

Bone Mineral Density of Indian Children and Adolescents with Cystic Fibrosis

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Objective: To document bone mineral density of children and adolescents with cystic fibrosis.

Design: Cross-sectional study.

Setting: Tertiary-care center of Northern India, July 2012 to August 2015.

Participants: 52 children aged 6-18 years with cystic fibrosis and 62 healthy controls of similar age and sex.

Methods: Both patients and controls were stratified into two groups, as pre-pubertal and peri-/post-pubertal, and compared for whole body bone mineral density, measured using dual energy X-ray absorptiometry. Serum levels of calcium, phosphate, alkaline phosphatase, 25-hydroxyvitamin D and parathyroid hormone were measured in children with cystic fibrosis.

Results: Compared with controls, the mean (SD) bone mineral density of children with cystic fibrosis was significantly lower in both the pre-pubertal (0.7 (0.1) g/cm² vs 0.9 (0.1) g/cm²;

$P < 0.001$) and peri-/post-pubertal groups (0.9 (0.1) g/cm² vs 1.1 (0.1) g/cm²; $P < 0.001$). Also, the mean (SD) bone mineral apparent density of pre-pubertal and peri-/post-pubertal cystic fibrosis patients was lower than the controls ($P < 0.001$ and $P = 0.01$, respectively). Thirty-seven (71.2%) cystic fibrosis patients had serum 25-hydroxyvitamin D level below 15 ng/mL.

Conclusion: Bone mineral density of children with cystic fibrosis was significantly lower than controls; majority of them were vitamin-D deficient. Intervening at an early stage of the disease and providing optimal therapy involving simultaneous management of the several factors affecting bone mineral accretion may be beneficial in improving bone health of these patients.

Keywords: Bone health, Chronic illness, Dual energy X-ray absorptiometry, Vitamin D.

With increased survival, individuals with cystic fibrosis (CF) develop disease-related long-term complications, among which, one is low bone mineral density (BMD). Factors that contribute to low BMD in CF include malnutrition, chronic respiratory inflammation, delayed puberty, reduced physical activity, exocrine insufficiency and glucocorticoid usage [1].

Clinical profile of Indian CF patients is almost similar to Caucasians but the disease severity is higher, mainly due to delayed diagnosis and delayed implementation of appropriate management resulting from lack of awareness about the disease or lack of diagnostic facilities [2,3]. Studies on children and adolescents with CF from developed countries suggest low BMD [4-6]. Over past two decades, there is increasing awareness about CF in India but there are no studies on BMD in Indian children with CF. We conducted this study with the objective of documenting BMD in children with CF being followed up in a large tertiary-care hospital of Northern India.

METHODS

This study was carried out in the Department of Pediatrics, All India Institute of Medical Sciences in New Delhi, India, from July 2012 to August 2015. Children (age 6-18 y) with confirmed diagnosis of CF (clinical phenotype with sweat chloride ≥ 60 mEq/L), not having any primary bone disease were included in this cross-

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sectional study. Healthy controls included children of similar age and sex, not having any chronic illness affecting bone density or a period of immobility of more than two weeks in the preceding 12 weeks. They were either friends of CF participants or children of healthcare personnel of the center. Study was approved by the Institutional Ethics Committee of AIIMS. Written informed consent was obtained from parents and guardians of all eligible children.

All participants' weight was measured using

calibrated scales, and height was measured to nearest 0.1 cm using a standardized stadiometer. Body mass index (BMI) was calculated. Z scores were calculated using WHO Anthroplus software [7]. Pubertal development was determined by a self-assessment questionnaire using drawings and written descriptions of Tanners' breast, genital and pubic hair classification [8].

Whole body dual energy X-ray absorptiometry (DXA) scan (Hologic QDR 4500A, Hologic Inc., Bedford, MA, USA) was performed and the measurements included whole body bone mineral content (in g), area (in cm²), and areal density (in g/cm²). Since there are substantial changes in bone dimensions during childhood and BMD measured by DXA scan does not take into account thickness of bone, bone mineral apparent density (BMAD), which is bone mineral content normalized to a derived bone reference value, was calculated using methods suggested by Katzman, *et al.* [9]. BMAD minimizes effect of bone geometry and allows comparisons of mineral status among bones of similar shape but different size.

In addition, use of inhaled and oral bronchodilators and other medication was recorded in children with CF. Fasting blood samples of patients were collected to assess bone-related biochemical parameters. Serum calcium, phosphorous, and alkaline phosphatase were measured by Hitachi Modular P 800 autoanalyser, and 25-hydroxyvitamin D (25 (OH) D) was measured using chemiluminescent immunoassay (DiaSorin LIAISON, Minnesota, USA). Parathyroid hormone (PTH) levels in serum were measured with electrochemiluminescence method (Roche COBAS e411, Japan). The reference range of serum 25 (OH) D was taken as: sufficient (20-100 ng/mL); insufficient (15-19 ng/mL); and deficient (<15 ng/mL) [10].

Spirometric parameters (FEV₁ and FVC (% predicted) were measured in the CF patients with a portable spirometer (Super Spiro Micromedics, Rochester, UK), using standard method for test performance recommended by American Thoracic Society and European Respiratory Society [11]. Physical activity level of these patients was estimated using Habitual Activity Estimation Scale (HAES) [12]. Participants were asked to recall one usual weekday and one usual weekend day separately from past two weeks. Sum of 'somewhat active' and 'active' scores was taken as total activity in hours.

Statistical analysis: As accelerated bone acquisition occurs during the initial few years of life and then during the pubertal spurt, children were divided into prepubertal and peri-/post-pubertal strata based on self-assessed

Tanner stage. Stratified analysis was performed to compare demographic characteristics and DXA parameters between CF patients and controls separately in the two strata of children. Multivariable linear regression analysis was performed to assess factors associated with whole body BMD, which was the dependent variable. Independent covariates were presence of CF, age, sex and height.

RESULTS

Fifty-two children with CF and 62 controls were enrolled in the study. Baseline characteristics of participants are shown in **Table I**. Mean (SD) age of CF group was 149.8 (37.8) months with 30 boys and that of controls was 148.6 (36.3) months with 35 boys. There were 28 patients and 29 controls in the pre-pubertal stratum and 24 patients and 33 controls in the peri-/post-pubertal stratum. The height, weight and the BMI of patients were significantly lower as compared to controls (**Table I**).

Children with CF had median (IQR) vitamin D levels of 9.0 (6,15.5) ng/mL, PTH 47.3 (38.6, 73.9) pg/mL, and serum alkaline phosphatase 528.5 (376.5, 674.5) IU/L. Mean (SD) serum calcium and phosphate were 9.2 (0.5) mg/dL and 4.7 (0.7) mg/dL, respectively. Mean (SD)

TABLE I BASELINE CHARACTERISTICS OF PATIENTS WITH CYSTIC FIBROSIS SUBJECTS AND HEALTHY CONTROLS

Group/ Characteristics	Cystic fibrosis (n = 52)	Controls (n = 62)	P value
<i>Pre-pubertal</i>			
Number	28	29	
Age (months)	122.8 (25.9)	116.3 (18.1)	0.28
Female/Male (n)	9/19	12/17	0.47
Weight (kg)	22.9 (7.7)	36.3 (10.2)	<0.001
Height (cm)	128.0 (11.4)	140.9 (9.3)	<0.001
Height for age Z score	-1.8 (1.4)	-0.4 (1.2)	<0.001
BMI (Kg/m ²)	13.6 (2.7)	18.2 (4.2)	<0.001
BMI for age Z score	-2.5 (1.7)	0.3 (1.4)	<0.001
<i>Peri-/post-pubertal</i>			
Number	24	33	
Age (mo)	181.3 (21.4)	176.9 (21.5)	0.44
Female/Male (n)	13/11	15/18	0.52
Weight (kg)	36.0 (9.9)	55.4 (12.5)	<0.001
Height (cm)	149.7 (9.4)	158.8 (10.0)	<0.001
Height for age Z score	-1.9 (1.2)	0.4 (1.2)	<0.001
BMI (Kg/m ²)	15.8 (3.1)	21.9 (4.7)	<0.001
BMI for age Z score	-2.3 (2.0)	0.5 (1.7)	<0.001

All values as Mean (SD) unless specified; BMI: Body mass index.

FEV₁ and FVC (% predicted) were 61.2 (24.6)% and 67.3 (20.1)%, respectively. Three (5.7%) children with CF were vitamin-D sufficient, 12 (23.1%) had insufficiency and 37 (71.2%) were deficient.

Thirty-three (63.5%) children had FEV₁ (% predicted) <70%, twenty-seven (52%) were colonized with *Pseudomonas* and 20 children were moderately severely malnourished (height for age Z score <2). Using HAES, average activity level of CF boys was 6.5 hours and of girls was 5.8 hours. Of 52 enrolled CF children, 46 were taking inhaled glucocorticoids, 5 were on long term oral glucocorticoids and 5 had taken oral corticosteroids for short durations.

Compared with controls, mean (SD) whole body BMD Z score was significantly lower in CF patients, difference being more marked in peri-/post-pubertal stratum (**Table II**). 'Z' score of -2 and below was present in 11 (38%) children and 14 (58%) adolescents with CF. Ten (36%) children and 5 (21%) adolescents with CF had Z score between -1 and -2.

Controls had a significantly higher mean (SD) BMD as compared to CF patients, both in pre-pubertal and peri-/post-pubertal strata (**Table II**). As there was a significant difference in height between the two groups, BMD was adjusted for age, sex and height and difference in means between controls and CF for the two strata was reported (**Table II**). Mean (SE) BMD of controls was significantly higher than CF in both strata. Also mean BMAD of pre-pubertal, as well as, peri-/post-pubertal controls remained significantly higher than that in CF patients (**Table II**).

DISCUSSION

In our study, children with CF were relatively leaner, shorter and lighter than their healthy counterparts. Whole body BMD of both children and adolescents with CF was significantly lower than that of controls, even after adjustment for height, age and sex.

The major limitation of the study was that blood sampling of healthy controls could not be carried out for comparison with CF patients' bone-related biochemical parameters, especially vitamin D status. Also, the referral nature of hospital might have introduced bias of inclusion of CF patients with more severe disease profile.

Literature regarding low bone mass in children with CF is controversial, with most of the studies reporting normal BMD in well-nourished CF children. Hardin, *et al.* [13] found that total body BMD of 13 pre-pubertal CF children was significantly lower when compared with age- and gender-matched controls, but no difference was found when compared with controls matched for lean

TABLE II COMPARISON OF WHOLE BODY BONE MINERAL PARAMETERS IN PATIENTS WITH CYSTIC FIBROSIS AND HEALTHY CONTROLS

Parameter	Cystic fibrosis	Control	P-value
Pre-pubertal (n)	28	29	
Bone mineral density 'Z' score	-1.8 (1.1)	1.1 (0.9)	<0.001
Difference (95% CI)	-2.9 (-3.5, -2.4)		
<i>Bone mineral density (g/cm²)</i>			
Unadjusted	0.7 (0.1)	0.9 (0.1)	<0.001
Adjusted, Mean (SE)	0.7 (0.0)	0.8 (0.0)	<0.001
Difference (95% CI)	-0.1 (-0.2, -0.1)		
Bone mineral apparent density (g/cm ³)	0.08 (0.01)	0.09 (0.01)	<0.001
Peri-/post-pubertal (n)	24	33	
Bone mineral density 'Z' score	-2.4 (1.9)	0.9 (1.0)	<0.001
Difference (95% CI)	-3.4 (-4.2, -2.6)		
<i>Bone mineral density (g/cm²)</i>			
Unadjusted	0.9 (0.1)	1.1 (0.1)	<0.001
Adjusted, Mean (SE)	0.9 (0.0)	1.1 (0.0)	<0.001
Difference (95% CI)	-0.2 (-0.3, -0.1)		
Bone mineral apparent density (g/cm ³)	0.09 (0.01)	0.1 (0.01)	0.01

All values as Mean (SD) unless specified; Difference in means calculated by (CF - Control) with 95% CI; Adjusted for age, sex and height; SE: Standard error.

tissue mass, height, age and gender. Buntain, *et al.* [14] found that age-, sex- and height-adjusted total body, lumbar spine and femoral neck BMD of 32 well-nourished CF children was comparable to 40 controls of similar age and sex.

Some studies suggest that bone mineral deficit in CF children starts in early childhood. Ujhelyi and colleagues [15] observed lower Z scores of BMD in lumbar spine and femoral neck of 11 children with CF. In a Canadian study including 81 CF children, Z score for bone mineral content between -1 and -2 was observed for whole body in 38% of children and for lumbar spine in 28% of children; Z score of less than -2 was observed in 7 children for both the measures [16]. Findings of our study are consistent with the observations of these studies, where the whole body BMD Z score of -2 and below was observed in 11 (38%) children and 14 (58%) adolescents with CF as compared to none in healthy cohort.

Bone mass in CF patients is inversely related to disease severity, wherein, decrease in FEV₁ is associated with

WHAT IS ALREADY KNOWN?

- There is reduced bone mineral density in children with cystic fibrosis.

WHAT THIS STUDY ADDS?

- Majority of Indian children with cystic fibrosis are vitamin D - deficient and have low bone mineral density.

decrease in bone mass and increased glucocorticoid usage is associated with decreased bone mass [5,6,14]. Studies on Indian CF patients report increased disease severity due to delayed diagnosis [2]. Studies also suggest that pulmonary involvement is the most prominent and the severest manifestation in Indian CF patients [2,17]. Majority (63.5 %) of CF children enrolled in present study had moderate to severe lung disease and a large number of them were malnourished. These factors might have contributed to low BMD in our patients. As the disease severity observed in Indian CF patients and our study population is higher than Caucasians, use of glucocorticoids could be higher than the western world, which may be compromising the bone mineral accretion of our CF group. The oral glucocorticoids use showed a negative relation with mean whole body BMD and whole body BMD Z score. Using habitual activity estimation scale, in our study, average activity level of boys with CF was 6.5 hours and of girls was 5.8 hours which was lower than the documented activity levels by Boucher, *et al.* [18]. The lower activity levels of our CF cohort may be due to increased disease severity that might have affected bone mineral accretion. Some rare and novel mutations identified in Asian population could be another contributing factor to increased disease severity and thereby reduced bone mass of our CF population [2,19].

Influence of suboptimal levels of vitamin D and calcium homeostasis on bone metabolism of CF patients remains unclear. In the current study it was observed that majority of CF subjects were vitamin D-deficient and in 15 patients parathyroid hormone levels were above the normal limits. These findings are consistent with findings of Haworth, *et al.* [4] and Aris, *et al.* [20], and could be contributing to low bone mass in the present CF cohort.

The results of this study stress on implementation of vigorous strategies to manage skeletal health of CF children from early childhood. We conclude that in children with CF, several factors concomitantly adversely affect bone mineral accretion. The beneficial effects of improving/altering any one factor on bone mass may be masked by other factors. Therefore, intervening at an early stage of the disease and providing optimal therapy involving simultaneous management of several factors

affecting bone mineral accretion in CF patients may have a positive impact on their skeletal health.

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