Sasigarn A. Bowden*, Chiazor I. Akusoba, John R. Hayes and John D. Mahan

Biochemical markers of bone turnover in children with clinical bone fragility

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Abstract

Background: The role of biochemical bone turnover markers (BTMs) in assessing low bone mass and monitoring bisphosphonate treatment in pediatric patients with clinical bone fragility is not well established. The aim of the study was to examine the correlations of BTMs and the bone mineral density (BMD), and evaluate the effects of bisphosphonates therapy on BTMs in children with clinical bone fragility.

Methods: Clinical data of 115 patients with clinical bone fragility (mean age 9.7±5.8 years), 102 of whom received bisphosphonates, were studied. Serum alkaline phosphatase (ALP), osteocalcin (OC), urine pyridinoline (PD) and deoxypyridinoline (DPD), BMD at baseline and subsequent years were analyzed.

Results: There was a significant negative correlation between urine PD and lumbar BMD (slope=−0.29, $p<0.001$). There were no correlations between BTMs and lumbar BMD Z-score. There was a significant positive correlation between serum OC and serum ALP, urine PD and DPD ($p<0.001$). Serum OC, urine PD and DPD index, as expressed as measured value/upper limit of normal value for age, decreased during the first 3 years of bisphosphonate therapy.

Conclusions: In children with clinical bone fragility, BTMs correlated with each other, but not with lumbar BMD Z-score. While they were not reliable predictors of degree of low BMD, the bone markers showed suppression during bisphosphonate therapy and may be helpful in monitoring the response to therapy.

***Corresponding author: Sasigarn A. Bowden,** MD, Nationwide Children's Hospital, Division of Endocrinology, 700 Children's Drive, Columbus, OH 43205, USA, Phone: 614-722-4118; Fax: 614-722-4440, E-mail: sasigarn.bowden@nationwidechildrens.org
Chiazor I. Akusoba: The Ohio State University College of Medicine, Columbus, OH, USA
John R. Hayes: Pacific University, Forest Grove, OR, USA
John D. Mahan: Division of Nephrology, Department of Pediatrics, Nationwide Children's Hospital, Columbus, OH, USA

Keywords: bone markers; bone mineral density; bone turnover; deoxypyridinoline; osteocalcin; pyridinoline.

Introduction

Osteoporosis is defined as a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone, leading to bone fragility and fractures. Once known as a disease of the aging, osteoporosis has now been recognized in significant debilitating conditions affecting pediatric patients starting at very young age. The causes of osteoporosis in children and adolescents are divided into two groups: primary osteoporosis or genetic brittle bone diseases, such as osteogenesis imperfecta (OI) or juvenile idiopathic osteoporosis (JIO), and secondary osteoporosis due to underlying chronic illnesses.

Anti-resorptive or bisphosphonate therapy has been used to treat children and adolescents with a variety of causes of bone fragility to improve bone density and reduce fracture rate. Many studies have demonstrated beneficial outcomes with pamidronate treatment in children with OI [1–3], as well as other bone fragility conditions such as idiopathic juvenile osteoporosis [4], spastic quardriplegic cerebral palsy [5–7], neuromuscular disorders [8], steroids-induced osteoporosis [9], and fibrous dysplasia [10]. Bisphosphonates decrease bone turnover by decreasing bone resorption. They have a direct effect on decreased recruitment and function of osteoclasts and an indirect effect by stimulating osteoblasts to produce an inhibitor of osteoclast formation [11, 12].

Bone metabolism is a dynamic process between bone formation and bone resorption. In adults, bone is continuously being maintained by bone remodeling, which is important for repair of microfractures as a result of wear and tear in response to stress and biomechanical forces. Bone metabolism in children is more complex, and greatly differs from that of adults as it reflects both skeletal growth and modeling. During remodeling, which is seen in adults, bone formation and resorption are tightly coupled. In contrast, bone modeling during growth in children results in new bone formation at a different site

than the site of resorption and at a faster rate, leading to alterations in bone shape and bone mass accrual [13]. Interpretation of biochemical markers of bone turnover [bone turnover markers (BTMs)] in children is therefore more challenging than that in adults as biomarkers of bone metabolism represent the combined effects of bone formation, skeletal growth and bone remodeling.

Several biochemical assays are available that measure BTMs. These biochemical assays measure collagen breakdown products and other molecules released from osteoclasts and osteoblasts during the process of bone resorption and formation. Markers that are specific to bone formation include alkaline phosphatase (ALP), and osteocalcin (OC); markers specific to bone resorption include urine pyridinoline (PD), urine deoxypyridinoline (DPD), serum C-terminal cross-linking telopeptide of type I collagen (CTX) and N-terminal cross-linked telopeptide. In normal state of health, half of the ALP activity in serum is derived from bone, where it is produced by osteoblasts during bone formation, so called bone-specific ALP isoenzyme. The rest comes mainly from liver, with some from the intestine. Bone-specific ALP is a widely used bone formation marker in research as it is more specific for bone and therefore, more sensitive in detecting subtle changes in bone formation. Total ALP is considered to be an adequate marker for assessment of metabolic bone disease and it is often used due to convenience and low cost in evaluating and monitoring patients without liver disease. Total ALP is stable, with a relatively long half-life and no significant circadian variations [14]. Serum OC is derived from the most abundant non-collagenous protein in bone and is thought to be secreted exclusively by osteoblasts and thus correlates with osteoblastic activity, bone formation, and mineralization [15]. OC is elevated in high bone turnover states and is reduced in low bone turnover states [15]. PD is present predominantly in articular cartilage and to a lesser extent in bone, whereas DPD is most abundant in mineralized tissues such as bone and dentine [14]. During the process of bone resorption, type I collagen, the most abundant organic component of bone, is fragmented into smaller peptides which are then released into the blood and eventually excreted into the urine.

Several studies have shown that these markers could provide indices of bone turnover in patients with osteoporosis. In adults, biomarkers of bone turnover predict fracture risk and changes in bone mineral density (BMD) [16] and can be used to monitor the effectiveness of bisphosphonate treatment [17]. The evaluation of bone metabolism using such biochemical markers in children and adolescents is complicated by the variation by age, gender, or puberty with rapid growth [13, 15]. The role of biochemical BTMs in the care of pediatric patients with clinical bone fragility is not well established. Published literature contains limited data on the clinical usefulness of these markers in assessing osteoporosis and monitoring the efficacy of anti-resorptive therapy in pediatric patients. The aims of this study were (1) to evaluate associations between the markers of bone formation and bone resorption, (2) to examine the correlations between BTMs (serum ALP, serum OC, urine PD and DPD) and BMD, and (3) to evaluate the effects of bisphosphonate therapy on BTMs in children with bone fragility.

Materials and methods

Subjects and treatment protocols

Clinical data of 115 pediatric patients with clinical bone fragility osteoporosis of various etiologies followed by the Pediatric Metabolic Bone Clinic at Nationwide Children's Hospital from 1999 to 2010 were retrospectively reviewed. Clinical information including demographic data, medical history, and bisphosphonate therapy were collected. Dual-energy X-ray absorptiometry (Hologic DXA, Waltham, MA, USA) was performed at the time of referral or first visit and annually throughout the course of follow up while on bisphosphonate therapy. Blood chemistries including BTMs were obtained on all patients before the treatment initiation and annually during the course of bisphosphonate treatment and follow up per our routine protocol.

The patients were treated with bisphosphonates based on clinical bone fragility confirmed by significantly low lumbar BMD Z-scores. At the start of the study, pamidronate was the only parenteral long-acting bisphosphonate available and most patients received this agent. More recently, zoledronic acid was used in a few patients due to its ease of administration. In addition, oral bisphosphonates consisting of alendronate (maximal dose 70 mg weekly) or residronate (35 mg weekly) were used in some children due to poor intravenous access or the preference of the family.

Intravenous pamidronate, at 0.5 mg/kg/day, was administered for three consecutive days during the first cycle and repeated at 1 mg/kg/day × 3 days at 3 month intervals during the first year. The maximal dose was 60 mg. Intravenous zoledronic acid, at 0.025 mg/kg/dose, was given at the first dose, followed by 0.025–0.05 mg/kg/dose at 3–4 month intervals in the first year (total 0.1–0.15 mg/kg at 1 year). Frequency of both pamidronate and zoledronic treatment was reduced to every 4, 6, or 12 months depending on BMD response. Treatment was discontinued when lumbar BMD Z-score was near or above zero. No patients had treatment discontinued due to medication side effects. This retrospective study was approved by the Committee for the Protection of Human Subjects Internal Review Board at Nationwide Children's Hospital, and complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.

Bone turnover markers (BTMs)

Biochemical markers for bone turnover included serum ALP, serum OC and urine PD and DPD (second void urine specimen) were obtained in the morning during the Metabolic Bone Clinic visit. This consistent time of morning specimen collection minimized any bias from diurnal variation. Serum ALP was measured by a Hitachi multichannel analyzer. Serum OC was measured by radioimmunoassay at Esoterix Laboratory, Calabasas Hills, CA, USA. Intra-assay coefficient of variation (CV) was 6% and inter-assay CV was 13% for OC. The normal range of OC is 0.7–9.2 nmol/L (5–60 ng/mL) for 1–7 years, 4.6–15.8 nmol/L (30–103 ng/mL) for 8–9 years, 5.7–23.7 nmol/L (37–154 ng/mL) for 10–11 years, 6.4–34.6 nmol/L (42–225 ng/mL) for 12–15 years, and 0.3–2.4 nmol/L (2–22 ng/mL) for adults. Urine PD and DPD were measured by HPLC (high-performance liquid chromatography) at Mayo Clinic, Rochester, MN, USA. Inter- and intra-assay CVs were 5.1% and 3.8% and 6.2% and 4.2%, respectively. The normal range of PD is 158.4–441.9 nmol/mmol creatinine for 2–10 years, 106.9–397.6 nmol/mmol creatinine for 11–14, 42–200.5 nmol/mmol creatinine for 15–17 years, 20.0–60.8 nmol/mmol creatinine for adult males, and 22.1–88.9 nmol/mmol creatinine for adult females. The normal range of DPD is 31.1–111.7 nmol/mmol creatinine for 2–10 years, 16.6–100.8 nmol/mmol creatinine for 11–14 years, and 58.7 or less nmol/mmol creatinine for 15–17 years, 3.7–18.7 nmol/mmol creatinine for adult males, and 4.4–21.1 nmol/mmol creatinine for adult females. These bone resorption markers were used due to availability of pediatric reference data at that time.

Due to various normal reference values for different age group in the pediatric population, we calculated an index for serum OC, urine PD and urine DPD values, expressed as a ratio of measured values to upper limit of normal values for age.

Bone mineral density measurement

Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (Hologic Delphi, Waltham, MA, USA). The Z-score of the lumbar spine BMD was determined using our institution's normative data based on the report by Southard et al. from our institution that studied 218 healthy children, age 1–19 years [18]. The lumbar BMD is adjusted for weight and pubertal status, as multiple regression analyses in this study showed that Tanner stage and weight were the best predictive indicators of bone mass and BMD in children [18]. Total body bone mineral content (BMC) was determined for patients without hardware (e.g. ventriculoperitoneal shunt, intra-osseous rods).

Three patients with orthopedic hardware in the lumbar spine, which falsely elevates the BMD, were excluded from the analysis. In two patients with overlying gastrostomy tube over one vertebral body segment, three lumbar vertebral body segments (excluding the obscured segment) were used in each BMD measurement to increase accuracy of the data [19].

Statistical analysis

Analyses over time were conducted with a maximum likelihood within subjects analysis of variance and covariance (SPSS Proc Mixed) in order to account for missing data. An autoregressive covariance matrix was used for variance estimation. Pearson's correlation and regression were performed to assess the relationship between bone biochemical markers and different bone parameters. Data were reported as means and standard deviations. A p-value <0.05 was considered to be statistically significant. All statistical analyses were conducted by using Windows SPSS 14.0 (SPSS, Chicago, IL, USA).

Results

Clinical data of 115 patients (mean age 9.7±5.8 years, 72 males) with clinical bone fragility and low lumbar BMD were included in the study. Patients were classified as the following diagnoses: OI (41 patients), immobilization from various neuromuscular disorders (57 patients), juvenile osteoporosis (10 patients), and steroid-induced osteoporosis (7 patients). One hundred and two patients received bisphosphonates for bone fragility. Of these, 95 received pamidronate, three zoledronic acid, two residronate and two alendronate. Mean duration of bisphosphonate therapy was 4.0±1.9 years. Mean duration of follow-up was 8.0±2.9 years. Clinical data at baseline of 13 patients who did not receive bisphosphonate treatment were included in the analysis. As shown in Figure 1, the mean lumbar BMD Z-score increased steadily during the first 3 years of bisphosphonate therapy and then plateaued from the 4th to the 7th year.

There were positive correlations between lumbar BMD and age ($r=0.6$, $p<0.001$), and also between total BMC and age ($r=0.374$, $p=0.006$), demonstrating that lumbar BMD and BMC increase with age, as expected. In contrast, serum ALP, OC and urine PD had significantly negative correlation with age ($r=-0.445$, $p<0.0001$; $r=-0.282$, $p=0.032$; $r=-0.533$, $p<0.001$, respectively).

Associations between the markers of bone formation and bone resorption

There were significant positive correlations between serum OC and each of the following: serum ALP ($r=0.31$, $p<0.015$), urine PD ($r=0.32$, $p<0.026$), and urine DPD ($r=0.48$, $p<0.001$) (Figure 2), indicating that high bone formation and bone resorption markers correlated with high bone turnover state. There was no significant correlation between serum OC and serum parathyroid hormone (PTH).

Association between BTMs and lumbar BMD

There was a significant association between lumbar BMD and log PD (slope=−0.19, 0.04SE, $p<0.001$) while controlling for baseline lumbar BMD (slope=0.75, 12SE, $p<0.001$). Similarly there was a significant correlations between lumbar BMD and log DPD (slope=−0.13, 0.04SE, $p<0.001$)

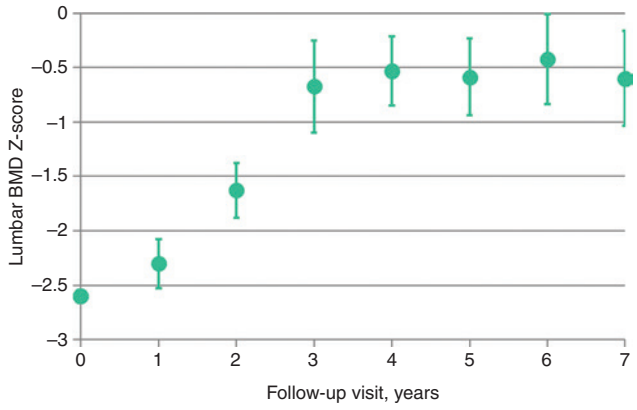

Figure 1: Lumbar vertebral BMD Z-scores in children with bone fragility and bisphosphonate therapy.
Lumbar Z-score increased significantly during the first 3 years of bisphosphonate therapy and then plateaued from year 4 to year 7 (as therapy was typically decreased or discontinued). Lumbar Z-score increased significantly during the first 3 years of bisphosphonate therapy and then plateaued from year 4 to year 7. (Numbers of patients were 102 at baseline, 102, 93, 84, 69, 52, 31, and 23 at 1, 2, 3, 4, 5, 6, and 7 years, respectively.)

and log ALP (slope=−0.18, 0.06SE, p=0.002) controlling for baseline lumbar BMD (slope=0.77, 0.12SE, p<0.001 and slope=−0.80, 0.12SE, p<0.001, respectively). Log transformations were used because of the right skewed nature of the BTM distributions, however all three BTM measures were significant (all p<0.005) with the arithmetic scale though the F-values were smaller due to larger variance caused by the tails of the distributions.

There were no significant associations between lumbar BMD Z-score and any of the absolute values of BTMs: serum ALP, serum OC, urine PD, or urine DPD. However, when using OC index (expressed as a ratio of measured OC value to upper limit of normal value for age) for analysis, there was a trend toward significant association between serum OC index and lumbar BMD Z-score (r=0.12, p=0.089) (Figure 3). Other indices of PD (r=−0.135, p=0.097) and DPD (r=−0.048, p=0.477) were also not significantly associated with lumbar BMD Z-score.

The effects of bisphosphonate therapy on BTMs

The indices of serum OC, urine PD and DPD over time are depicted in Figure 4. Mean urine PD index was >1.0 at baseline (absolute values being higher than upper limit of normal), indicating high bone turnover in this cohort. Similarly, urine DPD was also at the upper end of normal limit (having index >0.8). On the other hand, mean serum

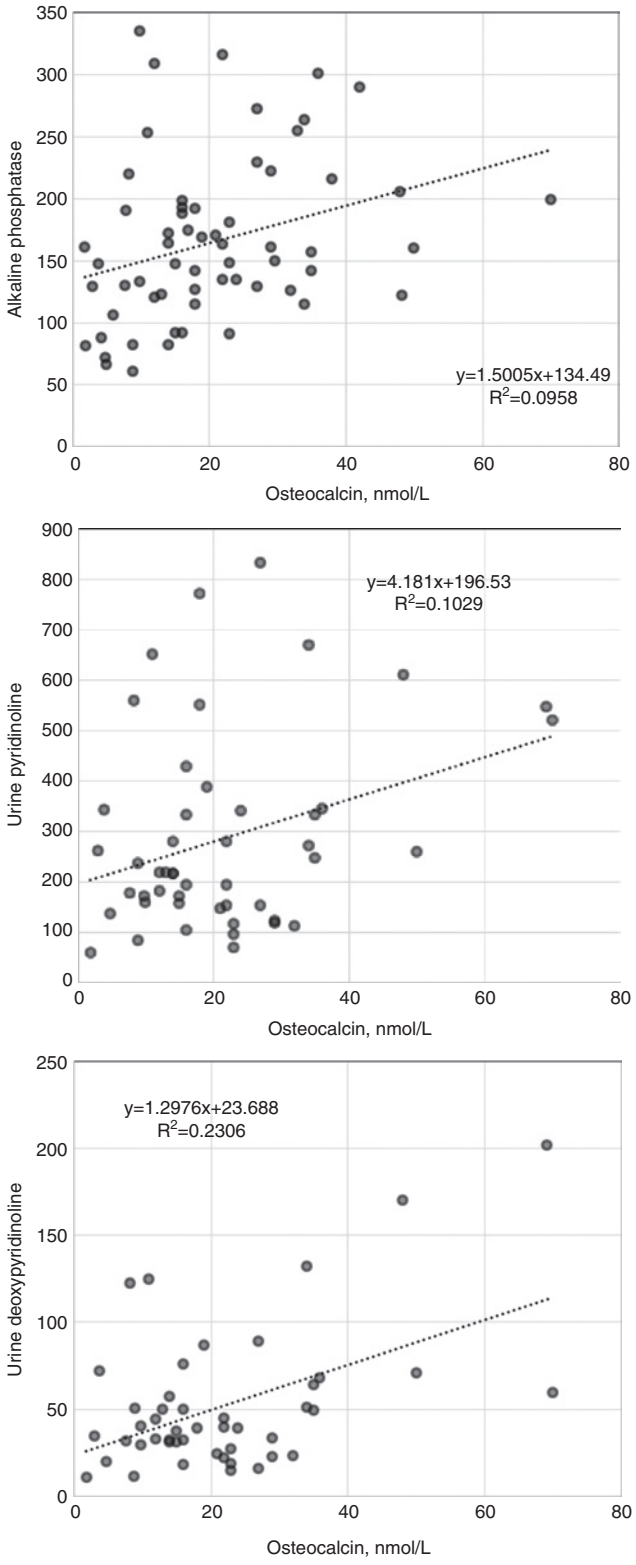

Figure 2: Serum osteocalcin in relation to serum alkaline phosphatase, urine pyridinoline and deoxypyridinoline in children with low bone density.
Serum osteocalcin was positively correlated with serum alkaline phosphatase, urine pyridinoline and urine deoxypyridinoline.

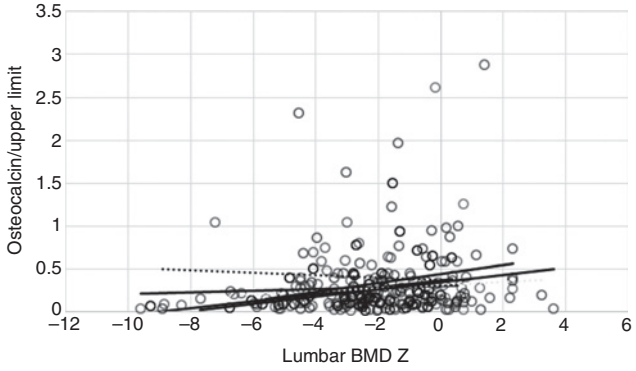

Figure 3: Serum osteocalcin index and lumbar BMD Z-score in children with low bone density.
There was a trend toward significant correlation between serum osteocalcin index and lumbar BMD Z-score. Osteocalcin index was expressed as a ratio of measured osteocalcin value to upper limit of normal value for age. Lines represent linear trend for each follow-up year to illustrate the consistent small upper trend each year. The dotted line was the baseline year.

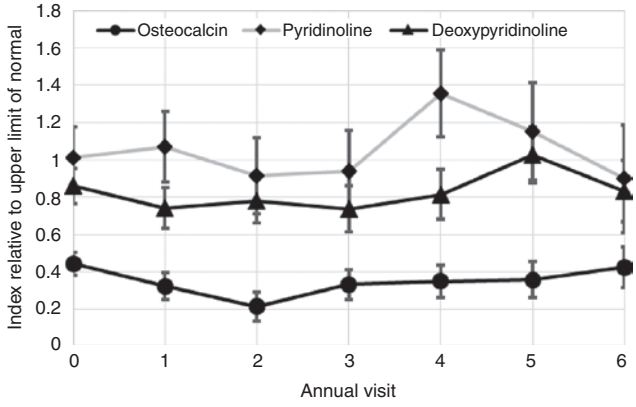

Figure 4: Bone turnover marker indices on bisphosphonate therapy in children with low bone mass.
Bone marker indices at baseline and while on bisphosphonate therapy. Index was expressed as a ratio of measured values and upper limit of normal range for age. Error bars are constructed so that non-overlapping bars are equivalent to an unadjusted $p<0.05$ (Fisher's least significant difference test). (n=89, 66, 57, 50, 44, 35, 29, and 23 at baseline, 1, 2, 3, 4, 5, 6, and 7 years, respectively.)

OC index was around half of the upper limit value at baseline. All OC, PD, and DPD indices trended to decrease (only OC was significant) during the first 3 years of bisphosphonate therapy, corresponding to the increasing lumbar BMD Z-scores observed during that time. Urine PD index was the first to increase to or above baseline value by year 4. Similarly, urine DPD index began to increase above baseline value by year 5, and then decreased back to baseline value. Serum OC index remained lower than baseline value for much longer time than PD or DPD index, and started to rise above baseline value by year 6.

There were no significant differences in bone turnover marker indices among patient classifications based on diagnoses.

Discussion

In this study, we found association between bone formation and bone resorption markers in pediatric patients with metabolic bone disease, as shown by positive correlations between serum OC and ALP, and between serum OC and urine markers for bone resorption. This indicates that high bone formation and resorption markers are both correlated with high bone turnover state. This is similar to studies in healthy children [20, 21], suggesting that the bone markers derive from and reflect the same biological processes of remodeling, modeling and epiphyseal growth, even in the setting of underlying metabolic bone disease, similar to that seen in healthy children.

We also found significantly negative associations between bone resorption markers (urine PD and DPD) and lumbar BMD, in this pediatric cohort with clinical bone fragility. This is similar to other studies in healthy children that showed significant correlations between bone mass and BTMs [20–23]. A study by Jürimäe et al. [21] showed significantly negative associations between BTMs and lumbar spine BMD, which remained significant after adjustment for age and pubertal stages. However, in this study, after controlling for age, we did not find any significant correlation between bone resorption markers and lumbar BMD Z-scores. After the variance accounted for by age was removed, there was no shared variance between BMD and BTMs. This suggests that BTMs do not predict the degree of low BMD, which is in agreement with a study by van der Sluis et al. [24] who found that BTMs cannot predict BMD in children.

It is well-recognized that BTMs in children are influenced by age, gender and pubertal stage, with increasing levels during pubertal growth spurt, paralleling the pattern of the standard height velocity curve, and declining during late puberty to adult levels [20, 25, 26]. This age-related changing pattern of BTMs results in different sets of pediatric normal reference values. Thus, unlike adults, in whom the assessment of BTMs is very straightforward, as the BTMs can be readily compared to baseline values, interpreting BTMs in growing children and adolescents is indeed much more challenging. Comparing the absolute values obtained at various times without taking into account of different age-related normal reference values can lead to misinterpretation of the results. Bisphosphonate therapy suppresses bone turnover, decreasing

BTMs. As raw bone marker levels decline during late adolescence, it is impossible for clinicians to differentiate therapy-induced changes from physiologically age-related changes. Therefore, in this study, we utilized the index of bone markers, expressed as a ratio of measured values to upper limit of normal values for age. This gives more meaningful interpretation of the results and better comparison relative to the physiologically changing reference ranges for the longitudinal assessment of BTMs. Calculating standard deviation scores of bone markers have been suggested by Rauchenzauner et al. [27] to evaluate the changes of bone markers. However, this would require a mean value of normal reference with a standard deviation, which is not available to clinicians. Using the index of bone markers as described in our study is a more practical way to track changes of bone markers to monitor the response to bisphosphonate therapy. In addition to age-adjusted values, puberty-adjusted normative values may be more helpful in assessing BMTs in pediatric patients with chronic diseases that may have alteration in their pubertal development.

We observed a decrease in indices of both bone formation and bone resorption markers, with the gain in lumbar BMD Z-score during the first 3 years of active bisphosphonate treatment, indicating suppression of BTMs that occurred during anti-resorptive therapy. These findings are in agreement with what has been well documented in adults [28], as well as many studies in children [1, 29, 30]. Clinical usefulness of BTMs in monitoring therapy has been well recognized in adults, as changes in bone marker levels in response to treatment are more rapid and dynamic than changes in BMD [28]. The absence of change in bone marker levels following the osteoporosis treatment initiation usually indicates non-compliance with oral bisphosphonates therapy, and occasionally a lack of response due to new development of other bone pathologies with high bone turnover such as osteomalacia, primary hyperparathyroidism, hyperthyroidism, bone metastases and multiple myeloma that can be seen in adults [28]. More studies are needed in the pediatric population to see if the biochemical bone markers, repeated at much shorter intervals, can be useful for early assessment of effectiveness of therapy long before changes in bone mass can be ascertained.

We also observed a rise in urine PD and DPD in year 4–5 which coincides with the cessation of bisphosphonate therapy. Rauch et al. observed a steady increase in urinary N-telopeptide but still less than pretreatment baseline values within 2 years after pamidronate discontinuation in patients with OI [31]. BTM levels returned to baseline values rapidly after discontinuation of oral risedronate treatment in adults [32], whereas, after discontinuation of intravenous ibandronate in adults, bone resorption markers returned progressively to the initial level followed by a similar increase in bone formation [33]. By contrast, BTM levels did not return to baseline values even after 3–5 years following cessation of 5 years of oral alendronate treatment [34]. It has been suggested from many adult studies that the increase in bone turnover rate and the parallel decrease in BMD after treatment discontinuation depends on the types or the accumulated dose of bisphosphonates, which also reflects the difference in the degree of inhibition of bone turnover or the difference in the degree of accumulation of drugs in bone matrix [15]. It is unknown whether these observations in adults can be generalized to the pediatric population. Due to the small number of subjects treated with oral bisphosphonates, we could not accurately assess any differences in effects between oral and intravenous agents. There has been no consensus for the dose or how long bisphosphonate should be treated or whether or when the patients should be converted to low dose or oral bisphosphonate therapy in children with clinical bone fragility. More studies are needed to evaluate the clinical utility of BTMs in establishing the optimal dose, determining the duration of bisphosphonate treatment or the timing of retreatment after cessation of therapy in children.

An association between BTMs and fracture risk has also been observed in adults. A greater decrease in BTM levels during the first year of anti-resorptive treatment is associated with greater anti-fracture efficacy over 3 years of treatment [15]. The goal of optimal therapy in adults is that the BTMs should be within the lower end of normal range in adults [15]. A prospective study in pediatric patients to determine the correlations of BTMs with fracture risk would require a large sample size, which may prove to be challenging.

Conclusions

The results of this study showed that there was a consistent relationship between the bone formation and bone resorption markers in children and adolescents with clinical bone fragility, similar to that in healthy children. These biochemical BTMs were correlated with lumbar BMD but not lumbar BMD Z-score. While the BTMs were not reliable predictors of degree of low BMD, the bone markers showed suppression compared to baseline during bisphosphonate therapy. Further studies are needed in children to expand the clinical utility of BTMs in monitoring the response to therapy, assessing anti-fracture efficacy, and

in determining the optimal dose or length of bisphosphonate therapy.

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