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Research Article

Biochemical and Dietary Indicators of Bone Health in Rural Adolescent Girls from Konkan Region of Maharashtra, India (DERVAN-5)

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Abstract

Adolescent period is marked by bone modeling and remodeling and leads to accrual of peak bone mass. Ideal peak bone mass depends on diet, hormones, genetic influence and environment and has consequences on bone health in adulthood. We measured biochemical indicators of bone health in rural adolescent girls.

Methods: Five hundred fifty adolescent girls from longitudinal DERVAN cohort study from Indian state of Maharashtra underwent anthropometry. Biochemical parameters (intact parathyroid hormone, vitamin D, calcium, phosphorus and alkaline phosphatase) were measured.

Results: Prevalence of underweight & stunting was 28.8%, 30.7% respectively. More than 56% were thin & only 5% were obese. Median body fat% & bone mass measured by bio-impedance were 23.3 and 1.6 Kg respectively. More than 80% were vitamin D deficient and 12.0% were calcium deficient. Median dietary calcium intake was 158.5mg/day which was far below recommended 850 mg/day. Median parathyroid hormone concentration was 8.49pmol/L and 66.7% had elevated concentrations (> 6.89pmol/L). Elevated phosphorus and alkaline phosphatase were observed in 23.3% and 23.0%. Parathyroid hormone was inversely associated with age ($p<0.001$) and vitamin D ($p<0.001$) and directly with phosphorus and alkaline phosphatase ($p<0.05$) for both. On multivariate analysis elevated parathyroid hormone was associated with low vitamin D ($p<0.001$).

Conclusion: The adolescent girls of KONKAN are undernourished and vitamin D deficient. Despite poor dietary calcium intake the serum calcium levels were maintained at the cost of elevated parathyroid hormone. Thus parathyroid hormone may be used as a marker of bone health. This could be useful in planning early interventions to improve bone health.

Keywords: Intact parathyroid hormone; Vitamin D; Adolescence; Undernourished; Konkan

Abbreviations

PTH: Parathyroid Hormone; DEXA: Dual Energy X-Ray Absorptiometry; ALP: Alkaline Phosphatase; RDA: Recommended Dietary Allowance; BMD: Bone Mineral Density

Introduction

Adolescent period is the most crucial in human life cycle where foundations of subsequent health in the adulthood are laid down. It is also a period when bone modeling and remodeling takes place leading to skeletal development reaching peak bone mass [1]. Attainment of ideal peak bone mass depends on diet, hormones, genetic influence and environment. About 40% of the peak bone mass is achieved during adolescence [2,3]. However, in many children and adolescents lack of optimal bone mineralization leads to insufficient bone mass resulting in brittle bones and risk of fractures in later adulthood [4]. Indian adolescents have lower bone mass when compared to Caucasians [5]. Parathyroid Hormone (PTH) and 25-Hydroxyvitamin D (Vitamin D) are the key players in bone metabolism and calcium homeostasis. A disturbed calcium homeostasis will result in poor bone mass and likely to increase the risk of hyperparathyroidism. To achieve calcium homeostasis, PTH mobilizes calcium from skeletal stores and increases dietary absorption and decreases urinary excretion of calcium [6].The role of vitamin D in calcium homeostasis is well established. Vitamin D deficiency is very common in children and adolescents especially in females. Seasonality is also an important factor. The best way to know the bone health is bone biopsy or Dual Energy X-Ray Absorptiometry (DEXA). But this may not be always possible especially in resource limited settings. Thus PTH could be used as a surrogate marker.

This manuscript explores the bone health of adolescent girls from cohort study (DERVAN cohort) using biochemical indicators. The study is taking place in the Konkan region of western Indian state of Maharashtra [7].

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Methodology

Subjects and methods

The details of DERVAN cohort study have been already reported [7]. It plans to recruit more than 1500 adolescent girls (age16-18 years) from three tehasils (administrative divisions) of Ratnagiri district. The girls are brought for 3 days residential camp for various investigations which comprise physical examination, nutritional assessment, and cognitive testing and blood investigations.

Anthropometry and nutrition

Height and weight are measured using standardized protocol and instruments (wall mounted stadiometer and electronic weighing scale). Body fat % and bone mass were measured using segmental bio-impedance (MC-780, TANITA Corporation Japan). Calcium intake estimation and frequency of consumption of milk and milk products, cereals and pulses was based on 24 hour recall.

Laboratory measurements

Fasting blood was collected in supine position. Hemogram was done from whole blood and remaining blood was centrifuged at 3000rpm for 15 minutes at 4°C in a cooling centrifuge. Serum sample were stored at -80°C for further analysis. 25-Hydroxyvitamin D (Vitamin D) and serum intact Parathyroid Hormone (iPTH) were measured by Abbott Architect i1000 SR Germany (chemiluminescent microparticle immunoassay). Intra and inter batch Coefficient of Variation (CV) for vitamin D and iPTH were (4.62, 6.55) and (7.81, 2.14) respectively. Serum calcium, phosphorus, Alkaline Phosphatase (ALP) was measured by Erba 200 (colourimetric). Intra and inter batch CV for calcium, phosphorus and ALP were (2.85, 6.18), (5.39, 8.25), (3.19, 7.39) respectively.

Classifications

Underweight and stunting were defined using WHO growth charts [8] while BMI was categorized using International Obesity Task Force (IOTF) standard [9]. Normal range for iPTH was 1.06-6.8965 pmol/L [10]. Those with vitamin D concentrations ≤49.92 nmol/L were classified as deficient, 49.92 -99.84 nmol/L insufficient and >99.84nmol/L sufficient. Normal range for calcium is 2.12-2.55 mmol/L and < 98U/L for alkaline phosphatase. Daily calcium requirement is 850 mg/day as per ICMR guidelines.

Statistical methods

Data has been represented as median and 25th-75th centiles. The categorical data has been represented as percentage. Interrelationships between biochemical parameters were analyzed by Pearson's partial correlation controlling for age. Univariate and multivariate logistic regression was used investigate factors associated with elevated iPTH. Odds Ratios (OR) & 95% confidence interval (CI) were obtained. Statistical software SPSS V25.0, STATA 13.0 was used for the analysis.

This manuscript is based on 550 adolescent girls recruited before onset of second wave of COVID-19 pandemic.

Results

Anthropometry and body composition (Table 1)

The median age, weight, height, BMI of the girls was 16.7y, 40.7kg, 151.5cm, 17.6kg/m^2 respectively. Prevalence of underweight was 28.8% and that of stunting 30.7%. Using IOTF standard more than 56% were classified as thin and only 5% as obese. Median body fat% was 23.3 and median bone mass 1.6kg.

Table 1: Anthropometry, body composition, biochemical parameters of bone health and diet (n=550).

Anthropometry	
Age (years)	16.7 (16.0-17.3)
Weight (kg)	40.7 (36.7-45.5)
Underweight (%)	28.8
Standing Height (cm)	151.5 (148.0-155.7)
Stunted (%)	30.7
BMI (kg/m^2)	17.6 (16.0-19.9)
Thinness (%)	56.4
Normal (%)	38.4
Overweight or Obese (%)	5.2
Body Composition	
Fat %	23.3 (19.7-28.3)
Fat Mass (kg)	9.3 (7.1-12.6)
Lean Mass (kg)	30.6 (28.6-33.0)
Bone mass (kg)	1.6 (1.4-1.8)
Biochemical Parameters of Bone Health	
Intact parathyroid hormone (pmol/L)	8.49 (6.20-11.15)
(%) >6.8965pmol/L	66.7
Vitamin D (nmol/L)	35.4 (26.96-44.43)
(%) ≤49.92nmol/L	84.5
Calcium (mmol/L)	2.32 (2.2-2.45)
(%) <2.12mmol/L	12
(%) 2.12-2.55mmol/L	76.6
(%) >2.55mmol/L	11.5
Phosphorus (mmol/L)	1.26 (1.13-1.42)
(%) >1.45mmol/L	23.3
Calcium- Phosphorus product	36.5 (32.5-42.3)
(%) ≤40	65.5
Alkaline Phosphatase (U/L)	76.0 (59.0-96.0)
(%) >98U/L	23
Diet (24hr. recall)	
Calcium intake (mg/day)	158.5 (114.8-281.0)
(%) consuming milk and milk products	11
(%) consuming cereals and pulses	99

Median (25th-75th) centile or %.

Biochemical parameters of bone health (Table 1)

Vitamin D and calcium deficiencies were observed in 84.5% and 12.0% girls with median concentrations of 35.4nmol/L and 2.32mmol/L respectively. Only one girl had sufficient vitamin D concentration. Elevated phosphorus and ALP were observed in 23.3% and 23.0% with median concentrations of 1.26mmol/L and 76.0U/L respectively. Overall 66.7% had elevated PTH concentration with a median of 8.49pmol/L.

Table 2: Partial correlations.

	Age (years)	Intact parathyroid hormone (pmol/L)	Vitamin D (nmol/L)	Calcium (mmol/L)	Phosphorus (mmol/L)	Alkaline Phosphatase (U/L)
Age (years)	1					
Intact parathyroid hormone (pmol/L)	-0.168***	1				
Vitamin D (nmol/L)	0.016	-0.231***	1			
Calcium (mmol/L)	-0.113***	-0.01	0.072	1		
Phosphorus (mmol/L)	-0.214***	0.108*	-0.028	0.126**	1	
Alkaline Phosphatase (U/L)	-0.296***	0.474***	-0.169***	0.095*	0.04	1

Other than age other correlations adjusted for age.

Diet (Table 1)

The median calcium intake was 158.5mg/day. Only 11% of the girls consumed either milk or milk products while almost all they consumed cereals and pulses.

Interrelationships of biochemical indicators of bone health (Table 2)

Intact PTH was inversely associated with age ($p<0.001$). After adjusting for age, iPTH was inversely associated with Vitamin D ($p<0.001$) and directly with phosphorous ($p<0.05$) and ALP ($p<0.001$). Vitamin D was inversely associated with ALP ($p<0.001$). Calcium concentrations directly associated with phosphorous ($p<0.01$) and ALP ($p<0.05$).

Risks for Elevated PTH

Univariate (Table 3)

Odds ratios for elevated iPTH was significantly lower [OR: 0.44, 95% CI (0.27-0.72), p=0.001] in older adolescent girls. Elevated iPTH was also predicated by those who are vitamin D deficient [OR: 2.0, 95% CI (1.25-3.20), p=0.004], higher calcium [OR: 2.92, 95% CI (1.22-7.0), p=0.016] and higher ALP concentration [OR: 2.76, 95% CI (1.65-4.61), p=0.000].

Table 3: Odds ratios for elevated intact parathyroid hormone (>6.8965pmol/L) (Univariate).

Exposures	Categories	odds with 95% confidence intervals	P value
Age (years)	15-16 (ref)	1	
	16-17	0.63 (0.38-1.04)	0.07
	17-18	0.44 (0.27-0.72)	0.001*
Vitamin D (nmol/L)	>49.92 (ref)	1	
	≤49.92	2.00 (1.25-3.20)	0.004*
Calcium (mmol/L)	<2.12 (ref)	1	
	2.12-2.55	1.32 (0.77-2.23)	0.307
	>2.55	2.92 (1.22-7.00)	0.016*
Phosphorus (mmol/L)	≤1.45 (ref)	1	
	>1.45	1.27 (0.75-2.16)	0.375
Calcium- Phosphorus product	≤40 (ref)	1	
	>40	1.59 (0.97-2.58)	0.063
Alkaline Phosphatase (U/L)	≤98 (ref)	1	
	>98	2.76 (1.65-4.61)	0.000*
Anthropometry	Overweight/ obese (ref)	1	
	Normal	0.28 (0.08-0.96)	0.044*
	Thin	0.25 (0.07-0.85)	0.026*

Ref: Reference category.

Multivariate (Table 4)

On multiple logistic regression, elevated iPTH was independently predicted by lower age [OR: 0.47, 95% CI (0.23-0.99), p=0.046] for 16-17y, [OR: 0.31, 95% CI (0.15-0.66), p=0.002] for 17-18y, vitamin D deficiency [OR: 2.46, 95% CI (1.42- 4.27), p=0.001]. ALP was also independently but marginally related [OR: 1.89, 95% CI (0.99-3.61), p=0.052].

Discussion

This report is the first attempt to measure biochemical indicators of bone health among adolescent girls from KONKAN region. Our results showed that iPTH values were significantly higher in adolescents than adults. Mean iPTH was 9.08pmol/L which was much higher than reported in other studies [11-13]. Maintaining normal blood calcium level is the primary role of PTH and this can be achieved in various ways. Low concentration of ionized calcium stimulates the secretion and synthesis of PTH to increase the absorption of dietary calcium and decrease renal clearance. PTH stimulates the release of bone calcium and phosphate into the blood. [14,15]. Vitamin D plays a key role in calcium homeostasis. Vitamin D3 (cholecalciferol) is a biologically active form. Low levels of vitamin D reduce the intestinal calcium absorption, which again results into increased secretion of PTH. Another cause of elevated PTH is dietary calcium deficiency. We observed median dietary calcium

Table 4: Odds ratios for elevated intact parathyroid hormone (>6.8965pmol/L) (Multivariate).

Exposures	Categories	Odds with 95% confidence intervals	P value
Age (years)	15-16 (ref)	1	
	16-17	0.47 (0.23-0.99)	0.046*
	17-18	0.31 (0.15-0.66)	0.002*
Vitamin D (nmol/L)	>49.92 (ref)	1	
	≤49.92	2.46 (1.42-4.27)	0.001*
Calcium (mmol/L)	<2.12 (ref)	1	
	2.12-2.55	1.49 (0.73-3.06)	0.272
	>2.55	2.23 (0.79-6.32)	0.131
Phosphorus (mmol/L)	≤1.45 (ref)	1	
	>1.45	0.97 (0.54-1.73)	0.91
Alkaline Phosphatase (U/L)	≤98 (ref)	1	
	>98	1.89 (0.99-3.61)	0.052

Ref: Reference category

intake of 158.5mg/day which is far below than RDA of 850mg/day as per guidelines of ICMR [16]. We could not see association of low dietary calcium intake and elevated PTH in our study which has been reported by others [12,17]. The dietary calcium intake reported in these studies was far higher than ours. Milk and milk products are the main source of calcium but the consumption is very poor. The main reasons are lack of availability in the region and unaffordability by the population. The diet is rich in cereals and pulses which compromises intestinal calcium absorption. We have found more than 80% vitamin D deficiency in our girls. Vitamin D deficiency has been attributed to malnutrition and inadequate sun exposure. Consumption of milk and milk products are very less in rural India and even if they are consumed they are not fortified [18]. In a comparative study of post menarchal girls from Pune and Manchester (UK) it was observed that both the groups have almost same Vitamin D deficiency, though Pune girls are exposed to abundant sun shine. Pune girls showed secondary hyperparathyroidism, low calcium and skeletal deformities which were attributed to low dietary calcium intake and higher consumption fiber and pulses, cereals which compromises calcium absorption [9]. For Indians minimum 45 minutes daily direct sun exposure to skin is required with bare face, arms and legs [18,19]. But this is not possible due to cultural as well as social norms. In our study there was a strong inverse association between iPTH and age. In a study by Steven et al rise in PTH was contributed by rapid bone-forming activity during mid-puberty and low vitamin D status to meet calcium needs [20]. Biochemical indicators of bone health include ALP, PTH and vitamin D. The most accurate measure of Bone Mineral Density (BMD) is done by DEXA. But it is not convenient in our rural setting. So we used biochemical indicators to assess the bone health in adolescent girls. Bone specific ALP is widely used as a bone formation marker in a research but total ALP is considered to be adequate marker for bone health due to its convenience, stability and low cost in individuals with normal Liver Function Test (LFT) [21].

In our study all the girls had normal LFT. We also found significant positive association between ALP and iPTH. We have used bio-impedance for measurement of bone mass. We could not find any association of bone mass to iPTH. D. Bonofiglio showed that low dietary calcium intake in adolescent girls did not show any immediate adverse effect on radial density but showed elevated PTH levels supporting the need for adequate calcium intake to achieve peak bone mass [17]. Intact PTH levels in our girls are very high. This could be due to triple burden of adolescence, poor dietary calcium intake and vitamin D deficiency. This can have adverse effect on bone health in adulthood. So adequate calcium and vitamin D intake should be provided at this stage, as good nutritional intake during first two decades of life contributes to attainment of maximum peak bone mass.

Our study has much strength. It is community based and is done in the region among adolescents girls with poor BMI status.

The major limitation is lack of Bone Mineral Density (BMD) measurements using X-ray or DEXA. Other limitation is single gender.

Conclusion

To summarize adolescent girls of KONKAN are undernourished and vitamin D deficient. Despite poor dietary calcium intake the serum calcium levels were normal as a result of over secretion of parathyroid hormone. Thus parathyroid hormone may be used as surrogate marker of early bone health. This could be useful in planning early interventions to improve bone health to reduce the risk of osteoporosis in adulthood.

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Ethics

Informed and written consent was obtained from all the participants to use the data. For the adolescents below 18 years of age, informed written assent was obtained from adolescents and in addition parental consent was taken. The study was approved by the Institute Ethics Committee of BKL Walawalkar Rural Medical College and Hospital. Our institute ethics committee is registered with the Government of India. Registration code is EC/755/INST/MH/2015/RR-18.

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