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Original Investigation

Changes in Bone Matrix Mineralization After Growth Hormone Treatment in Children and Adolescents With Chronic Kidney Failure Treated by Dialysis: A Paired Biopsy Study

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Background: Patients with chronic kidney disease (CKD) develop renal osteodystrophy with alterations in bone turnover, mineralization, and volume (TMV). A specific skeletal complication in children is growth impairment, which currently is treated by recombinant human growth hormone (rhGH). The effects on bone material properties are poorly understood. This study assesses the effects of rhGH treatment on bone matrix mineralization.

Study Design: Observational study.

Setting & Participants: 18 short children and adolescents (aged 3.6-16 years) with CKD on dialysis therapy.

Predictor: rhGH treatment for 1 year.

Outcomes: Tetracycline-labeled bone biopsy classified according to the TMV system.

Measurements: Bone mineralization density distribution (BMDD) was evaluated by quantitative backscattered electron imaging in trabecular and cortical compartments. Additional data for patients' height and biochemical bone serum parameters were obtained.

Results: Prior to rhGH treatment, our cohort showed low bone turnover and high mineralization densities versus reference data: Ca_{mean} (weighted mean calcium content) in cancellous bone, +3.3% ($P = 0.04$); Ca_{mean} in cortical bone, +6.7% ($P < 0.001$); Ca_{peak} (mode of the BMDD) in cancellous bone, +5.0% ($P < 0.001$); Ca_{peak} in cortical bone, +8.2% ($P < 0.001$); Ca_{width} (heterogeneity in mineralization), no significant difference for cancellous ($P = 0.2$) and cortical ($P = 0.1$) bone; Ca_{high} (portion of fully mineralized bone) in cancellous bone, 5-fold greater ($P < 0.001$); Ca_{high} in cortical bone, 14-fold greater ($P < 0.001$); Ca_{low} (portion of low mineralized bone) in cancellous bone, +23.9% ($P = 0.02$); Ca_{low} in cortical bone, –22.2% ($P = 0.05$). After rhGH treatment, height increased by 9.1 cm ($P < 0.001$) and bone turnover indices to normal values or beyond. Matrix mineralization was lesser and more heterogeneous compared to baseline: Ca_{width} for cancellous bone, +15.3% ($P < 0.001$); Ca_{width} for cortical bone, +34.1% ($P < 0.001$). Ca_{mean}, Ca_{peak}, and Ca_{high} for cancellous bone and Ca_{mean} and Ca_{peak} for cortical bone were no longer significantly different from reference data. Ca_{high} for cortical bone dramatically decreased after treatment but was still substantially greater than reference data.

Limitations: Low case number per TMV subgroup, no measurements of fibroblast growth factor 23.

Conclusions: Children and adolescents with CKD and growth deficiency are at risk of having low bone turnover. rhGH treatment improves height and concomitantly bone modeling/remodeling, which appears beneficial for bone matrix mineralization.

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INDEX WORDS: Bone mineralization density distribution (BMDD); quantitative backscattered electron imaging (qBEI); bone histomorphometry; renal osteodystrophy; children and adolescents with chronic kidney disease (CKD); growth hormone therapy.

Skeletal alterations, termed renal osteodystrophy, occur early during a decrease in kidney function and are common in patients with chronic kidney disease (CKD).[1-4] Renal osteodystrophy is considered as a multifactorial disorder of bone remodeling and is diagnosed in bone biopsies as histologic types classified from low bone formation (adynamic bone) to increased bone turnover (osteitis fibrosa), with or without features of abnormal mineralization of the bone matrix.[3,5,6]

Alteration in mineralization has been shown to be the first skeletal abnormality in children and adolescents with CKD stage 2 and the prevalence increases with progression of CKD.[1,5,7-10] Affected patients

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also often experience growth retardation due to disturbance of the somatotropic hormone axis, leading to a relative growth hormone (GH) insensitivity and functional IGF-1 (insulinlike growth factor 1) deficiency. As a result, chondrocyte differentiation and their subsequent expansion within the epiphyseal growth plate are impaired.[11-13] Recombinant human GH (rhGH) has been used successfully for many years to compensate growth failure in children and adolescents with CKD who are on conservative or dialysis treatment, as well as to reverse the growth-depressing effects of glucocorticoids after kidney transplantation.[2,12,14-16] However, to evaluate the impact of CKD on bone development and the therapeutic effect of GH therapy, not only bone mass and bone microarchitecture, but also bone material quality should be assessed. A key determinant of stiffness, strength, and toughness of bone material is the degree of mineralization of the bone matrix, which can be determined by quantitative backscattered electron imaging in biopsy samples prepared for histology and histomorphometry.[17] Hence, bone mineralization density distribution (BMDD) describes the degree and distribution of mineral content of the bone matrix (which reflects the rate of bone turnover) and the mineralization kinetics (speed of mineral deposition within the newly formed osteoid).[18,19] Moreover, a recently established set of BMDD reference data enables comparison of mineralization status between children, adolescents, and young adults with and without bone disorders.[20]

In the present study, we focused on bone material properties in 18 children and adolescents on dialysis therapy before and after 1 year of rhGH treatment. Bone histomorphometry was performed on paired biopsy specimens and renal osteodystrophy was characterized according to the recently recommended TMV system that addresses bone turnover, mineralization defects, and abnormalities in bone volume.[1] Furthermore, we evaluate BMDD before and after rhGH treatment and compared all data to the reference cohort.[20]

METHODS

Study Population

The present study population comprised 18 whites with CKD stage 5D, 11 treated by continuous peritoneal dialysis and 7 treated by hemodialysis,[21] and is part of a larger cohort reported previously.[9] We reused all available paired biopsy samples with intact bone compartments and sufficient residual bone material for histomorphometry and quantitative backscattered electron imaging analysis. All patients were treated with calcium carbonate as phosphate binder, alfacalcidol administered at a mean dosage of 0.008-0.3 μg/kg body weight per week (daily or in intermittent doses), and calcium carbonate at a dosage of 55-334 mg/kg body weight per day. Indication for "pulse" doses of alfacalcidol was according to parathyroid hormone (PTH) level (abnormally high) as evaluated during clinical monitoring. Calcium carbonate was administrated individually according to phosphorus level.

None of the patients had fractures or received a kidney transplant between bone biopsies. Plain radiographs of the left hand were obtained to confirm delayed skeletal age according to the Greulich-Pyle scale.[22] Subsequently, children and adolescents were treated for 12 months with rhGH (daily subcutaneous application, 1.0-1.1 IU/kg/wk). Relevant clinical data are listed in Table 1.

Bone Serum Parameters

The following relevant bone parameters were assessed in fasting blood serum at the day of bone biopsy: calcium, phosphorus, intact PTH (detection of the peptide containing amino acids 1-84 by immunoradiometric assay [Incstar Corp]),[23] and alkaline phosphatase (ALP; by the kinetic method). Serum concentrations of osteocalcin were determined in 11 patients by radioimmunoassay (Incstar Corp).[23]

Bone Biopsy, Histomorphometry, and TMV Classification

Prior to each bone biopsy, tetracycline was administered orally in a dosage of 10 mg/kg/d for 3 days, with a 10-day interval for dynamic measurements of bone formation. Transiliac bone biopsies were performed 4-6 days after the last dose of tetracycline with a Bordier trocar (6-mm inner diameter). Undecalcified bone samples were embedded in polymethylmethacrylate.[20] Consecutively, 3-μm sections were stained with a modified Goldner's trichrome method, and 10-μm sections were used for fluorescent microscopy. Histomorphometric analyses were performed with original magnification $\times$100 for structural and $\times$200 for static and dynamic parameters (ocular $\times$10 and objective $\times$10 or $\times$20, respectively) by means of an Axiophot microscope (Zeiss) equipped with an AxioCam videocamera (Zeiss). The images obtained were analyzed further using NIH Image software versions 1.62 and 1.63 (developed at the US National Institutes of Health and available at rsb.info.nih.gov/nih-image). Bone histomorphometry was performed according to Parfitt et al.[24]

Classification of renal osteodystrophywas assessed by interpretation of histology and histomorphometry indexes according to the TMV system[1,2] as previously done by Bakkaloglu et al.[3] All parameters were compared with published references from Glorieux et al.[25]

Quantitative Backscattered Electron Imaging

The remaining tissue blocks were prepared by grinding and polishing, and surface planes containing bone tissue were coated by carbon for quantitative backscattered electron imaging analysis in the scanning electron microscope as reported elsewhere.[17,26] Trabecular and cortical BMDD were determined using a digital scanning electron microscope (DSM 962; Zeiss) equipped with a 4-quadrant semiconductor backscattered electron detector.[17,26,27] BMDD parameters are shown in Fig 1. The outcomes were compared to the published reference BMDD of healthy children, adolescents, and young adults.[20]

Statistical Evaluation

Statistical analysis was performed using SigmaStat for Windows, version 2.03 (SPSS Inc). Because sample sizes within the patient cohort and in particular within the TMV subgroups were relatively small, all outcomes (clinical, histomorphometry, and BMDD) were given as median values with interquartile ranges. Comparisons after treatment versus baseline and cortical versus cancellous BMDD variables were based on Wilcoxon signed tests.