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Markers of Bone Turnover Are Associated With Growth and Development in Young Subjects With Sickle Cell Anemia

Ellen B. Fung, PhD, RD^{1,*}, Deborah A. Kawchak, MS, RD², Babette S. Zemel, PhD², Alisha J. Rovner, PhD², Kwaku Ohene-Frempong, MD³, and Virginia A. Stallings, MD²

¹Department of Hematology, The Children's Hospital & Research Center, Oakland, California

²Department of Pediatrics, Divisions of Gastroenterology, Hepatology and Nutrition, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

³Department of Pediatrics, Division of Hematology, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Abstract

Children with sickle cell anemia (SCA) have low bone mass though bone turnover has not been well described. In this study, growth and pubertal development were assessed twice, 1 year apart, in 80 young subjects with type-SS SCA, while whole body bone mineral content (BMC) and density were measured in a subset (n = 46). Markers of bone turnover were measured at the second visit. Bone formation (alkaline phosphatase) was elevated, whereas bone resorption (deoxypyridinoline) was decreased compared to published data in healthy children. Markers of bone turnover correlated with growth velocity and pubertal development but not with changes in bone mass.

Keywords

bone markers; bone mineral content; bone mineral density; growth; sickle cell anemia

INTRODUCTION

Children with sickle cell anemia (SCA) have low bone mass compared to healthy children [1-3]. Low bone mass in these children is apparent even after adjusting for age, height, pubertal development, and lean body mass, suggesting that the deficits cannot be fully explained by short stature, delayed puberty, or altered body composition [3]. Chronic hemolytic anemia and the resulting erythroblastic hyperplasia may contribute to bone demineralization in SCA [4, 5]. Furthermore, reduced physical activity [6], decreased circulating growth hormone [7], vitamin D deficiency, [8] and poor dietary intake of bone-forming nutrients [8,9] are likely contributing factors.

Suboptimal peak bone mass acquisition in childhood may contribute to the development of osteoporosis in later life [10]. Studying the process and factors influencing the acquisition of bone mass in chronic diseases of childhood allows clinicians to design treatments to optimize bone health.

There are few studies of biochemical markers of bone turnover in relation to bone mineral density (BMD) in children with SCA. BMD is a result of bone formation and resorption;

*Correspondence to: Ellen B. Fung, HEDCO Health Sciences Center, Children's Hospital & Research Center, Oakland, 5700 Martin Luther King Jr. Way, Oakland, CA 94609. E-mail: efung@mail.cho.org

therefore, analysis of these separate but related processes can lead to an improved understanding of why bone demineralization may be present in SCA. The aims of this pilot study were to describe bone turnover in children with SCA and to determine whether a relationship exists between these markers of bone turnover and the change in bone density and/or growth parameters over a 1-year period.

RESEARCH DESIGN AND METHODS

Children with homozygous type-SS SCA were recruited from The Children's Hospital of Philadelphia to participate in a longitudinal study of nutrition and growth as previously described [3]. Height and weight were measured, and pubertal development (Tanner stage) determined by a validated self-assessment question-naire [11] at two annual visits (years 4 and 5). Whole body BMD, bone area (BA), and bone mineral content (BMC) were also determined twice by dual energy X-ray absorptiometry (DXA: Hologic QDR 2000, Bedford, MA) [3] in a subset of subjects ($n = 46$). There were no differences in age, gender, or growth between those who had DXA measured at one versus both time points. Morning blood samples were drawn on all subjects at the second visit, and either first or second morning void urine samples were collected and stored at -80°C until analysis. This protocol was approved by the Institutional Review Board, and informed written consent was obtained from the parent/guardian and assent from each subject older than 7 years.

Bone formation markers measured in serum included: bone specific alkaline phosphatase (BSAP) using an immunoradiometric assay (Alkphase-B®, Metra Biosystems, Mountain View, CA), and osteocalcin (OC) using a monoclonal antibody assay (Novo-Calcein®, Metra Biosystems). Bone resorption markers included urinary deoxypyridinoline (DPD) crosslinks, with a competitive enzyme immunoassay (Pyrilinks-D®, Metra Biosystems) normalized for creatinine, and intact serum parathyroid hormone (iPTH) by enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories, Inc., Webster, TX). Reference values were obtained from the literature which utilized similar analytical techniques [12-14]. Bone mineral and growth Z-scores were calculated as previously described [3]. Data were analyzed using standardized techniques (STATA version 9.0, Stata Corp, College Station, TX).

RESULTS

Eighty subjects (40 male) are included in this report (Table I). Male subjects had lower weight-for-age ($P = 0.01$) and height-for-age Z-scores ($P = 0.005$) compared to females, and exhibited a decline in height-for-age Z-score over the 1-year period. Approximately 63% of the total sample (4.5- 14.3 years) were pre-pubertal. There were no significant gender differences in the biochemical markers of bone formation and resorption. As has been observed in healthy children [15], markers of bone formation varied by stage of puberty. In female subjects, 38% of the variability in OC ($P = 0.005$), and 45% of the variability in BSAP ($P < 0.001$) were attributed to stage of pubertal development. However, pubertal development was not as clearly associated with bone formation in male subjects. Markers of bone formation were positively correlated with each other in the group as a whole: BSAP and OC ($r = 0.36$, $P = 0.001$). Height velocity was positively correlated with markers of bone formation [BSAP ($r = 0.57$, $P < 0.001$)] in all subjects, and with bone resorption only in female subjects: DPD ($r = 0.56$, $P = 0.008$). The observed gender differences may be related to disruption in regulatory mechanisms of growth and bone in males with SCA due to the more severe growth failure.

Subjects had reduced whole body BMC corrected for age (At year 5: Z-score, Males: -1.4 ± 1.3 ; Females: -0.6 ± 0.9) and height (Z-score Males: -0.6 ± 0.9 ; Females: -0.6 ± 0.8). BMC for age Z-score decreased by 32% over the year in male subjects ($P = 0.038$). Though unexpected in healthy children, this decrease was correlated with short stature (Height-for-age Z-score: r

= 0.55, $P = 0.02$) and height velocity in SCA males (Z-score: $r = 0.62$, $P = 0.005$), but not with change in height-for-age Z-score. BSAP was negatively correlated with total body BMC and BMD, ($P < 0.007$); however, there were no associations between markers of bone turnover and BMC or BMD Z-scores, nor between markers of bone turnover and change in BMD or BMC.

DISCUSSION

Evaluating these results in relation to published data was a challenge due to differences in assays and a paucity of ethnic specific pediatric reference data. Compared to reference data from a group of healthy Caucasian children from Los Angeles (126 Male, 143 Female), we found no differences in OC levels and higher levels of BSAP in children with SCA in Tanner stages 1 to 3 (Fig. 1) [12]. However, when compared to healthy Thai children (9-18 years), children with SCA had higher OC levels, and girls with SCA in Tanner stages 3 to 5 had higher BSAP [14]. As for bone resorption, children with SCA, regardless of Tanner stage had significantly lower DPD compared to healthy German children (116 Male, 126 Female) (Fig. 1) [13].

From these comparisons, we suggest that subjects with SCA in this sample had elevated markers of bone formation with decreased markers of bone resorption when compared to healthy Caucasian and Asian children. Unfortunately, there are no published reference data for bone markers from healthy black children. Therefore, we were unable to determine if the differences observed were due to the disease and/or ethnicity. Additionally, the small numbers of children in Tanner stages 2 to 5 included in this sample limit the generalizability of the results among more mature children.

Few studies have assessed bone turnover in patients with SCA and explored its relation to DXA [1], ultrasound [16], or protein metabolism [17]. Some [17,18], but not all [1,16] reported elevations in markers of bone formation (BSAP, C-terminal propeptide of type I collagen) in SCA compared to black controls. Reports of markers of bone resorption in pediatric subjects with SCA are equally divided: one reported increased urinary pyridinium [17] and the other reported decreased serum N-terminal telopeptide of type 1 collagen in serum compared to controls [16]. The only report in SCA relating DXA bone densitometry to bone markers found no significant relationships [1].

Since there is an expectation that biochemical markers reflect bone turnover, the variability in comparisons among studies may be explained by differences in assays, ethnicity, nutritional status, age of pubertal onset, and/or other sampling differences. Because pediatric subjects with SCA often have delayed puberty, our results were analyzed by pubertal stage when reference data were available. These data must be considered in the presence of possible suboptimal nutritional status; 65% of the present sample had inadequate vitamin D status [8]. We observed a positive correlation between 25 hydroxy vitamin D and BSAP in male SCA subjects ($r = 0.36$, $P = 0.046$), and it is known that vitamin D status influences OC synthesis within osteoblasts [19]. Additionally, we have shown that many young subjects with SCA are at risk for zinc deficiency [20]. Alkaline phosphatase isozymes are dependent upon zinc for function; therefore, some variability in our sample may be due to underlying nutritional deficits.

Bone turnover markers have advanced significantly such that they are now used to assist with the diagnosis and monitoring of osteoporosis therapy in adults. Some have suggested that the usefulness of these markers in children is not in the clinical diagnosis of low bone mass but rather in the longitudinal assessment of individuals to assess the effects of interventions on bone and growth [15]. However, their clinical usefulness in pediatric patients is limited by the paucity of reference data according to ethnicity and Tanner stage.

In conclusion, in these young subjects with SCA, biochemical markers of bone turnover appear to reflect changes in growth and pubertal status rather than changes in bone indices (BMC and BMD) over a 1-year period. However, until robust pediatric, ethnic specific reference data become available for commonly used bone biomarkers, it is difficult to use these markers to predict clinical outcomes in individual pediatric patients with SCA.

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Fig. 1

. Bone biomarkers by Tanner stage in children with SCA compared to published reference ranges, Mean $\pm$ SD. **A, B:** Osteocalcin (ng/ml) in male and female SCA versus healthy subjects (Reference [12]); **(C, D)** Bone specific alkaline phosphatase (IU) in male and female SCA versus healthy subjects (Reference [12]); **(E, F)** Urinary deoxypyridinoline (nmol/mmol creatinine) in male and female SCA versus healthy subjects (Reference [13]).

Summary of Growth, Development, Bone Turnover Markers and Bone Density Characteristics in Young Subjects With Sickle Cell Disease, Mean ± SD (Range)^{*}

	Males	Females
Age, years	N = 40	N = 40
Weight-for-age Z-score ^a	11.2±4.6 (4.5 to 19.0)	10.0±3.8 (4.5 to 19.1)
Height-for-age Z-score ^b	-1.2 ± 1.1 (-3.2 to 1.1)	-0.5 ± 0.9 (-2.1 to 1.5)
Height velocity, cm/year ^c	-0.9 ± 1.1 (-3.2 to 1.7)	-0.1 ± 1.0 (-2.7 to 1.8)
BMI Z-score	4.6±2.2 (0.0 to 8.1)	5.1±2.5(0.3 to 9.2)
Pre-pubertal: Tanner stage 1, # (%)	-0.9±1.1 (-3.6 to 1.2)	-0.6±1.0 (-4.2 to 1.6)
Post-pubertal: Tanner stage 2, 3, 4, # (%)	23 (57.5)	27 (67.5)
Post-pubertal: Tanner stage 5, # (%)	13 (32.5)	9 (22.5)
Female subjects menstruating, # (%)	4 (10.0)	4 (10.0)
Average age at menstruation ^d , years	8/12	(66.7%)
Bone formation		12.7±1.3
Serum osteocalcin, ng/ml ^e		13.5±5.1 (3.6 to 23.5)
Serum bone specific alkaline phosphatase, U/L	13.8±5.3 (5.6 to 25.2)	85.2±32.6 (24.2 to 154.4)
Bone resorption	85.4±34.3 (33.3 to 160.0)	
Urinary deoxypyridinoline, nmol/nmol creatinine		
Serum intact parathyroid hormone, pg/mL ^e	3.6±1.7 (0.8 to 8.2)	4.2±2.3 (0.2 to 9.7)
Changes in growth and bone mass ^f	42±29 (1 to 100)	39±44 (1 to 170)
Change in height-for-age Z-score	(N = 23)	(N = 23)
BMC, g/year	-0.2±0.4 (-1.1 to 0.2)	0.0±0.2 (-0.4 to 0.4)
BMD, g/cm2/year	82±97	105±52
BMC for age Z-score/year	0.012±0.036	0.024±0.02
BMC for height Z-score/year	-0.32±0.61	0.005±0.27
Bone area Z-score/year	0.02±0.41	0.03±0.40
	0.03±0.53	-0.07±0.38

^{*} Data in this table are from year 5 of the study unless stated otherwise

^a Significant difference observed between male and female subjects, *P*=0.01

^b Significant difference observed between male and female subjects, *P*=0.005

^c Height velocity calculated from height obtained at years 4 and 5, annualized

^d Age at menstruation provided for female subjects who had achieved Tanner stage >1 by the second study time point (year 5)

^e Two subjects had osteocalcin levels and 12 subjects had parathyroid hormone levels below the assay sensitivities and are not included in these reported means

^f Change in bone density parameters calculated from subjects who had DXA scans at both years 4 and 5, annualized (n=46)