Bone 73 (2015) 176–180

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Areal bone mineral density in children and adolescents with Marfan syndrome: Evidence of an evolving problem

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ARTICLE INFO

Article history:
Received 20 August 2014
Revised 31 October 2014
Accepted 9 December 2014
Available online 13 December 2014

Edited by Bente Langdahl

Keywords:
Marfan syndrome
Bone mineral density
Children

ABSTRACT

Marfan syndrome (MFS), an autosomal dominant disorder of connective tissue, is due to defective fibrillin-1. Defects involve the cardiovascular system, the eye, the lungs, and the skeleton. The aim of the current study was to characterize the bone mineral status in children and adolescents with MFS. We performed an observational cross-sectional study and a longitudinal follow-up of two years.

We enrolled 73 young patients with MFS (3–17 years). A subset of 44 patients participated in the longitudinal study. Healthy children were studied as controls for biochemical analyses. Bone mineral density (BMD) was measured at lumbar spine, femoral neck and total femur by dual-energy X-ray absorptiometry. BMD values were expressed as Z-scores adjusted for height using height-for-age Z-scores. BMD measurements corrected for height were significantly lower than reference at all skeletal sites ($P < 0.0001$). Patient on cardiac treatment with losartan had lower BMD measurements corrected for height compared to non-treated patients. Total femur BMD decreased significantly over time ($P = 0.027$). BMD at the other two skeletal sites did not change significantly during follow-up, but remained significantly low compared to reference ($P < 0.0001$). In conclusion, young patients with MFS have markedly low BMD at the lumbar spine and femur, and values show a tendency to decrease over time in the peripheral skeleton. Because increased life expectancy of MFS patients, the reduced BMD during childhood may lead to a low peak bone mass, increasing the fracture risk during adult life.

Introduction

Marfan syndrome (MFS; OMIM #154700) is a genetic disorder of connective tissue affecting about 2–3 per 10,000 individuals [1]. Cardinal clinical manifestations include cardiovascular, musculoskeletal, neurologic and ocular abnormalities with an extremely variable phenotype and age of onset. The onset of clinical signs is often age-dependent, and therefore the diagnosis of MFS in pediatric patients may be difficult and delayed [2,3]. The diagnosis of MFS is made according to the Ghent criteria, codified in 1996 [4] and revised in 2010 [5].

The disease is caused by mutations in the gene encoding fibrillin-1, located on chromosome 15. Fibrillin-1, a glycoprotein of the extracellular matrix, forms the backbone of microfibrils in tissues as vessels, skin and bone. The altered fibrillin-1 impairs integrity of connective tissue [6]. Fibrillin is also known to modulate growth factors playing a critical role in bone metabolism [7]: it regulates osteoblast maturation by controlling transforming growth-factor-beta (TGF-β) and bone morphogenetic protein (BMP) availability. A marked alteration of TGF-β activity and signaling was demonstrated in the fibrillin-1 deficient mouse [8].

Skeletal involvement in MFS includes overgrowth of long bones, responsible for the disproportionate stature, protrusion of acetabuli, and scoliosis, but decreased bone mineral density (BMD) has also been reported in adult patients [9–12]. Studies in children are few, and limited to small number of observations [10,13,14] and they provide controversial results; moreover there are no longitudinal perspective studies available.

Therefore, the aim of the present study was to assess bone status in a large pediatric population with MFS by measurements of BMD and serum markers of bone metabolism. We also report the data of a longitudinal survey on a subgroup of children and adolescents with MFS.

Subjects and methods

Subjects

Eligible for the study were patients with Marfan syndrome, attending the Marfan Clinics at L. Sacco Hospital. Patients were diagnosed by the use of the Ghent nosology revised criteria [5].

Abbreviations: MFS, Marfan syndrome; $BMD_{HAZ}Z$, bone mineral density adjusted for height for age Z-score

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http://dx.doi.org/10.1016/j.bone.2014.12.006
8756-3282/

Exclusion criteria included disorders or medications affecting bone metabolism, except those for cardiovascular treatment. Excluded were also patients with Marfan syndrome phenotype, but lacking one criterion according to the Ghent nosology [5]. Patients who underwent spinal surgery to correct severe scoliosis or had severe scoliosis were also excluded. All patients were Caucasian, lived at the same latitude (41–45°N) and moderately active, avoiding contact sports because of the risk of damaging the aorta and the eyes. All subjects had appropriate pubertal development for age and nobody received therapy to accelerate epiphyseal fusion and reduce final height. We enrolled 73 patients with Marfan syndrome (35 girls and 38 boys), whose age ranged from 3 to 17 years. The characteristics of the patients are shown in Table 1. At the time of the study the patients had height measurements markedly higher than reference population, with a mean height standard deviation score (SDS) of 1.9 (1.4). Conversely, patients had BMI values significantly lower than reference ($t = -7.1$; $P < 0.0001$). At the time of the study 33 patients were on cardiac treatment either with losartan (30 patients), enalapril (2 patients) or atenolol (1 patient). The remaining patients were not receiving treatment. Scoliosis was present in 41 patients (64%). A subgroup of 44 patients (22 girls and 22 boys) with Marfan syndrome was studied at baseline and after 1 and 2.3 years (longitudinal study). The age at baseline ranged from 3 to 15 years, and the characteristics of the participating patients are shown in Table 4. The weight SDS increased modestly over time. Similarly, we observed a modest increase of height SDS values during the observation period.

We also enrolled healthy unrelated children to compare biochemical measurements. None had a history of endocrine, nutritional, growth or renal problems. The control group for biochemical measurements consisted in 26 healthy children (aged 4 to 17 years). Their mean weight was 44.9 (15.2) kg, their mean height was 1.56 (0.2) m, and their BMI was 17.9 (3.6) kg/m^2.

Informed consent was obtained from each patient's and healthy control's legal guardian and from the patients when appropriate, before enrollment. The study was approved by the ethical committee of the L. Sacco Hospital.

Study design

We report the results of an observational cross-sectional study conducted on a large group of patients with Marfan syndrome. A subgroup of 44 patients had bone mineral measurements performed also after 1 and 2.3 years from baseline (longitudinal study).

Table 1
Age, sex, anthropometric and bone mineral measurements of the 73 patients with Marfan syndrome enrolled in the observational cross-sectional study.

Variable		Value
Age	(Years)	10.0 (3.8)
Sex		Girls = 35, Boys = 38
Weight	(kg)	36.9 (13.4)
	(SDS)	0.02 (1.2)
Height	(m)	1.5 (0.2)
	(SDS)	1.9 (1.4)
BMI	(kg/m^2)	15.9 (2.5)
	(SDS)	−1.3 (1.4)
Lumbar spine BMD	(g/cm^2)	0.616 (0.156)
	(BMD$_{HAZ}$ Z-score)	−1.9 (1.0)
Femoral neck BMD	(g/cm^2)	0.616 (0.152)
	(BMD$_{HAZ}$ Z-score)	−1.03 (1.7)
Total femur BMD	(g/cm^2)	0.688 (0.132)
	(BMD$_{HAZ}$ Z-score)	−1.4 (1.2)

Values are expressed as mean (SD). SDS = standard deviation score; BMI = body mass index; BMD = bone mineral density; and BMD$_{HAZ}$ = BMD corrected for height-for-age Z-score.

Clinical and anthropometric assessment

All patients enrolled in this study underwent physical examination to obtain anthropometric measures. Body weight was measured to the nearest 0.1 kg on a balance beam scale (Seca, Hamburg, Germany) and height was measured to the nearest millimeter using a wall-mounted stadiometer (Holtain Ltd., Crosswell, U.K.) Body mass index (BMI) was then calculated as weight on height^2 (kg/m^2). Standard deviation scores of anthropometric measurements for study subjects were calculated using specific Italian standards [15].

Bone mineral measurements

Bone mineral measurements were obtained at the L1–L4 vertebrae level, femoral neck and whole femur by dual-energy X-ray absorptiometry (DXA). The instrument (Hologic mod Discovery W 12.4.2) was calibrated on a daily basis according to the manufacturer's instructions. The longitudinal coefficient of variation for our instrument using daily measurements of a spine phantom was 0.4%.

Bone mineral measurements are expressed as areal bone mineral density (BMD in g/cm^2 and Z-scores), according to the recommendations of the International Society for Clinical Densitometry (ISCD, [16]). Reference data used were those provided by the DXA manufacturer. Patients with Marfan syndrome are taller than healthy individuals, and therefore we adjusted bone mass measurements for height using a recently proposed method [17]. Briefly, BMD Z-scores were re-calculated considering height-for-age Z-scores (HAZ). The BMD$_{HAZ}$ Z-scores were then obtained by the following equation: BMD$_{HAZ}$Z = BMD$_{age}$Z − HAZ-predicted BMD Z-score. By this equation, if the bone Z-score is appropriate for a subject's height status, the adjusted Z-score is zero. If the bone Z-score is less than expected given the height status, the HAZ-adjusted Z-score is negative.

Biochemical assessments

Blood was drawn by venipuncture for biochemical measurements, and serum separated after centrifugation. All serum samples were stored at −30° until assayed.

Parathyroid hormone (PTH) serum concentration was measured by an electro-chemiluminescence assay (ECLIA) on a Cobas® apparatus (Roche Diagnostics GmbH, Mannheim, Germany). Intra-assay variability was 1.7% and inter-assay variation was 6.5%. Sensitivity of the assay was 6.0 pg/mL. The serum concentration of osteocalcin (OC) was measured by ECLIA (Cobas®, Roche Diagnostics GmbH, Mannheim, Germany). Intra-assay variability was 2.2%, and inter-assay variation was 3.3%. Sensitivity of the assay was 0.5 ng/mL. The serum concentration of 25 hydroxyvitamin D (25OHD) was measured by ECLIA (Cobas®, Roche Diagnostics GmbH, Mannheim, Germany). Intra-assay variability was 7.8%, and inter-assay variation was 10.7%. Sensitivity of the assay was 4.0 ng/mL. Bone resorption rate was evaluated by serum measurements of the C-terminal telopeptide of type I collagen (CTX) by ECLIA (Cobas®, Roche Diagnostics GmbH, Mannheim, Germany). Intra-assay variability was 4.7%, and inter-assay variation was 8.4%. Sensitivity of the assay was 0.07 pg/mL. Alkaline phosphatase (ALP) concentration was measured by standard procedures.

Statistical analyses

Distribution of variables has been examined by the Shapiro–Wilk methods. All statistical tests have been conducted at an alpha level of 0.05, and were two-tailed. Data are shown as mean (SD), as otherwise stated. One-group tests were performed by t-test or Wilcoxon test, according to variable distribution. Changes over time of the study variables were tested by paired t-test or paired Wilcoxon test.