Original Article

Bone Mineral Density and Serum Bone Turnover Markers among Post-Menarchal Girls from Rural South India

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Abstract

Background: The data on the bone mineral density (BMD) and bone turnover markers (BTMs) in Indian adolescents are limited. **Objectives:** To assess BMD at lumbar spine (LS, L1-L4) and femoral neck (FN) in South Indian post-menarchal girls and correlate it with dietary calcium intake (mg/day), physical activity score and post-menarchal years. The study also assessed serum BTMs and their correlation with chronological age in the study population. **Methods:** This cross-sectional study included apparently healthy post-menarchal adolescent girls aged 12–16 years randomly selected from the community. Participants with vitamin D deficiency were excluded. The data on calcium intake and physical activity were obtained using validated questionnaires. All participants were evaluated with serum calcium, 25-hydroxy vitamin D, parathyroid hormone, N-terminal propeptide of type 1 collagen (P1NP) and Beta-CrossLaps (CTx) and BMD at LS and FN using dual X-ray absorptiometry (DXA). **Statistical Analysis:** EpiData version 3.1 was used for the data entry. The data analysis was done using Statistical Package for Social Sciences (SPSS) version 21. Continuous variables were expressed as mean ± SD. Pearson's correlation coefficient (r) was calculated, and two-tailed Kendall's tau-b test was used for assessing correlation of all nonparametric measures. **Results:** A total of 103 participants were screened, and data from 77 were analysed. There was a significant positive correlation of BMD at LS with chronological age (r: +0.235, $P = 0.036$), but not at FN. Positive correlation of BMD with increase in post-menarchal years was also noted at LS (r: +0.276, $P = 0.015$). There was no significant association of BMD with calcium intake and physical activity scores at both sites. There was a significant negative correlation of serum BTMs with age CTx (r: -0.596, $P = 0.0001$) and P1NP (r: -0.505, $P = 0.0001$). **Conclusion:** This study provides insight into the reference BMD range at LS spine and FN in South Indian rural post-menarchal adolescent girls. BMD positively correlated, whereas BTMs negatively correlated with age. The study also provides the first Indian reference range for serum BTMs in this age group.

Keywords: Adolescent health, BMD, bone health, bone mineral density, bone turnover markers, BTM's, rural community

Introduction

Bone is a solid organ which undergoes constant modelling and remodelling. The bone turnover is highest during the period of rapid bone growth in childhood and adolescence. Peak bone mass is attained at the end of skeletal maturation. It has been proven by bone densitometry studies that children accrue nearly half of their peak bone mass during two years around puberty, and in girls, up to 53% of total bone mineral content is established during pre-menarchal period.[1] Nearly 90% of adult height in girls is attained during pre-menarchal period.[2] The rate of bone growth and mineralization depend on various factors like genetic makeup, gender, hormonal action, nutrition, sunlight exposure and physical activity. Genetic factors contribute to 46–62% of the variance in bone mineral density (BMD).[3] Several countries have established normative BMD data for the paediatric population.[4-6] In India, an initial study among healthy Indian school children (10–18 year of age) was published in 2005 which demonstrated that forearm BMD was significantly higher among upper socioeconomic status (SES) children compared to those with lower SES

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in urban New Delhi.[7] A later study from Pune provided gender- and age-specific reference charts on BMD parameters for affluent urban children 5–17 years of age.[8] There is no Indian data on BMD among children from rural community and none from South India. There are ethnic differences among populations from various regions within India. Hence, studies to assess BMD among South Indian children are essential.

Calcium intake, vitamin D levels, exposure to sunlight and physical activity play a role in attaining adequate BMD.[9-11] Recent literature has referred to the importance of serum and urinary bone turnover markers (BTM), which reflect the dynamic process of modelling and re-modelling more realistically than DXA.[12] Markers of bone formation and resorption increase as linear growth accelerates during early puberty, then decrease during late puberty.[13,14] A negative correlation between the markers and BMD during puberty has been described and is emerging as a new tool in management of adults with osteoporosis.[14-16]

The aim of this study was to measure BMD and its association with calcium intake, physical activity and post-menarchal years, among post-menarchal girls aged 12–16 years from our South Indian community who are normocalcemic and do not have vitamin D deficiency. The study also evaluated the two commonly used BTMs in serum, viz. P1NP and CTX in the study population.

Methods

This is a cross-sectional study done in a tertiary care centre after obtaining approval from the Institutional Review Board (IRB Ref. No. 12277) meeting all ethical standards. Study subjects belonged to the rural community of Thirupatthur Taluk, Vellore from Southern India. Our social worker residing at this rural community received oral consent from healthy post-menarchal girls of 12–16 years of age and their parents. They were then brought to the hospital, as a non-portable hospital-based DXA machine was used for this study. The principal investigator (PI) at the hospital obtained a written consent from the parents/guardians and assent/consent from the adolescents after confirming their age by checking school identity card/Aadhaar card. Menarchal age was also confirmed with the accompanying parent. The study recruitment was done from November 2019 to February 2020. The PI ensured that accurate measurement of height, weight, body mass index (BMI) and their Z-scores were calculated using the revised Indian Academy of Pediatrics (IAP), 2015 growth charts. Ensuring appropriate privacy in the consultation room outside the DXA room, blood pressure (BP) and sexual maturity (Tanner staging) were assessed in all participants using standardized charts by the PI. For this study, systolic BP of >130 mmHg and/or diastolic BP of >90 mmHg was considered as hypertension. Patients with hypocalcaemia, vitamin D deficiency [25-hydroxy vitamin D (25OHD): <12 ng/ml], hypertension, weight or height >2 SDS or <-2 SDS for age and sex, chronic systemic illness, malnutrition, spine deformity or participants receiving/recently received glucocorticoids or other hormonal therapy, calcium/vitamin D supplements were excluded. Dietary history was taken using 24-hour dietary recall method, and the dietary calcium intake was estimated using Indian standardized chart published by ICMR.[17] The physical activity scoring was done by the participants using IPAQ-A questionnaire. Twenty-three physical activity parameters are included in the questionnaire, and the assessment for previous 7 days was made. Ten relevant parameters were selected, and the score was calculated as validated in a previous Indian study.[18] Mean score of less than 1 indicates a low physical activity, and score of 5 suggests high activity. Based on PAQ scores, the participants were divided into two groups: 1–2.9 score and 3–5 score for analysis.

The biochemical and hormonal parameters were measured in fasting blood sample as per the standard in-house lab guidelines. Serum calcium, phosphorus, alkaline phosphatase, albumin and creatinine were measured using Roche Cobas 704 automated chemistry analyser, Basel, Switzerland. Intact plasma parathormone level was analysed using Advia Centaur chemiluminescence immunoassay instrument from Siemens, GMBH, Germany. Serum 25OHD, CTx and P1NP were analysed using Roche Cobas e602 automated electrochemiluminescence (ECLIA) immunoassay analyser, Basel, Switzerland. The latter two parameters were assayed in EDTA plasma samples according to the manufacturer's instructions. Inter- and intra-assay coefficients of variation (CV) and range for the hormonal parameters are described in Annexure 1. BMD at the lumbar spine and femoral neck was measured by Hologic machine Discovery A series by a trained technician. Daily quality control was performed with a phantom provided by the manufacturer, and machine was calibrated. Study participants underwent BMD examination after ensuring the calibrated value was within the normal range. A coefficient of variation of <1% was noted at both sites (LS and FN) over the study period.

Sample size calculation

Based on earlier published data[8] of mean LS BMD (g/cm^2) for girls aged 12 to 16 years, respectively, using a precision of 0.05 the sample size calculated was 29, 27, 23, 12 and 16 girls in each age group (12, 13, 14, 15 and 16 years of age, respectively), i.e., a total of 107. Institutional review board clearance was obtained to include 110 participants.

Statistical analysis

EpiData version 3.1 was used for the data entry. The data analysis was done using SPSS version 21. Continuous variables were expressed as mean ± SD. Pearson's correlation coefficient (r) was calculated, and two-tailed Kendall's tau-b test was used for assessing correlation of all nonparametric measures (BMD with age, height, post-menarchal years). Association of physical activity score group (1–2.9 and 3–5) with BMD was done using Student's t-test. P value <0.05 was considered significant.

Ethical clearance statement

This study was approved by the Institutional Review Board of the Christian Medical College and Hospital, Vellore, vide

letter IRB Min. No. 12277 dated 08.10.2019. Written informed consent was obtained for participation in the study and use of the patient data for research and educational purposes. The procedures follows the guidelines laid down in Declaration of Helsinki 2008.

Results

A total of 103 girls were recruited of which four did not satisfy age criteria and 22 had vitamin D deficiency (serum 25OHD <12 ng/ml). The baseline data on 77 study participants are represented in Table 1. The weight, height and BMI Z-scores were normal. Serum calcium, phosphorus, creatinine and albumin levels were normal for age in all participants with no significant difference between the age groups.

- **Bone mineral density and its correlation with age**
  Figure 1 demonstrates the BMD at LS and FN. In all age groups, LS BMD was higher than FN BMD. There was a significant positive correlation of BMD with age at LS (r: 0.235, p: 0.036) but not at FN (r: 0.034, p: 0.766). Maximal percentage increase in BMD occurred from age 15 to 16 (6% at FN and 7.6% at LS). The highest value of BMD was seen at age 16 at LS (0.914 ± 0.090 g/cm2) and age 14 in the FN (0.796 ± 0.096 g/cm2).
- **Correlation of BMD with height and post-menarchal years**
  There was significant positive correlation of height with BMD at LS (r = +0.247, *P* **0.006***)* but not at FN. Fifty-seven per cent of the study population (41/77) had Tanner stage 3, and 35% (27/77) had Tanner stage 4 of sexual maturity rate (SMR). The mean age of menarche of the study population was 12.3 (range: 9–15) years. A significant positive correlation in LS-BMD with post-menarchal years was also noted (r: 0. 276,6, *P* = 0.015) [Table 2].
- **Association of BMD with calcium intake and physical activity**
  Among 77 participants, 31 had dietary calcium intake of less than 200 mg/day, 43 had intake between 200 and 400 mg/day, whereas three had intake of >400 mg/day. There was no significant association of calcium intake with BMD at FN (p: 0.488) or LS (p: 0.51). Most (73/77) of the participants qualified to have low-moderate activity profile (PAQ score: 1–2.9). There was also no significant association of physical activity with BMD at FN (p: 0.58) or LS (p: 0.10).
- **Serum Bone turnover markers**
  The serum BTMs, CTX and P1NP, were measured and noted to be decreasing with age as seen in Table 3.

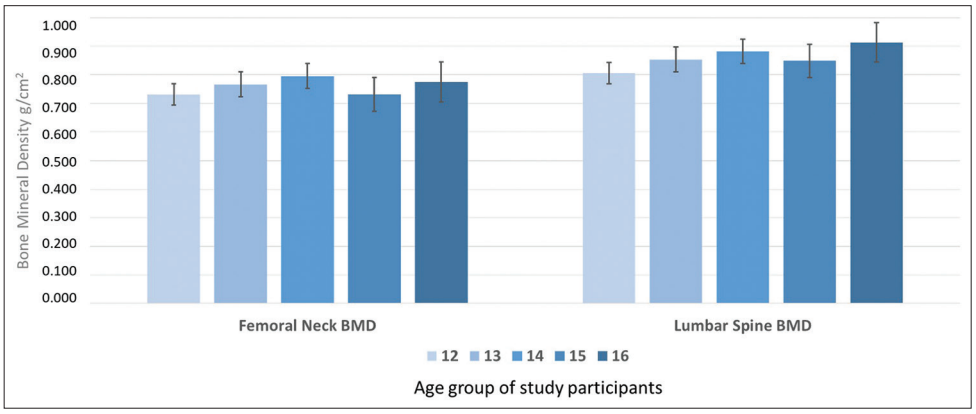

Figure 1: BMD at femoral neck and lumbar spine

Table 1: Baseline anthropometry and biochemical parameters

Parameter	Age in years				
Age (years)	12	13	14	15	16
Frequency, (n)	9	18	13	24	13
Weight (kg)	45.3±5.8	45±9.5	48±7.6	44.9±11.6	48.8±13.6
Weight *Z*-score	0.27±0.74	0.01±0.89	-0.12±0.85	-0.64±1.24	-0.36±1.44
Height (cm)	154±6	154±5	157±5	157±5	155±4.8
Height *Z*-score	0.56±0.43	0.12±0.68	0.13±0.87	-0.08±0.82	-0.36±0.77
BMI (Kg/m^2)	19.2±2.8	19.2±3.2	19.5±2.9	18.4±4.4	20.2±5.1
BMI *Z*-score	0.06±0.85	-0.06±0.85	-0.2±0.9	-0.74±0.29	-0.23±0.47
Calcium (mg/dl) * (8.4-10.2)	9.88±0.32	9.66±0.33	9.73±0.24	9.77±0.35	9.64±0.41
Phosphorus (mg/dl)$^{\#}$ (3.3-5.4)	4.9±0.3	4.9±0.5	4.4±0.6	4.6±0.4	4.5±0.4
25-hydroxy vitamin D$^+$ (ng/ml) (20-30)	18±4	17±5	19±3	20±6	19±5.7
Alkaline phosphatase (IU/L)	186±9	153±1	128±33	111±42	96±4.3
PTH pg/ml (14-75)	62.61±25.8	72.65±23.3	61.66±19.3	57.89±35.3	60.58±20.84

P: 0.37*, 0.23$^{\#}$, 0.72$^+$

This negative correlation noted on two-tailed Pearson's correlation test was significant for both (r = -0.596 and -0.505, respectively, with *P* 0.001 using Kendall's tau-b test). The level of both the serum BTMs showed decline from Tanner stage 3 to lowest at Tanner stage 5. However, the fall in CTX and P1NP with advance in sexual maturity was not significant on statistical analysis.

Discussion

This is the first study to report BMD and serum BTMs among healthy adolescent South Indian post-menarchal girls aged 12–16 years randomly selected from the community. The prevalence (22/103, 21%) of vitamin D deficiency (<12 ng/ml) in our study population is comparable to that reported in healthy children from another Indian rural setting.[11] In the cohort of 77 whose data were analysed, there was a progressive significant increase in mean BMD at LS with advance in age from 12 to 16 years [Figure 1]. The maximum BMD of 0.914 ± 0.09 g/cm^2 was attained at 16 years at LS spine. At FN, the maximal BMD was noted at 14 years of age, and although positive correlation was noted with advancement of age from 12 to 16, this was not significant. The maximum LS BMD in a previous Indian study was also noted at age 16.[8] However, the maximal percentage increase in BMD from the previous age-year occurred from 12 to 13 in the Pune study and from 15 to 16 in our study. The possible reason for this could be a later age of menarche in our cohort (mean: 12.3 years); however, this is only a speculation as the mean age of menarche in the previous study is not mentioned. When we compare this with the Thai population, the maximal annual increase in LS BMD was also noted at an earlier age of 11–12 years, the peak BMD was still similar being attained at 17 years.[6] The Brazilian girls had an increase in bone mass accrual after 10 years till 13 years with maximum annual increase in BMD at 11 years which was much earlier as compared to our study population.[4] Thus, it becomes evident that the age at maximal annual BMD increase is variable across ethnicities and socioeconomic backgrounds. Age at menarche could have a significant bearing on this which needs further evaluation. Also, as highlighted by our study, there could be intra-country differences based on regional/economic status distribution. This further authenticates need for region- and ethnicity-specific data for BMD.

Table 2: Distribution of bone mineral density at lumbar spine (L1-L4) and femoral neck based on post-menarchal years

Post-menarchal years	Lumbar spine (g/cm^2)*	Femoral neck (g/cm^2)#
<1 year (*n*=13)	0.815±0.146	0.748±0.119
1-3 years (*n*=42)	0.851±0.085	0.747±0.091
3-5 years (*n*=17)	0.925±0. 075	0. 791±0.133
>5 years (*n*=5)	0.910±0.103	0.749±0.133

*r: $^{+}$0.276, P=0.015, $^{\#}r$: $^{+}$0.074, P=0.398

The overall calcium intake in our study population was much lower than the RDA of 500–1000 mg/day. There was no significant change in BMD with the calcium intake. Indian girls belonging to upper socioeconomic strata have been shown to have a mean calcium intake of 764 mg per day in keeping with the RDA.[19] Our population had a calcium intake ranging from 200 to 250 mg/day. Boot *et al*.[20] showed better correlation of calcium intake with TB BMD than LS BMD in boys and no significant association in girls. With calcium supplementation, there was 1.4% increase in BMD at all sites.[21] There has been varying results on the influence of calcium on the BMD, and our study did not show any significant correlation. Similarly, there was no association between physical activity and BMD noted in the present study though there are interventional studies proving positive correlation between the two.[22] Both these modifiable factors were confounded by low intake/scores in our cohort. A follow-up prospective study with supplemental calcium intake and set physical activity scheduling could help determine their roles in BMD increment in our population better.

The serum BTMs (CTx and P1NP) were measured among the post-menarchal girls. P1NP is an osteoblastic marker, and CTx reflects osteoclastic activity. It is known that during childhood and adolescence, the osteoblastic activity will outweigh the osteoclastic activity although a dynamic process of linear growth and remodelling is happening. Numerically, this was clearly reflected in the serum measurements [Table 3]. During adolescence and after puberty onset, the BTM values gradually decrease and BMD gradually increases.[12,15] This reflects the fact that maximal dynamic change in bone metabolic activity happens around puberty, and post-pubertal phase is mainly characterized by bone mineral accrual with low BTMs. In the present study, there is a significant decline in both serum BTMs with age [Table 3]. Our results are similar to the data described among American adolescents and data from the

Table 3: Age-specific data of serum bone turnover markers—CTX and P1NP and their correlation with age

Age	*n*	CTX (pg/ml) *Mean±SD	CTX (pg/ml) Interquartile reference range	P1NP (ng/ml) #Mean±SD	PINP (ng/ml) Interquartile reference range
12	9	1787±737	1171-1976	550±324	289-700
13	18	1799±661	1362-1929	471±267	293-489
14	13	1254±417	1093-1327	236±89	172-280
15	24	1055±386	888-1227	233±180	145-275
16	13	1024±368	1007-1225	169±58	129-181

*r-0.596, P 0.001; $^{\#}r$-0.505, P 0.001

UK.[13,15] These vary from the Japanese data where increase in BTM was noted from age 12 to 18 years after which the values remained fairly constant during 19–30 years of age.[23] More studies are warranted to evaluate the effect of Tanner stage, age at menarche and gender on BTMs. As such interpretation of BTMs remains most valuable with retesting at follow-up, further Indian studies with follow-up BMD and BTMs' post-intervention may give more lucidity to these data. Our study also provides the first reference range [Table 3] for the serum BTMs in this Indian cohort which will be of enormous value in the management of bone disorders among children and adolescents. Following the study, we have ensured that the community social worker has educated these girls on adequate calcium intake and moderate physical activity.

Limitations

Our study population from the community was deficient in calcium intake as well as had poor physical activity score, and thus, these data may not represent a 'normative' standard. However, this highlights the need for adolescent health education in our community schools. A future comparative study after calcium supplementation and appropriate physical activity profile will be beneficial to establish the role of these modifiable risk factors. Recruitment of more participants would have been ideal to get robust data. However, with the COVID crisis, we decided not to bring in any more healthy adolescents and expose them to hospital environment during the study period. The study did not include boys, and this is being planned in the future.

Conclusions

This study provides insight into BMD data at LS spine and FN for South Indian rural post-menarchal girls 12–16 years of age. There was progressive increase in BMD with age from 12 to 16 years, with LS BMD being the best site to demonstrate these changes. Maximum BMD was attained at age 16 at the LS site. Positive correlation of BMD with height and post-menarchal years was noted. This is the first Indian study analysing serum BTMs among paediatric cohort and also provides first insight regarding their reference data on this specific subgroup. A significant negative correlation of serum BTMs with advancing age from 12 to 16 was demonstrated in our cohort.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Key message

Ethnicity-specific bone mineral density data for healthy post-menarchal girls from rural South India were generated by this study which also provides the first reference for serum bone turnover markers for this age group.

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The study was funded by the institutional research office after institutional review board clearance.

Conflicts of interest

There are no conflicts of interest.

References

1. Lloyd T, Rollings N, Andon MB, Demers LM, Eggli DF, Kieselhorst K, *et al*. Determinants of bone density in young women. I. Relationships among pubertal development, total body bone mass, and total body bone density in premenarchal females. J Clin Endocrinol Metab 1992;75:383–7.
2. Bailey DA. The Saskatchewan Pediatric Bone Mineral Accrual Study: Bone mineral acquisition during the growing years. Int J Sports Med 1997;18(Suppl 3):S191-4.
3. Krall EA, Dawson-Hughes B. Heritable and life-style determinants of bone mineral density. J Bone Miner Res 1993;8:1–9.
4. Fonseca ASM, Szejnfeld VL, Terreri MT, Goldenberg J, Ferraz MB, Hilário MOE. Bone mineral density of the lumbar spine of Brazilian children and adolescents aged 6 to 14 years. Braz J Med Biol Res 2001;34:347–52.
5. Jeddi M, Roosta MJ, Dabbaghmanesh MH, Omrani GR, Ayatollahi SMT, Bagheri Z, *et al*. Normative data and percentile curves of bone mineral density in healthy Iranian children aged 9–18 years. Arch Osteoporos 2013;8:114.
6. Nakavachara P, Pooliam J, Weerakulwattana L, Kiattisakthavee P, Chaichanwattanakul K, Manorompatarasarn R, *et al*. A normal reference of Bone Mineral Density (BMD) measured by dual energy X-Ray absorptiometry in healthy Thai children and adolescents aged 5–18 years: A new reference for Southeast Asian Populations. PLoS One 2014;9:e97218.
7. Marwaha RK, Tandon N, Reddy DRHK, Aggarwal R, Singh R, Sawhney RC, *et al*. Vitamin D and bone mineral density status of healthy schoolchildren in northern India. Am J Clin Nutr 2005;82:477–82.
8. Khadilkar AV, Sanwalka NJ, Chiplonkar SA, Khadilkar VV, Mughal MZ. Normative data and percentile curves for Dual Energy X-ray Absorptiometry in healthy Indian girls and boys aged 5-17 years. Bone 2011;48:810–9.
9. Kadam NS, Chiplonkar SA, Khadilkar AV, Fischer PR, Hanumante NM, Khadilkar VV. Modifiable factors associated with low bone mineral content in underprivileged premenarchal Indian girls. J Pediatr Endocrinol Metab 2011;24:975–81.
10. Thacher TD, Fischer PR, Strand MA, Pettifor JM. Nutritional rickets around the world: Causes and future directions. Ann Trop Paediatr 2006;26:1–16.
11. Sahu M, Bhatia V, Aggarwal A, Rawat V, Saxena P, Pandey A, *et al*. Vitamin D deficiency in rural girls and pregnant women despite abundant sunshine in northern India. Clin Endocrinol (Oxf) 2009;70:680–4.
12. Rauchenzauner M, Schmid A, Heinz-Erian P, Kapelari K, Falkensammer G, Griesmacher A, *et al*. Sex- and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years. J Clin Endocrinol Metab 2007;92:443–9.
13. Harel Z, Gold M, Cromer B, Bruner A, Stager M, Bachrach L, *et al*. Bone mineral density in postmenarchal adolescent girls in the United States: Associated biopsychosocial variables and bone turnover markers. J Adolesc Health 2007;40:44–53.

14. Yilmaz D, Ersoy B, Bilgin E, Gümüşer G, Onur E, Pinar ED. Bone mineral density in girls and boys at different pubertal stages: Relation with gonadal steroids, bone formation markers, and growth parameters. J Bone Miner Metab 2005;23:476–82.
15. Walsh JS, Henry YM, Fatayerji D, Eastell R. Lumbar spine peak bone mass and bone turnover in men and women: A longitudinal study. Osteoporos Int 2009;20:355–62.
16. Shetty S, Kapoor N, Bondu JD, Antonisamy B, Thomas N, Paul TV. Bone turnover markers and bone mineral density in healthy mother–daughter pairs from South India. Clin Endocrinol (Oxf) 2016;85:725-32.
17. Table - Nutritive value of Indian foods. C. Gopalan, B.V Rama Sastri, S. C Balasubramianian. National Institute of Nutrition, ICMR, Hyderabad. Table 1. 1989.
18. Rani MA, Sathiyasekaran BWC. Behavioural determinants for obesity: A cross-sectional study among urban adolescents in India. J Prev Med Public Health 2013;46:192–200.
19. Sanwalka NJ, Khadilkar AV, Mughal MZ, Sayyad MG, Khadilkar VV, Shirole SC, et al. Study of calcium intake and sources of calcium in adolescent boys and girls from two socioeconomic strata, in Pune, India. Asia Pac J Clin Nutr 2010;19:324-9.
20. Boot AM, de Ridder MAJ, Pols HAP, Krenning EP, de Muinck Keizer-Schrama SMPF. Bone mineral density in children and adolescents: Relation to puberty, calcium intake, and physical activity. J Clin Endocrinol Metab 1997;82:57–62.
21. Johnston CC, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC, *et al*. Calcium supplementation and increases in bone mineral density in children. N Engl J Med 1992;327:82–7.
22. Meyer U, Romann M, Zahner L, Schindler C, Puder JJ, Kraenzlin M, *et al*. Effect of a general school-based physical activity intervention on bone mineral content and density: A cluster-randomized controlled trial. Bone 2011;48:792–7.
23. Orito S, Kuroda T, Onoe Y, Sato Y, Ohta H. Age-related distribution of bone and skeletal parameters in 1,322 Japanese young women. J Bone Miner Metab 2009;27:698–704.

Annexure 1: Assay details of the biochemical analytes

Test	Inter-assay CV (%)	Intra-assay CV (%)	Assay range
Alkaline phosphatase	3.8	1.7	5-1200 U/L
Phosphorus	2.36	1.0	3-20 mg/dl
Calcium	2.2	1.0	0.8-20 mg/dl
N terminal propeptide of type 1 collagen	5.3	3.1	5-1200 ng/ml
Albumin	2.66	1.5	0.2-6.8 g/dl
Creatinine	4.0	1.5	0.2-24.9/dl
25-hydroxy vitamin D	5.4	3.8	3-70 ng/ml
Parathormone	5.8	3.4	5.-2000 pg/ml
Beta-CrossLaps	4.1	2.3	10-6000 pg/ml