural factors. We, therefore, conducted this study with the following specific objectives: (1) to assess bone health (areal bone density and bone geometry) using DXA and pQCT in Indian adolescent girls and young women residing in slum vs nonslum areas and (2) to identify factors associated with poor bone health with respect to bone mass and geometry as measured by DXA and pQCT.

Materials and Methods

Study design and participants: In this cross-sectional, observational study, 110 apparently healthy unmarried, nonpregnant, nonlactating, postmenarchal adolescent girls and young women aged 11 to 24 years residing in urban slums were selected by random sampling. Candidates meeting the criteria were chosen through a lottery method. Age- and gender-matched apparently healthy participants (n = 110) from nonslum areas (age range 11–24 years) were selected by approaching schools, colleges, and offices in the urban area of Pune (Western Maharashtra, India).

Public schools in slum areas catering to the LSEC population and private schools catering to USEC, depending on school fee structure and area of residence, were randomly approached.[10] All adolescent girls aged 11 years and above who had already attained menarche were offered participation in the study after obtaining permission from school authorities. Written informed consents were obtained from all the participants/parents and assent from participants under 18 years. Participants who did not consent to participate and subjects with a history of any prior medical conditions such as type 1 diabetes mellitus, growth hormone deficiency and bone-related disorders were excluded from the study. All the study participants were assessed by a paediatrician/physician for any concurrent illness and medical history was recorded using a prevalidated questionnaire.

None of the participants had a history of any medication such as steroid intake. *Post hoc* power analysis using a PS sample size calculator with an independent sample t test indicated that a sample size of 110 subjects per group was sufficient to observe a 5% difference (delta) in lumbar spine bone mineral density (LSBMD) values in comparison with a previous study (0.906 ± 0.016 g/cm^2), at an alpha of 0.05 and power of 0.8.[9]

Anthropometric parameters, Clinical and Biochemical evaluation

Standing height and weight were measured using standardized protocols and equipment (Seca Portable stadiometer, Hamburg, Germany, up to 0·1 cm accuracy and Seca 876 Flat scale, Hamburg, Germany, up to 100 g accuracy, respectively). Body mass index (BMI) was computed using the formula—BMI = weight (kg)/height (m) ^{2}. Standard deviation scores (Z scores) for height, weight and BMI were computed using Indian reference data.[13] A medical examination was performed by a paediatrician along with the assessment of the clinical history of each participant. Fracture history was also noted using a questionnaire. Haemoglobin concentrations were assessed using HemoCue equipment by the finger prick method described previously.[14]

Physical activity

Using structured and prevalidated physical activity questionnaires, activity performed during the day was recorded for all the participants. Outdoor sports activities, such as

playing football/basketball/gym activities, were classified as vigorous activities.[15]

Nutrient Intakes

Dietary intakes were recorded for three nonconsecutive days using the 24-h recall method. Nutrient intakes were estimated using the C-diet program based on a cooked and raw food database.[16-19] Age-matched recommended dietary allowances were calculated using recommended dietary allowance (RDA tables).[20]

Bone parameters

All the study subjects were measured on the GE-Lunar iDXA (GE Healthcare, Madison, Wisconsin, USA) fan-beam dual-energy X-ray absorptiometry (DXA) scanner with a 64-channel detector (software version encore 16) for bone density (g/cm^2) at total body, lumbar/anteroposterior spine and dual femur (both limbs including femoral neck). pQCT measurements were performed on the subjects' nondominant hand (radius) using an XCT 2000 scanner (Stratec, Pforzheim, Germany). Trabecular bone density was measured at 4% site, while cortical density, muscle area, cortical thickness, total bone area and stress–strain index (SSI) were measured at 66% site. The manufacturer provided reference data to generate Z scores of all pQCT parameters.[21] Detailed procedures for measuring DXA and pQCT-derived parameters are published elsewhere.[22]

Statistical analysis

SPSS software for Windows (v26, IBM statistics data editor, IBM Corp, 2019) was used for data analysis. The normality of the variables used for analysis was checked using the one-sample Kolmogorov–Smirnov test. Normally distributed parameters are presented as mean ± SD, whereas nonnormal variables are described as median (IQR). Appropriate tests were applied for analysis depending on the normality of the data. Student t tests, correlations (Spearman rho), univariate general linear model and linear regression (ANCOVA) were used to analyse and present the data. Regression models were adjusted with covariates such as age at menstruation, calorie-adjusted calcium and protein intakes, sunlight exposure, height Z scores, vigorous physical activity, and haemoglobin concentrations, and DXA-derived muscle mass or pQCT-derived fat to muscle density. The level of significance was set at $P < 0.05$.

Ethical aspects

This study was approved by the ethics committee jehangir clinical development center pvt. ltd.(EC registration number:ECR/352/Inst/MH/2013) dated 22 December 2015 before commencing study. Written informed consent was obtained from every participant and the study was in compliance with Declaration of Helsinki 1964 and revised thereafter.

Results

We studied 220 menarchal adolescent girls and young women (unmarried), of which 110 were residing in slums (mean age 15.9 ± 2.9 years), and 110 age-matched girls were enrolled from nonslum areas (mean age 16.2 ± 3.8 years). The demographic characteristics of both groups are described in Table 1.

The slum girls were significantly shorter and lighter than the nonslum girls ($P < 0.05$). Dietary intakes for calcium and protein of participants living in slum areas were significantly lower than in nonslum areas even after adjusting for total calorie intake per day. Forty-eight per cent of nonslum-dwelling girls reported sunlight exposure of less than 15 min per day as opposed to 26% reported by slum-dwelling girls. Outdoor sports, such as football and badminton, were reported only in nonslum girls. The mean age at menstruation and prevalence of fractures (around 13%) were similar in both groups ($P > 0.1$).

Table 2 illustrates bone health parameters as assessed by DXA. Areal bone-mineral density at lumber spine, femur and total body (less head) were significantly lower in slum-dwelling participants as compared to the nonslum counterparts ($P < 0.05$). Bone-mineral apparent density (adjusted for volume)[23] at the lumbar spine was also significantly lower in girls residing in a slum than in nonslum ($P < 0.05$). Height-adjusted bone density at the femur was significantly lower in slum girls compared to their nonslum counterparts ($P < 0.05$).

We observed similar trends in bone strength (SSI) and bone geometry parameters assessed by pQCT (cortical thickness or cortical area). Except for trabecular density and endosteal circumference at radius, nonslum-dwelling girls had higher strength and geometry parameters than slum girls ($P < 0.05$). The results remained unchanged after adjusting for radial length except for periosteal circumference ($P > 0.05$). These parameters are described in Table 3.

When compared with machine-derived data,[21] Z scores for all the parameters (such as trabecular density for age, cortical density for age, SSI POL, fat area, bone area, and muscle area) were below median except for bone for muscle parameter at radius [Figure 1].

Further, nonslum-dwelling girls had significantly higher Z scores at radius than slum girls ($P < 0.05$). As shown in Figure 2a and b, cortical thickness and muscle area were significantly higher in nonslum girls than in slum girls ($P < 0.05$) even after adjusting for forearm length.

Bivariate correlation analysis indicated that dietary calcium intakes were positively associated with cortical bone density (spearman rho = 0.142, $P = 0.036$), while trabecular bone density had no association with dietary calcium (data not shown).

To identify the factors associated with bone health, univariate regression analysis was performed. Figure 3a-c displays the unadjusted means and estimated marginal means after accounting for covariates such as age at menstruation, height Z scores, muscle mass, vigorous sports activity, dietary protein and calcium intake and sunlight exposure.

With LSBMD as a dependent variable, after adjusting for all the above-mentioned confounders, nonslum dwelling girls

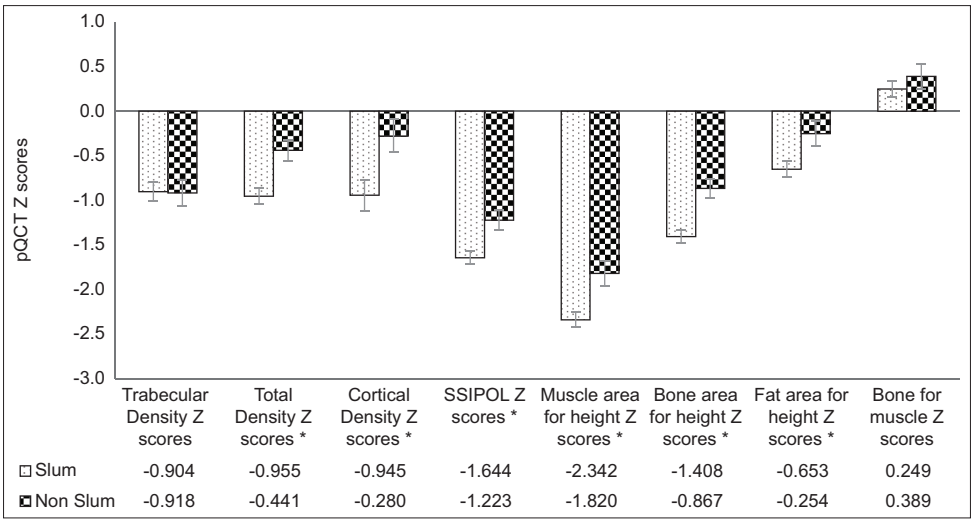

Figure 1: pQCT-generated Z scores for bone health parameters in slum and nonslum dwelling participants. *Footnote: *Indicates significant differences among slum and nonslum groups. Level of significance P* < 0.05

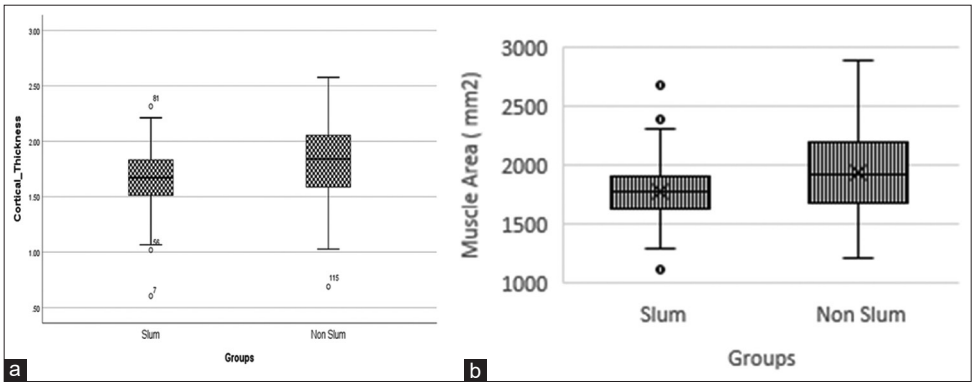

Figure 2: a and b: Cortical thickness and muscle area for slum and nonslum participants using pQCT. *Footnote*: *P* < 0.01 *for both parameters* {*unadjusted and adjusted with object length*}

Table 1: Demographic parameters of the study population

	Mean±SD		Significance, *P*
	Slum (*n*=110)	Nonslum (*n*=110)	
Age (years)	15.9±2.9	16.2±3.8	0.126
Age at menstruation (years)	12.9±1.1	13.0±1.2	0.418
Height *Z* score	−0.47±0.76	−0.16±0.90	0.007
Weight *Z* score	−0.68±1.01	−0.03±1.00	0.001
BMI *Z* score	−0.50±1.02	0.1±1.04	0.001
Haemoglobin (g/dL)	10.8±1.7	11.9±1.6	0.001
Calories (kcal/day)	1202±370	1570±483	0.001
Dietary calcium (mg/day)	276±104	414±225	0.001
Dietary phosphorous (mg/day)	614±195	824±267	0.001
Dietary calcium: phosphorus	0.46±0.12	0.51±0.20	0.035
Calcium intake per100 Kcal (mg/day)	23.4±6.3	27±12.4	0.007
Protein intake per 100 Kcal (g/day)	2.3±0.3	2.4±0.4	0.045
Fracture prevalence (%)	12.7%	13.8%	0.847
Sunlight exposure <15 min (%)	26%	48%	<0.05
Daily vigorous PA (%) (outdoor sports activity)	Not reported	48%	<0.05

Level of significance, *P*<0.05

had significantly higher BMD (1.022 ± 0.032 vs 0.945 ± 0.012 gm/cm^3) than slum girls (partial eta^2 = 0.036, *P* = 0.039). Height Z score and percent muscle mass were significant confounders impacting the BMD (partial eta^2 = 0.033,

$P = 0.048$ and partial eta$^2 = 0.118$, $P = 0.001$, respectively). Similarly, bone strength (the stress–strain index, SSI) and bone geometry parameters (cortical thickness) were significantly higher among nonslum girls than their slum counterparts even after adjusting for the above-mentioned confounders. The differences in both the groups were attributed to age at menstruation and height Z scores in SSI (partial eta$^2 = 0.031$, $P = 0.035$ and partial eta$^2 = 0.034$, $P = 0.028$, respectively) and cortical area (partial eta$^2 = 0.038$, $P = 0.02$ and partial eta$^2 = 0.06$, $P = 0.003$, respectively).

Table 2: Bone health parameters as assessed by DXA

DXA parameters	Mean±SD		*P*
	Slum	Nonslum	
L1–L4 BMD g/cm^2	0.940±0.13	1.042±0.15	0.001
L1–L4 BMC gm	38.37±8.36	44.30±9.31	0.001
L1–L4 Area cm^2	40.4±4.40	42.10±4.62	0.007
LSBMAD g/cm^3	0.295±0.04	0.319±0.04	0.001
Z LSBMAD	−0.873±1.21	-0.104±1.14	0.001
Dual femur BMD g/cm^2	0.860±0.11	0.911±0.12	0.002
Dual femur BMD g/cm^2 (height adjusted)!	0.862±0.011	0.905±0.011	0.009
TBLHBMD g/cm^2	0.804±0.08	0.872±0.11	0.001

BMD=Bone-mineral density BMC=Bone-mineral content, LSBMAD=Lumbar spine bone-mineral apparent density, TBLHBMD=Total body less head BMD.! = Values displayed are mean±SE after adjusting for height, Level of significance, $P<0.05$

Discussion

We report poor size-adjusted bone density (DXA-derived) and bone geometry (at 66% radius) among slum-dwelling Indian adolescent girls and young women compared to their nonslum counterparts. Slum-dwelling girls were shorter and lighter and had lower dietary calcium and protein intake with lower physical activity. Height significantly affected the BMD, SSI and cortical thickness. Muscle mass that significantly affected BMD, SSI and cortical thickness were significantly affected by age at menstruation and dietary calcium intake correlated significantly with cortical

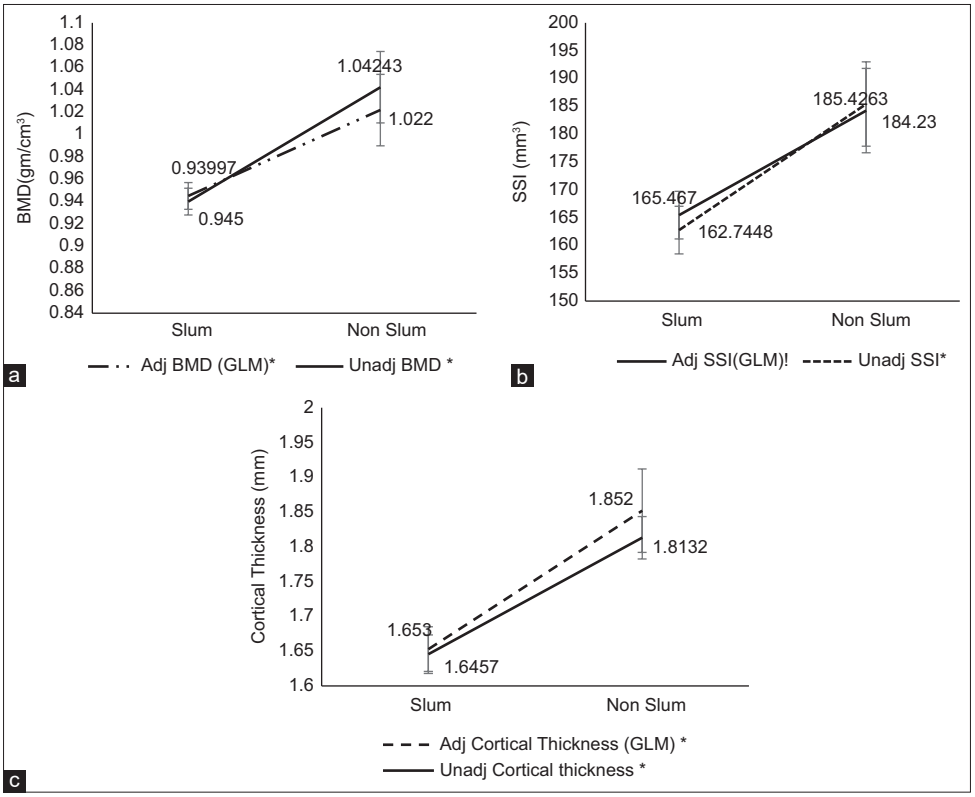

Figure 3: a): Bone-mineral density (DXA) (unadjusted and adjusted (ANCOVA) in slum and nonslum participants. *Footnote: Covariates appearing in the ANCOVA model (GLM) are evaluated at the following values: age at menstruation = 12.8 years, dietary calcium intake = 24.2 mg/100 kcal, dietary protein intake = 2.33 g/100 kcal, sunlight exposure >15 mins/day = 0.74, height Z score = -0.40, haemoglobin = 11 g/dl, vigorous sports activity (yes) =0.07, muscle mass (%) =70.6, level of significance - * P* < 0.05, b) pQCT-derived SSI (unadjusted and adjusted (ANCOVA)) in slum and nonslum participants. *Footnote: Covariates appearing in the ANCOVA model are evaluated at the following values: age at menstruation = 12.8, dietary calcium intake = 24.4 mg/100 kcal dietary protein intake = 2.33 g/100 kcal, sunlight exposure > 15 mins/day = 0.71, vigorous sports activity (yes) =1.8, height Z score = -0.36, haemoglobin = 11.1 g/dl, fat: muscle density = 50.9 (mg/cm3). Level of significance—for unadjusted SSI * P* < 0.05, *after adjustment for SSI ! P* < 0.1, c) pQCT-derived cortical thickness (unadjusted and adjusted (ANCOVA)) in slum and nonslum participants. *Footnote: Covariates appearing in the model are evaluated at the following values: age at menstruation = 12.8 years, dietary calcium intake = 24.3 mg/100 Kcal, dietary protein intake = 2.3 g/100 kcal, sunlight exposure > 15 mins/day = 0.71, height Z score = -0.36, haemoglobin = 11.1 g/dl, fat: muscle density = 51.1 (mg/cm3), vigorous sports activity (yes) =0.16. Level of significance—* P* < 0.05

Table 3: Bone density, strength and geometry parameters as assessed by pQCT

pQCT parameters	Mean±SD		Significance, *P*
	Slum	Nonslum	
Length of forearm (mm)	232±12	237±15	0.003
Trabecular density (At 4% radius-mg/cm3)	160.6±29.3	164.0±33.0	0.436
Cortical density (At 66% radius mg/cm3)	1074±65	1110±54	0.001
Muscle area (mm^2) (At 66% radius)	1788±278	1949±351	0.001
Muscle area (mm^2)! (At 66% radius)	1811±29	1927±29	0.006
Cortical area (mm^2) (At 66% radius)	49.2±8.6	54.8±9.4	0.001
Cortical area (mm^2)! (At 66% radius)	49.9±0.8	54.7±0.8	0.001
Cortical thickness (mm) (At 66% radius)	1.65±0.29	1.83±0.31	0.001
Adj.! Cortical thickness (mm) (At 66% radius)	1.66±0.03	1.82±0.03	0.001
Periosteal circumference (mm) (At 66% radius)	35.2±2.5	36.1±2.6	0.022
Adj.! Periosteal circumference (mm) (At 66% radius)	35.5±0.23	35.9±0.23	0.167
Endosteal circumference (mm) (At 66% radius)	24.9±3.1	24.6±3.3	0.671
Adj.! Endosteal circumference (mm) (At 66% radius)	25.0±0.3	24.5±0.3	0.219
SSI (at 66% radius-mm^3)	163±38.9	187±41.0	0.01
Adj.! SSI (at 66% radius-mm^3)	166±3.6	184±3.6	0.001

! adjusted for radial length and corresponding values are displayed in mean±SE, level of significance, $P<0.05$

density. Although the difference in BMD or bone geometry parameters was small after adjusting for confounders like sunlight exposure, anthropometric parameters and dietary calcium intake, the area of dwelling was still a significant factor contributing to the differences, which may be attributed to genetic/epigenetic adaptations to generations of chronic undernutrition.

Poor nutritional status of urban Indian slum-residing adolescent girls has been reported from various parts of the country. Data from slums in eastern India suggest around 30% prevalence of thinness and 42.2% prevalence of stunting.[24] Chronic malnutrition has been reported to be an underlying reason for poor anthropometric parameters. Studies from other parts of the country have also reported a very high prevalence of malnutrition in slum-dwelling adolescent girls and have attributed it to chronic energy deficiency.[25] Although there are no reports on the comparison of anthropometric parameters between slum vs nonslum dwelling adolescent girls from Maharashtra (the state where the current study was performed), a study has shown that childhood stunting and underweight in children, as well as adult women, was higher in slum population of Mumbai than in the nonslum residing population.[26]

We observed lower dietary calcium intake and low vigorous physical activity in urban slum-dwelling adolescent girls and young women. This may contribute to their poor bone health as the role of physical activity and dietary calcium intake in attaining peak bone mass has been previously reported. It has been suggested that bone mass is positively correlated with childhood activity and a positive association between activity and bone health of adolescents has been observed.[27] The detrimental effects of malnutrition on bone density in adolescents have also been reported. Calcium is critical for bone mineralization; hence, additional calcium in the diet may increase bone density by affecting bone turnover and the size of the remodelling space.[28] As adolescence is one of the most critical periods for bone-mineral accrual, the provision of optimal calcium intake to maximize peak bone mass has been advocated due to enhanced calcium absorption in puberty.[29] Similar to our study, others have also noted that the effect of calcium intake varies by skeletal site, with cortical bone appearing to respond more significantly than trabecular bone.[30] Further, other studies have also reported greater loss in cortical bone in adolescent girls and have suggested that this is an age-specific change in the characteristic of adolescent bone.[31,32]

We report muscle mass percentage and age at menstruation as significant predictors of DXA-derived BMD and PQCT-derived cortical area, respectively. A study on lean body mass and bone health in urban adolescents from northern India also observed a strong correlation between lean mass and BMC in both sexes.[33] It is also known that bone mass is strongly associated with muscle mass and during growth, mechanical loading causes a cascade of events that augment bone deposition, leading to changes in bone strength and cross-sectional muscle area and lean mass. Bone stress induces signals that stimulate osteoblast bone formation and reduces osteoclast-induced bone resorption and muscle mass is an index of the mechanical stimulation to bone. Thus, muscle mass is correlated with bone mass, density, and architecture.[34] As bone consolidation is related to an increase in oestradiol secretion at the beginning of menarche, time since menarche is a predictor of bone events in young females. Oestrogen-driven endosteal apposition of bone is responsible for the increase in the relative amount of cortical bone in premenopausal women, which begins at menarche. Some studies document lower BMD in adult women with a history of late onset of

menarche, probably due to inadequate oestrogen levels during skeletal development.[35]

To the best of our knowledge, ours is the first study comparing bone health (both bone density and geometry) between slum-dwelling and nonslum-dwelling young girls and women from a south-east Asian low-middle-income country (LMIC). Despite adjusting for modifiable environmental and behavioural factors, we report poor bone health among slum-dwelling young girls which further might be one of the causes for intergenerational transmission of risk of poor bone health status. Our study has very important public health implications. With the increase in slum population in India, poor bone health in girls and women could significantly add to the burden of health and impact the economy, especially in resource-limited settings.

Nonavailability of Indian reference data to compute Z scores of DXA and pQCT-derived parameters and the lack of biochemistry data on parameters such as parathyroid hormone and growth hormone are some of our limitations. Also, we have not been able to study biochemical markers of bone turnover and the role of genetic factors and epigenetic modifications in differences in bone health in slum and nonslum-dwelling girls. Further genetic studies are needed to identify the factors, leading to poor bone health in them.

Conclusion

In conclusion, poor bone density and geometry due to social determinants and lifestyle factors such as area of residence, dietary intake, and physical activity in slum-dwelling girls and young women are a matter of concern. Longitudinal studies are required to assess the consequences of these observations.

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Author contributions

Neha Avinash Kajale- Conceptualization, Methodology, Formal analysis, investigation, writing original draft, Review and Editing, Chirantap Oza- Formal analysis, Writing original draft, Writing - Review and Editing Dipali Ladkat- Methodology, Writing - Review and Editing Ketan Gondhalekar- Formal analysis, Writing - Review and Editing the manuscript. Tarun Katapalli- Conceptualization, data analysis, Manuscript Writing. Jasmin Bhawra- Conceptualization, Manuscript Writing and editing. Nina Mansukhani- Conceptualization, Manuscript Writing and editing. Anita Bapat- Conceptualization, data collection, Manuscript Writing - Review and Editing. Vaman V Khadilkar- Conceptualization, Writing - Review and Editing, Anuradha V Khadilkar- Conceptualization, Methodology, Formal analysis, Writing - Review and Editing. Manuscript has been read and approved by all authors.

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Conflicts of interest

There are no conflicts of interest.

Data availability

Data will be made available on reasonable request.

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