Bone Mineral Density in Prepubertal Children With β-Thalassemia: Correlation With Growth and Hormonal Data

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Patients with β-thalassemia major (β-thalassemia) frequently have bone disorders of multifactorial etiology. We attempted to analyze the relationship between the bone mineral density ([BMD] measured by dual-photon absorptiometry) and auxanologic parameters, degree of siderosis, function of the growth hormone (GH)/insulin-like growth factor-I (IGF-I)/IGF-binding protein-3 (IGFBP3) axis, calcium-phosphate balance, parathyroid hormone (PTH), and cytokines (interleukin-1β [IL-1] and tumor necrosis factor-alpha [TNF-α]) in 30 prepubertal children with β-thalassemia major and 15 age-matched children with constitutional short stature (CSS), who have normal glucose tolerance and thyroid function. Children with β-thalassemia had a significantly decreased BMD and mean BMD% for age and sex (0.75 ± 0.24 g/cm² and 71% ± 10%, respectively) versus children with CSS (1.06 ± 0.3 g/cm² and 92% ± 7%, respectively). Thalassemic patients had significantly lower circulating concentrations of IGF-I and IGFBP3 (49 ± 21 ng/mL and 1.2 ± 0.25 mg/L, respectively) compared with control children (153 ± 42 ng/mL and 2.1 ± 0.37 mg/L, respectively). The GH response to provocation by clonidine and glucagon was defective (peak GH < 7 μg/L) in 12 of the 30 thalassemic children. Serum concentrations of IL-1β and TNF-α did not differ among the two study groups. Hypocalcemia was detected in five of the 30 thalassemic patients: hypoparathyroidism was diagnosed in two of the five and rickets in the other three. BMD was highly correlated with the circulating concentrations of IGF-I and IGFBP3, as well as with the auxanologic parameters (age, weight, height, height standard deviation score [HSDS], and body mass index [BMI]). It is suggested that increasing the circulating IGF-I concentration through aggressive nutritional therapy and/or GH/IGF-I therapy with supplementation with vitamin D and/or calcium might improve bone growth and mineralization and prevent the development of osteoporosis and consequent fractures in these patients. Such therapy requires blinded controlled trials.

OSTEOPOROSIS is a disease characterized by loss of bone mass and microarchitectural deterioration, resulting in a reduced mechanical competence and consequent increased risk of fractures.[1] β-Thalassemia major is associated with significant bone disease.[2] These changes include bone marrow expansion of the medullary cavity, cortical thinning, trabecular coarsening with various striations or the appearance of cystic spaces, and coarsening of the bone pattern with a drop out of all but the mechanically most necessary trabeculae.[3]

With advances in transfusion management beginning in the 1960s, there has been marked improvement in terms of skeletal development and normal cosmetic facial and long bone appearance.[4] However, even well-transfused patients remain radiographically osteopenic. Prior to institution of aggressive transfusion regimens, fractures occurred primarily in the long bones and were associated with trauma.[5] After the introduction of aggressive transfusion, the pattern changed, with less involvement of long bone and an increased in vertebral compression fractures, especially in older patients.[6]

Although the etiology of the bone disease is still debatable, many factors can adversely affect bone accretion in thalassemic children. These include (1) chronic hypoxemia and medullary expansion, (2) defective growth affecting both height and weight, (3) abnormal calcium-phosphate homeostasis, (4) delayed or lack of pubertal development and decreased sex steroid secretion, (5) compromised nutritional status and increased energy expenditure, (6) abnormal growth hormone (GH)/insulin-like growth factor-I (IGF-I)/IGF-binding protein-3 (IGFBP3) axis, and/or (7) development of diabetes mellitus.[7-10]

The aim of this study was to investigate some factors affecting bone mineral metabolism in 30 children with β-thalassemia and to attempt to find a relationship, if any, between the degree of siderosis, calcium-phosphate balance, the GH/IGF-I/IGFBP3 axis, parathyroid hormone (PTH) secretion, and auxanologic data, on one hand, and bone mineral density (BMD) on the other.

SUBJECTS AND METHODS

Thirty-three prepubertal patients with β-thalassemia major randomly selected from those attending the outpatient Pediatric Hematology Clinic of Alexandria University Children's Hospital, Alexandria, Egypt, were the subjects of this study. All children underwent regular blood transfusions to keep the hemoglobin (Hb) concentration above 10 g/dL. All were taking folic acid supplements and iron chelation with daily intramuscular desferoxamine. Fifteen age-matched normal short children (constitutional short stature [CSS], height standard deviation score [HtSDS] ≤ −2, annual growth velocity [GV] ≤ 5 cm/yr, normal GH response to provocation, and delayed bone age) served as controls. None of the children had a history of intrauterine growth retardation, other systemic or endocrine disease or dysmorphic trait, or central nervous system irradiation. All had normal tolerance to an oral glucose load (1.75 g/kg dextrose). Three patients who had abnormal glucose tolerance were excluded from the study.

Informed consent for the testing procedures was obtained from the parents and, when appropriate, from the children before entering the study. The study protocol was approved by the ethics committee of Alexandria University.

All children were examined with a special emphasis on nutritional data. The auxanologic data included weight, height, and midarm circumference. Harpenden calipers and anthropometric measurements were used. The data recorded were the average of three sequential measurements determined by the same observer (A.T.S.). The HtSDS and body mass index (BMI) were calculated and recorded. The linear GV in centimeters per year was calculated for the past year. Normal

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population data were from Tanner et al.[11] The bone age was determined according to the Greulich and Pyle atlas.[12]

On the day of admission, venous blood samples were obtained for determination of the complete blood cell count, and serum concentrations of albumin, bilirubin, and alanine aminotransferase (ALT). Following an overnight fast (8 hours) venous blood samples were drawn through a polyethylene catheter inserted in a forearm vein between 8 and 9 AM. The serum was separated from the formed elements by centrifugation and kept frozen at −20°C until analyzed for GH, IGF-I, IGFBP3, free thyroxine (FT_4), thyrotropin (TSH), cortisol, PTH (intact molecule), calcium (Ca), phosphorus (PO4), alkaline phosphatase (ALP), ferritin, interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF = α) concentrations. After obtaining the basal samples, an oral dose of clonidine 0.15 mg/m^2 and intravenous dose of corticotropin (ACTH) (Synacthen; Ciba-Geigy, Basel, Switzerland) 1 μg/m^2 were given, and blood samples were collected every 30 minutes for 2 hours for measurement of GH levels and after 60 minutes for cortisol levels. On the next morning, a standard glucagon test for GH release was performed.

Human GH and IGF-I concentrations were measured by radioimmunometric assay using reagents purchased from Nichols Institute (San Juan Capistrano, CA). Mean intraassay coefficients of variation (CVs) were 5.8% and 7.6%, respectively, and interassay CVs were 7.8% and 8.5%, respectively, in the range of GH and IGF-I values detected. The IGFBP3 level was measured by radioimmunoassay in Serono Biochemical Laboratories (SCL) & Bioscience Services (London, UK) using reagents supplied by Mediagnost (Rome, Italy). The assay sensitivity is 0.06 μg/mL with intraassay and interassay CVs of 5.2% and 8.6%, respectively. The level of intact PTH was measured in the serum using an immunochemiluminometric method. Intraassay and interassay CVs were 3.6% and 6.2%, respectively. IL-1β and TNF-α levels were measured using an enzyme-linked immunosorbent assay technique (Biokine; T-Cell Diagnostics, Cambridge, MA; intraassay CVs, 8.4% and 6%, respectively; interassay CVs, 8.8% and 7%, respectively).

BMD of the lumbar spine (second, third, and fourth lumbar vertebrae) was measured by dual-photon absorptiometry using a Norland 2600 bone densitometer (Cambridge, UK). All children were scanned in the supine position. BMD data were expressed as grams per centimeter squared and were compared with BMD values of normal children of the same age.[13]

Statistical analyses were performed using the unpaired t test to compare mean analyte concentrations among the two study groups when the data were normally distributed, and the Wilcoxon test when they were not. Statistical significance was accepted at P less than .05. Multiple regression analysis was performed using BMD as the dependent variable and all of the other auxanologic and biochemical data as independent variables. Data are presented as the mean ± SEM.

RESULTS

Anthropometric and bone age data are presented in Table 1. The chronological age, HtSDS, GV, BMI, and bone age did not differ significantly between the two study groups. Biochemical, hormonal, and BMD data are shown in Tables 2 and 3. The circulating concentrations of albumin, creatinine, ALP, and PO4 did not differ significantly among the two groups. Hypocalcemia (Ca ≤ 1.4 nmol/L, 5.7 mg/dL) was detected in five patients. Three of them (aged 11, 12.5, and 14 years) had other biochemical evidence of hypoparathyroidism (high PO4, normal ALP, and low PTH concentrations). The other two patients (aged 9 and 11 years) had biochemical evidence of rickets (low PO4, high ALP and PTH, and low 25-hydroxyvitamin D_3 concentrations). Circulating concentrations of IGF-I and IGFBP3 were significantly lower in thalassemic children compared with controls. Their peak GH responses to provocation with clonidine and glucagon were significantly less than those for the controls. Twelve of the 30 children with β-thalassemia did not mount a GH response to provocation above 7 μg/L. Although basal levels of cortisol did not differ between the two groups, the cortisol response to a low-dose ACTH test was significantly lower in thalassemic children versus controls. Two children had mild chemical hypothyroidism (FT_4, 9.5 and 8.7 pmol/L; TSH, 8.6 and 10.3 μIU/mL). Both were treated with L-thyroxine for 1 month before testing their GH response to provocation.

Table 1. Anthropometric Data of the Patients and Controls

Group	HtSDS	GV (cm/yr)	Age (yr)	BMI (kg/m^2)
β-Thalassemia (n = 30)	1.95 ± 0.11	4.1 ± 0.24	8.8 ± 0.27	13.9 ± 0.24
CSS (n = 15)	2.6 ± 0.13	4.6 ± 0.03	8.2 ± 0.26	14.9 ± 0.013

*P < .05, β-thalassemia v CSS.

Dual-photon absorptiometry showed that children with β-thalassemia had a significant reduction of BMD (30% less) compared with the mean BMD for age-and sex-matched normal children, corresponding to a BMD of −1.5 to −2 SD. Thalassemic children had significantly lower BMD versus age-matched children with CSS. Correlations between BMD and different parameters are shown in Table 4 and Fig 1. BMD was correlated significantly (P < .01) with age, height, weight, and BMI, as well as with the circulating concentrations of IGF-I and IGFBP3. No significant correlations were found between BMD, on one hand, and PTH, PO4, Ca, or ALP concentrations on the other. IL-1β and TNF-α concentrations did not differ significantly between the thalassemic group (25.9 ± 11.4 and 399 ± 113 pg/mL, respectively) and controls (21.1 ± 6.4 and 383 ± 122 pg/mL).

DISCUSSION

From infancy through late adolescence, bone-forming activity exceeds bone resorption, resulting in a steady accumulation of bone mass. On average, most of the skeletal mass is accumulated by the age of 18 years.[14-18] Since the bone mass is one of the main determinants of fractures, a high bone mass at skeletal maturity (peak bone mass) is considered the best protection against age-related bone loss.[19] Small differences in bone mass at skeletal maturity of 5% to 10% could contribute to substantial differences in the incidence of osteoporotic fractures.[20]

Bone modeling and skeletal consolidation result from a complex sequence of hormonal changes in interaction with nutritional factors, where the concerted actions of GH, IGF-I, and sex hormones and their receptors, besides other factors, are responsible for the timing and attainment of skeletal consolidation. At puberty, circulating IGF-I concentrations correlate with sexual development. Specifically, the surge in sex steroids, in turn, increases the secretion of GH, which stimulates the production of IGF-I[21,22] and increases bone mass.[23] In addition, a large number of other factors interact at the level of the osteoblast, osteoclast, and other cells to regulate the balance between net resorption and formation. These include PTH, vitamin D, and cytokines.[23]

Table 2. Biochemical Data of the Patients and Controls (mean ± SEM)

Group	Albumin (g/dL)	ALT (IU/L)	Bilirubin (μmol/L)	Ca^{2+} (mg/dL)	PO4 (mg/dL)	ALP (IU/L)	Ferritin (ng/mL)	Hb (g/dL)
β-Thalassemia (n = 30)	3.8 ± .09	86 ± 5*	49 ± 2.7*	8.2 ± .15	5 ± .2	178 ± 10	880 ± 46*	8.2 ± .27*
CSS (n = 15)	3.9 ± .1	21 ± 3	15 ± 1.3	9.1 ± 0.3	4.4 ± 013	161 ± 7	89 ± 10	12.4 ± 0.3

*$P < .05$, β-thalassemia *v* CSS.

The GH/IGF-I/IGFBP3 Axis and Factors Affecting It

In this study, the hormonal profile of children with β-thalassemia showed a significant deficiency of circulating IGF-I and IGFBP3 (both are GH-dependent peptides). The significant correlation between the IGF-I levels and HtSDS and BMD supports a major role played by IGF-I in stimulating linear growth and bone mineralization.

Forty percent of these prepubertal thalassemic children had defective GH secretion after provocation by clonidine and glucagon. In concert with our findings, Danesi et al,[24] Saglamer et al,[25] and Pintor et al[26] reported a low GH response to provocation by insulin hypoglycemia, arginine, L-dopa, and GH-releasing hormone in many of their patients, denoting impairment of somatotroph function. This can explain the low IGF-I synthesis in some patients. However, the thalassemic children with normal GH secretion had low circulating IGF-I concentrations (50 ± 19 ng/mL) comparable to those seen with defective GH release (46 ± 24 ng/mL), suggesting that other factors contribute to low IGF-I synthesis in these children. Leger et al[27] found that decreased IGF-I secretion occurs before an alteration in GH secretion in response to GH-releasing hormone, arginine, or insulin. Other investigators reported the neurosecretory dysfunction of GH secretion to be responsible for decreased IGF-I synthesis in some patients with a normal GH response to provocation.[28,29] Some investigators indicated that decreased GH secretion may be due to an age-related deterioration of the hypothalamic-pituitary function secondary to progressive siderosis.[27,30] In support, Perignon et al[31] reported that their patients with β-thalassemia had a low IGF-I concentration that did not increase at puberty. Although the idea of a defect at the hepatic GH receptor or postreceptor level was suggested to explain the low IGF-I production,[26] Postel-Vinay et al[32] found no evidence for a defect in GH binding to liver membranes in thalassemic patients.

It is well recognized that the nutritional status has an important influence on the GH/IGF-I/IGFBP3 axis.[33] Fasting results in increased GH secretion and decreased IGF-I levels,[34] and proper nutrition increases IGF-I levels in malnourished children.[35] Our children with β-thalassemia had a BMI and MAC at or below the 10th centile for age and sex, suggesting a mild degree of undernutrition. Decreased food intake,[36] pancreatic exocrine dysfunction, hepatic cirrhosis,[37,38] and/or hypermetabolism secondary to bone marrow hyperactivity and increased cardiac work might compromise nutrition and growth in these children. In this study, circulating IGF-I concentrations were correlated significantly with ALT levels ($r = -.465$, $P < .01$), and 50% of the children were hepatitis B surface antigen carriers and had significantly elevated ALT concentrations. Clinically, 25 of 30 patients had cirrhotic livers. In one study,[36] nutritional intervention resulted in an improvement of weight for height and increased IGF-I concentration. In malnutrition[35] and hypercatabolic states,[39] low IGF-I production is associated with high basal and stimulated GH levels, denoting a normal sensitivity of the hypothalamic-pituitary axis to the low IGF-I level (normal feedback). In β-thalassemia, the low-normal GH levels despite low circulating levels of IGF-I prove a defective-feedback effect of decreased IGF-I on the pituitary (either due to lack of sensitivity or defective somatotroph function). We and others reported partial resistance to GH in thalassemic children as evidenced by low IGF-I generation in response to exogenous administration of GH and slow linear growth on GH therapy.[40-42]

During a normal pubertal growth spurt, sex steroids increase GH secretion with a subsequent increase of IGF-I levels. Sex steroids and GH each contribute approximately 50% of the height gain. Children with GH insufficiency not treated with exogenous GH attain only 50% to 66% of the expected growth spurt.[43-47] Reduction of the sex steroid concentration during gonadotropic-releasing hormone therapy decreases GH secretion and serum IGF-I concentrations.[48,49] Patients with β-thalassemia have a high incidence of failure of puberty (51% of boys and 47% of girls) and secondary amenorrhea (23%).[50] Defective gonadotropin secretion with subsequent sex steroid deficiency have been detected in these patients with low IGF-I levels.[51-53] Even those who enter puberty do not have the

Table 3. Hormonal Data of the Patients and Controls

Parameter	CSS (n = 15)	β-Thalassemia (n = 30)
FT_4 (pmol/L)	18.4 ± 0.155	15.2 ± 0.347*
TSH (μIU/mL)	1.6 ± 0.038	2.5 ± 0.347
GH-P-Clon (μg/L)	19.6 ± 0.697	6.9 ± 0.49†
GH-P-Glu (μg/L)	16.1 ± 0.82	7.4 ± 0.4†
IGF-I (ng/mL)	153 ± 10.85	49 ± 3.8†
IGFBP3 (mg/L)	2.1 ± 0.09	1.2 ± 0.045†
Cortisol-b (nmol/L)	466 ± 18.8	318 ± 11.5*
Cortisol-a (nmol/L)	788 ± 25.6	455 ± 17.4†
PTH (pmol/L)	7.8 ± 1.16	11.1 ± 2.88
BMD (g/cm²)	1.06 ± 0.08	0.75 ± 0.044*‡
BMD (%)	92 ± 0.2	71 ± 1.8*‡

Abbreviations: GH-P-Clon, GH peak after clonidine; Glu, glucagon; cortisol-b, before ACTH; a, after ACTH.

*$P < .05$, †$P < .01$, β-thalassemia *v* CSS.

‡$P < .05$, β-thalassemia *v* normal children.[13]

Table 4. Correlation Between BMD and Auxanologic and Biochemical Data (*r*)

	IGFBP3	IGF-I	BMI	HtSDS	Weight	Height	Age	Ferritin
BMD	.693†	.74*	.49†	.52†	.59†	.54†	.79†	−.27*
IGF-I	.72†	1*	.41*	.44†	.39*	.47†	.68†	−.45†

*$P < .05$.

†$P < .01$.

enhanced GH secretion and increased IGF-I synthesis pattern accompanying normal puberty,[30,31] denoting a major effect of sex steroid deficiency on the GH/IGF-I axis in peripubertal and pubertal children with β-thalassemia. Unlike children with a constitutional delay of puberty, who secrete normal GH in response to provocation after priming with sex steroid,[43] in our prepubertal thalassemic patients older than 12 years (n = 8), the peak GH response to provocation did not improve after (7.5 ± 2.1 μg/L) versus before priming with estrogen (6.5 ± 1.5 μg/L).

The reported prevalence of diabetes mellitus in treated β-thalassemia is about 16%, and the incidence of impaired glucose tolerance is approximately 60%. Islet cell destruction secondary to iron overload and/or exhaustion of B cells due to chronic insulin resistance and liver derangement are possible pathogenic factors.[54-58] Defective insulin secretion and an insulin-resistant state can impair hepatic IGF-I production.[59,60] Moreover, insulin plays an important part in determining the bioavailability of IGF-I through its action on IGFBP1. Therefore, defective insulin secretion or insulin resistance in thalassemic children can increase hepatic production of IGFBP1, leading to decreased bioavailability of IGF-I.[61,62] However, this factor can be excluded in our patients with normal glucose tolerance.

In summary, the markedly decreased hepatic production of IGF-I in our thalassemic prepubertal patients with normal

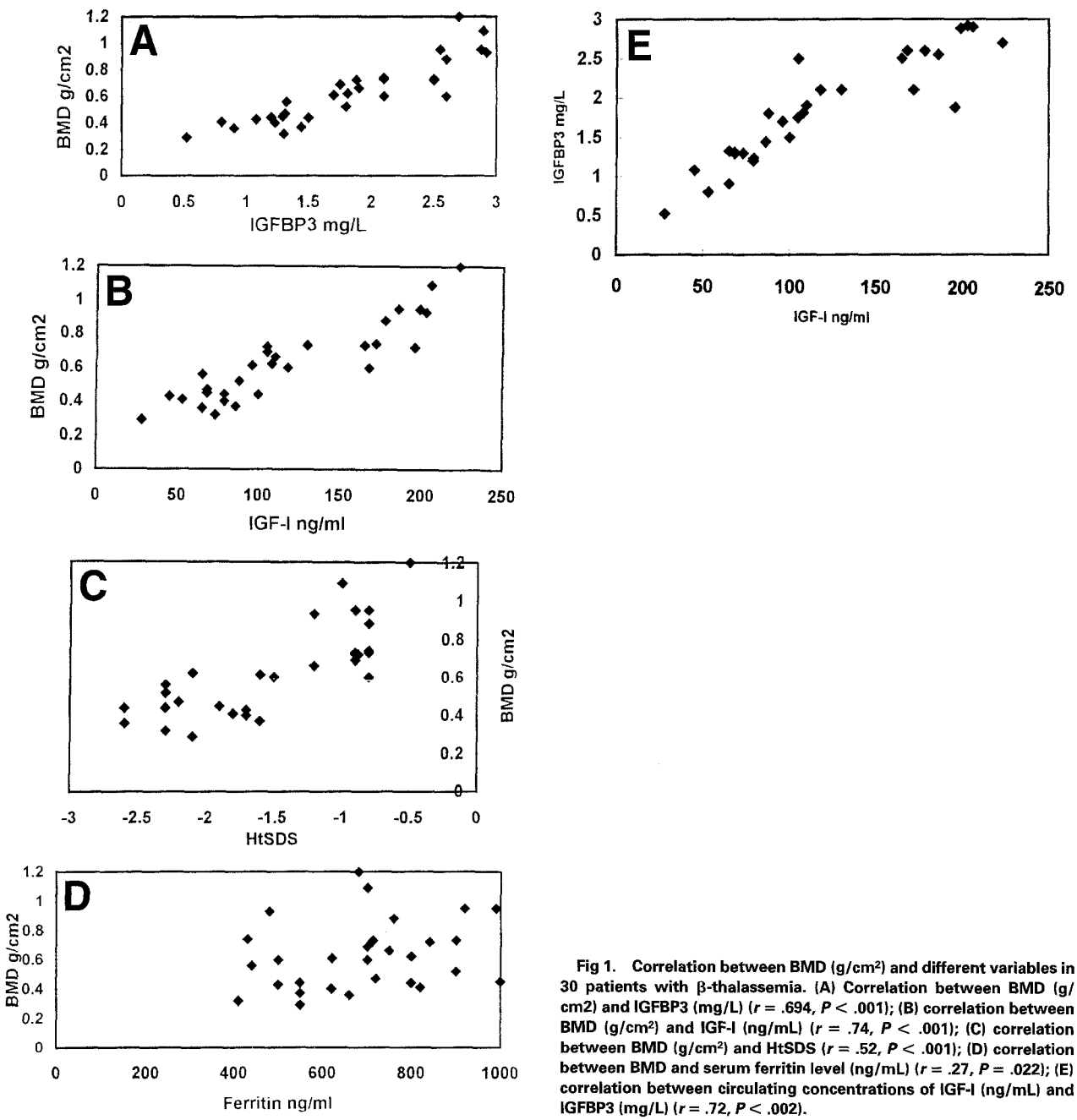

Fig 1. Correlation between BMD (g/cm²) and different variables in 30 patients with β-thalassemia. (A) Correlation between BMD (g/cm2) and IGFBP3 (mg/L) ($r = .694$, $P < .001$); (B) correlation between BMD (g/cm²) and IGF-I (ng/mL) ($r = .74$, $P < .001$); (C) correlation between BMD (g/cm²) and HtSDS ($r = .52$, $P < .001$); (D) correlation between BMD and serum ferritin level (ng/mL) ($r = .27$, $P = .022$); (E) correlation between circulating concentrations of IGF-I (ng/mL) and IGFBP3 (mg/L) ($r = .72$, $P < .002$).

glucose homeostasis can be attributed to defective GH secretion, hepatic cirrhosis (secondary to siderosis and/or chronic viral hepatitis), and/or GH resistance. The delay or lack of pubertal development and the occurrence of type I diabetes with advancing age might further impair the secretion and/or bioavailability of IGF-I.

IGF-I is a potent stimulator of linear growth and a major determinant of bone mineralization. Exogenous administration of IGF-I has been shown to increase growth and bone formation in humans and animals.[63-66] This effect is potentiated when IGF-I is combined with IGFBP3.[67,68] In our children with β-thalassemia, the decreased production of IGF-I and IGFBP3 and the significant correlation between these growth factors and BMD and linear growth (weight, height, and HtSDS) parameters suggested a major role for them in the pathogenesis of osteoporosis. GH or IGF-I therapy may improve linear growth and bone mineralization in these children, as seen in patients with GH deficiency/resistance and other diseases with osteopenia.[69-71] This hypothesis needs to be tested by a double-blind therapeutic trial of GH or IGF-I in these patients.

Calcium-Phosphate Balance and PTH

In this study, hypocalcemia occurred in five of 30 children with β-thalassemia. All five children had markedly decreased BMD. Analyses of the other biochemical parameters differentiated two possible disease entities. Two of the five patients had evidence of hypoparathyroidism (low Ca, high PO4, normal ALP, and low PTH). This can be explained by siderosis of the parathyroid gland.[72-75] In support of this view, Gertner et al[76] found a low PTH reserve to induced hypocalcemia in thalassemic patients. The other three patients had biochemical evidence of rickets (low PO4, low Ca, high ALP, high PTH, and low 25-hydroxyvitamin D_3). In concert with this finding, De Vernejoul et al[77] reported osteomalacia in their thalassemic patients. The cause of rickets/osteomalacia in these patients is a defective 25-hydroxylation due to hepatic impairment and/or decreased vitamin D absorption in these children. Impaired osteoblast function with diminished bone formation and a low serum concentration of 25-hydroxyvitamin D_3 with high PTH levels have been reported in patients with hemochromatosis and liver cirrhosis and in pigs overloaded with parenteral iron.[78-81]

The normocalcemic (25 of 30) patients with β-thalassemia had a slightly higher PTH concentration and significantly lower BMD versus normal children. In agreement with this finding, Pawlotsky et al[82] reported an elevated PTH concentration, normal serum calcium level, and increased bone resorption in their hemochromatic patients. It appears that both vitamin D deficiency and hypoparathyroidism might affect bone mineralization in thalassemic children.

Cytokines

Cytokines represent a group of factors influencing the balance between bone formation and resorption. Increased bone resorption induced by an overproduction of critical cytokines, such as IL-1, TNF, and GM-CSF, by the hyperactive marrow cells and monocyte/macrophage lineage is an attractive theory to explain the pathogenesis of osteoporosis seen in patients with β-thalassemia, as with other diseases. IL-1 and TNF are among the most powerful stimulators of bone resorption known and are well recognized inhibitors of bone formation.[83-87] However, we found normal serum levels of IL-1 and TNF in our thalassemic patients comparable to those for control children, which might rule out a significant role for these cytokines in the development of osteoporosis in these children.

Cortisol Secretion

In this study, children with β-thalassemia had a significantly lower cortisol response to provocation with low-dose ACTH. Other studies reported both low[88,89] and normal[90,91] cortisol responses to high-dose ACTH. Slate gray pigmentation that becomes progressively intense with time, poor weight gain, weakness, and absent adrenarche were significant signs in our thalassemic patients. However, the contribution of different factors including adrenal insufficiency, siderosis, and anemia in the production of these manifestations is difficult to assess. McIntosh[89] reported high circulating ACTH in β-thalassemia, and suggested that it is the cause of the pigmentation. In concert with these findings, the graded-dose adrenal cortical stimulation showed significant suppression of cortisol secretion. Although iron deposition in the adrenals might be the cause of adrenal insufficiency, it has been shown recently that IGF-I enhances the steroidogenesis and ACTH responsiveness of human adrenocortical cells in culture.[92-94] A deficiency of IGF-I synthesis in β-thalassemia might contribute to the defective cortisol production and possibly other adrenal androgens, which might explain the lack of or delay in adrenarche in thalassemic patients. These data suggest that replacement with physiological doses of hydrocortisone might improve some of the manifestations of the disease. In addition, increasing the IGF-I level might also improve the secretion of adrenal androgens necessary for adrenarche.

The question is, what possible therapeutic or preventive options are available that might influence bone mineralization and growth in thalassemic children? The data from this study support the development of a controlled clinical trial to evaluate several possible therapeutic interventions. In addition to proper and aggressive nutritional intervention, which should be an integral part of any treatment strategy, possible new therapeutic interventions would include the following: (1) GH and/or IGF-I replacement therapy, especially for those with GH and/or IGF-I insufficiency. These measures might increase the circulating IGF-I level and consequently increase bone formation and prevent osteopenia. Adding IGFBP3 to IGF-I and/or GH therapy might potentiate their effect on bone growth and mineralization.[67,69-71] (2) Treatment with vitamin D or vitamin D analogs at modest doses (800 to 1,500 IU/d vitamin D_3) may offer a safe and substantial contribution to the prevention of osteoporosis in these children. Positive correlations of vitamin D levels with BMD of the vertebrae and proximal femur have been found in young and old women with poor vitamin D status.[95-98] Patients with osteoporosis and biochemical evidence of rickets need higher doses of vitamin D_3 or its analogs for treatment. (3) Calcium supplementation, which has been shown to increase bone density in normal prepubertal children, is another good potential option.[99] (4) Initiation of puberty at an

appropriate age through the use of progressively increased doses of androgens or estrogens. These agents would prevent osteoporosis and increase the BMD,[23,100-102] forcing into consideration the risk of advancing the bone age faster than the height age.

In summary, prepubertal children with β-thalassemia and normal glucose tolerance have decreased BMD, delayed growth, and a defective GH/IGF-I axis. Biochemical evidence of hypoparathyroidism or rickets may be detected in thalassemic patients with hypocalcemia. It is logical to propose that treatment of these patients with GH and/or IGF-I with aggressive nutritional support and supplementation with vitamin D and/or calcium might improve the bone density and prevent the development of osteoporosis and subsequent fractures.

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