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Shwachman–Diamond syndrome is associated with low-turnover osteoporosis

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Abstract

Introduction: Shwachman–Diamond syndrome (SDS) is an autosomal recessive disorder characterized by exocrine pancreatic insufficiency and bone marrow dysfunction. These result in malabsorption and hematological abnormalities. A skeletal dysplasia is also an integral feature of SDS. The present study assessed prevalence and determinants of osteopenia and osteoporosis in patients with SDS and disease-causing mutations in the *SBDS* gene.

Materials and methods: Eleven patients (8 males) aged from 5 to 37 years (median 16.7 years) with a genetically confirmed diagnosis of SDS were assessed for fracture history, bone mineral content (BMC), lean tissue mass (LTM) and bone mineral density (BMD) (Hologic Discovery A), osteoporotic vertebral changes, and for blood biochemistry and hematological parameters. Iliac crest bone biopsies were obtained from four patients for histology and histomorphometry.

Results: The main findings were: (1) markedly reduced BMD *Z*-scores at the lumbar spine (median −2.1, range −4.4 to −0.8), proximal femur (median −1.3, range −2.2 to −0.7) and, whole body (median −1.0, range −2.8 to +0.6), and reduced *Z*-scores for height-adjusted BMC/LTM ratio (median −0.9, range −3.6 to +1.1); (2) vertebral compression fractures in three patients; and (3) blood biochemistry suggestive of mild vitamin D and vitamin K deficiency. Bone biopsies in four patients showed significant low-turnover osteoporosis with reduced trabecular bone volume, low numbers of osteoclasts and osteoblasts, and reduced amount of osteoid.

Conclusions: The results suggest that in addition to the skeletal dysplasia, SDS is associated with a more generalized bone disease characterized by low bone mass, low bone turnover and by vertebral fragility fractures. Osteoporosis may result from a primary defect in bone metabolism, and could be related to the bone marrow dysfunction and neutropenia.

Keywords: Shwachman–Diamond syndrome; *SBDS*; Osteoporosis; Neutropenia; Malabsorption

Abbreviations: SDS, Shwachman–Diamond syndrome; *SBDS*, Shwachman–Bodian–Diamond Syndrome gene; BMD, bone mineral density; BMC, bone mineral content; LTM, lean tissue mass; DXA, dual-energy X-ray absorptiometry; 25-OHD, 25-hydroxyvitamin-D; PTH, parathyroid hormone; CF, cystic fibrosis.

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Introduction

Shwachman–Diamond syndrome [1,2] (MIM# 260400) is a rare autosomal recessive disorder in which the symptoms arising from pancreatic insufficiency and bone marrow dysfunction dominate. The pancreatic dysfunction presents as impaired enzyme output and malabsorption of fat-soluble nutrients [3–5]; imaging studies show a fatty pancreas [6,7]. Bone marrow dysfunction is characterized by persistent or intermittent neutropenia, anemia or thrombocytopenia, and in bone marrow studies by hypocellular marrow, aplastic anemia, myelodysplasia and leukemia [8,9]. The *SBDS* gene that is associated with SDS has been recently identified [10] and several disease-causing mutations have been described [11–13]. Recent data [14–17] suggest that the SBDS protein is associated with RNA metabolism and ribosomal function, but the specific role of *SBDS* remains to be elucidated.

The association of a skeletal dysplasia with SDS has been reported in several studies [6,18–23]. According to a recent study [24], all patients with SDS and *SBDS* mutations have findings consistent with skeletal dysplasia but the localization and severity of these features vary with age, and even in patients with identical genotypes.

In addition to skeletal dysplasia, generalized osteopenia and even spinal compression fractures at an early age have been described in occasional case reports in children and adults with SDS [7,19,24–27]. Patients with SDS have several risk factors for osteoporosis. These include malabsorption and compromised nutrition due to pancreatic insufficiency, vitamin D and vitamin K deficiency, delayed puberty and infections. However, no previous studies have assessed the overall bone health in patients with SDS. In order to gain knowledge on the prevalence, etiology and characteristics of osteopenia and osteoporosis in SDS, we assessed 11 patients with SDS for several parameters of skeletal health. The results suggest that in addition to the previously characterized epiphyseal and metaphyseal skeletal changes, SDS is often associated with symptomatic osteoporosis.

Materials and methods

Patients

Study subjects were ascertained through the pediatric units of all five university hospitals in Finland. The inclusion criteria for this study were (i) exocrine pancreatic dysfunction associated with fatty pancreas in imaging studies, (ii) hematologic abnormalities, characterized by persistent or intermittent neutropenia, (iii) age >4 years, and (iv) verified mutations in the *SBDS* gene. All 11 patients meeting these criteria consented to participate. The study protocol was approved by the Research Ethics Board, Helsinki University Hospital and a written informed consent was obtained from each patient and/or guardian.

Clinical data

Data on the patients' previous medical history were collected from hospital records. A detailed history on present health, nutrition status, stature, growth and level of physical exercise, puberty, gastrointestinal and skeletal symptoms, history of fractures and medications, including vitamin substitutions, was obtained by patient interview and from hospital records. All the patients were clinically assessed for phenotypic features by a pediatric endocrinologist (OM). Height was measured with a Harpenden stadiometer (Holtain Limited, UK) and weight with an electric scale; values were compared with Finnish growth charts [28]. Height standard deviation score (height *Z*-score) was defined as deviation of height, in SD units, from mean height for age and sex [29].Weights were expressed as body mass index (BMI), derived from [weight (in kg)/by height^2 (in m^2)]. Pubertal maturation was assessed according to Tanner [30]. The presence or absence of pancreatic insufficiency was classified on the basis of serum trypsinogen concentration [25,31]; patients with values <6 μg/L were classified as having pancreatic insufficiency (PI) and patients with intermediate (6 to 16.6 μg/L) or normal (>16.6 μg/L) values were classified as having pancreatic sufficiency (PS). The pancreatic phenotype was confirmed with the measurement of serum isoamylase level [4].

Bone mineral density and radiographic assessment

Areal bone mineral density (aBMD) for the lumbar spine (L1–L4), proximal femur and whole body were measured with dual-energy X-ray absorptiometry (DXA, Hologic Discovery A®, Bedford, USA). The aBMDs were transformed into *Z*-scores by using age- and sex-specific reference data for the equipment. In addition, T-scores were calculated for adult patients by comparing the aBMD results with sex-specific reference data for the equipment. The *Z*-score is the number of standard deviations a given BMD measurement differs from the mean for age-, sex- and race-matched reference population. .The *T*-score is the number of standard deviations a given BMD measurement differs from the mean for sex-matched normal young adult reference population. Body composition, including bone mineral content (BMC), fat mass and lean tissue mass (LTM), was obtained with the same DXA scanner. Height-adjusted *Z*-scores for BMC/LTM were calculated using previously published reference data for children and young adults [32].

Standard anterior–posterior and lateral neutral radiographs of the thoracic and lumbar spine were taken in supine position. All thoracic and lumbar radiographs were reviewed by two radiologists. Changes in vertebral morphology were graded by inspection of digitized images (AGFA ImPacs System®) and classified according to the grading methods of Genant [33] for adults and of Mäkitie [34] for children to identify vertebral morphological changes suggestive of osteoporosis. Compression of 20% or more in the anterior, middle or posterior vertebral height was considered significant.

Bone biopsy

A transiliac bone biopsy was obtained from four patients with a bone biopsy needle of 7.5 mm inner diameter (Rochester Bone Biopsy, Medical Innovations Incorporation Inc., USA) following a double-labeling course with oral tetracycline, with a 10-day interval period. The biopsy was performed 4 days after the end of tetracycline administration. Bone histomorphometry analyses were conducted at the Bone and Cartilage Research Unit, University of Kuopio, Kuopio, Finland. All parameters were analyzed using a semiautomatic image analyzer (Bioquant Osteo, Bioquant Image Analysis Corporation, Nashville, TN, USA) and the results compared with normative data [35–37]. All nomenclature, abbreviations, and standard formulas follow the recommendations of the American Society for Bone and Mineral Research.

Mutation analysis

The *SBDS* coding regions were screened for mutations by restriction enzyme digestion of amplified exon 2 or by direct sequencing of PCR-amplified products of genomic DNA as previously described [10].

Biochemistry

Peripheral blood counts were obtained to assess hematological dysfunction. Anemia was defined as a hemoglobin concentration below the normal age and sex-specific reference range [38], neutropenia as a neutrophil count $<1500\times10^6$ cells/L, and thrombocytopenia as a platelet count of $<150\times10^9$ cells/L. Serum concentration of cationic trypsinogen (S-Tryp, reference range 16.6–42.6 μg/L) was determined by a double-antibody radioimmunoassay [39]. Serum pancreatic