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SHORT COMMUNICATION

Bone density, microarchitecture, and material strength in chronic kidney disease patients at the time of kidney transplantation

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Abstract
Summary Bone health is assessed by bone mineral density (BMD). Other techniques such as trabecular bone score and microindentation could improve the risk of fracture's estimation. Our chronic kidney disease (CKD) patients presented worse bone health (density, microarchitecture, mechanical properties) than controls. More than BMD should be done to evaluate patients at risk of fracture.
Introduction BMD measured by dual-energy X-ray absorptiometry (DXA) is used to assess bone health in end-stage renal disease (ESRD) patients. Recently, trabecular bone score (TBS) and microindentation that can measure microarchitectural and mechanical properties of bone have demonstrated better correlation with fractures than DXA in different populations. We aimed to characterize bone health (BMD, TBS, and strength) and calcium/phosphate metabolism in a cohort of 53 ESRD patients undergoing kidney transplantation (KT) and 94 controls with normal renal function.
Methods Laboratory workout, lumbar spine/hip BMD measurements (using DXA), lumbar spine TBS, and bone strength were carried out. The latter was assessed with an impact microindentation device, standardized as percentage of a reference value, and expressed as bone material strength index (BMSi) units. Multivariable linear regression was used to study differences between cases and controls adjusted by age, gender, and body mass index.
Results Among cases, serum calcium was 9.6 ± 0.7 mg/dl, phosphorus 4.4 ± 1.2 mg/dl, and intact parathyroid hormone 214 pg/ml [102–390]. Fourteen patients (26.4%) had prevalent asymptomatic fractures in spinal X-ray. BMD was significantly lower among ESRD patients compared to controls: lumbar 0.966 ± 0.15 vs 0.982 ± 0.15 (adjusted $p = 0.037$), total hip 0.852 ± 0.15 vs 0.902 ± 0.13 (adjusted $p < 0.001$), and femoral neck 0.733 ± 0.15 vs 0.775 ± 0.12 (adjusted $p < 0.001$), as were TBS (1.20 [1.11–1.30] vs 1.31 [1.19–1.43] (adjusted $p < 0.001$)) and BMSi (79 [71.8–84.2] vs 82. [77.5–88.9] (adjusted $p = 0.005$)).
Conclusions ESRD patients undergoing transplant surgery have damaged bone health parameters (density, microarchitecture, and mechanical properties) despite acceptably controlled hyperparathyroidism. Detecting these abnormalities may assist in identifying patients at high risk of post-transplantation fractures.

Keywords Bone mineral disease · Bone material strength index · Chronic kidney disease · Microindentation · Trabecular bone score

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Introduction

In the early period after transplant, mineral and bone disease experiences a deep change, with a readjustment of secondary hyperparathyroidism (HPT) parameters and an initial decrease in bone mineral density (BMD), up to 20% in the first 6 months, with further stabilization [1, 2]. According to this, a higher risk of fracture in kidney transplant (KT) population is observed in the first years after transplant, when also the dose of glucocorticoids administered is higher [3].

In order to prevent these fractures, predicting the population at risk has become a matter of concern for transplant community. The assessment of bone in end-stage renal disease (ESRD)-KT candidates is currently performed as in the general population, with BMD measured by dual-energy X-ray absorptiometry (DXA) because it is available and non-invasive. However, there are limitations for DXA use alone since bone quantity only captures the amount of mineral but cannot measure either microarchitecture or tissue characteristics (i.e., bone tissue quality) aspects relevant for the resistance to fracture in these patients [4, 5]. In order to improve the bone strength's estimation, other techniques have been developed, such as trabecular bone score (TBS) that can assess trabecular microarchitecture at the lumbar spine by analyzing DXA images with specific software [6, 7]. TBS has been applied in hemodialysis patients [8]. Reference point indentation (RPI) is another novel technique that claims to directly measure the mechanical properties of bone at a tissue level [9, 10]. RPI provides the resistance of cortical bone tissue to the opening of micro-cracks with a very fine probe, the phenomenon closely mimicking the initiating crack of the starting fracture [9, 11]. In previous studies, RPI has demonstrated better performance than DXA to discriminate osteoporotic fracture risk among healthy controls [9] and better clinical correlation with fractures in patients with atypical fracture compared to controls or patients with typical fractures [10], type 2 diabetes mellitus post-menopausal women compared to non-diabetic controls [12], or patients with osteopenia and fragility fractures [13]. Furthermore, we have recently reported the feasibility of RPI to better assess bone strength and risk of fracture in long-term stable renal allograft recipients [14].

We therefore aimed to analyze bone health (density, trabecular microarchitecture, and tissue-level quality/strength) in a cohort of ESRD patients at the time of undergoing KT surgery.

Material and methods

A cross-sectional study was performed in a sample of ESRD patients recruited between July/2012 and February/2014 during hospital admission for KT surgery. Written informed consent was obtained, and the Ethics Review Board in our institution approved the study protocol.

A general clinical history, physical examination, and routine laboratory measurements, including levels of intact parathyroid hormone (iPTH) (electrochemiluminescence, Roche Diagnostics GmbH, Penzberg, Germany) and 25-OH vitamin D (25[OH]D) (ELISA, IDS, Boldon, UK), were measured hours before the KT procedure. iPTH and 25[OH]D results are expressed as medians and interquartile ranges [IQR].

Bone health assessments including spine X-ray (anterioposterior and lateral), DXA scan, and bone microindentation were taken within the first week post-operatively (i.e., post-transplant). Spinal X-rays were informed by two independent observers using Genant's semiquantitative method [15] accepting grade I or above (loss >20% of one of the vertebral heights) as a fracture. Discrepancies were solved by consensus. BMD was measured using DXA scans at lumbar spine and proximal hip (Hologic QDR 4500 SL® (Hologic Inc. Bedford, MA, USA)). DXA-based TBS was evaluated using the same lumbar spine BMD measurements using iNsight® v 2.1 (Med-Imaps, Merignac, France). Bone microindentation was performed at the anterior face of tibia with a handheld reference point indenter device, Osteoprobe® (Active Life Scientific, Santa Barbara, CA, USA) following the recently described protocol [16]. In brief, after local anesthesia, a preload of 10 N followed by a 30-N indentation was performed with a test probe with a conic edge of 4 μm. Average values of eight indents were calculated by a computerized algorithm. Five calibration indents were then performed in a polymethylmethacrylate block (BMSi-100 Reference Material). Ratio between both tibia and the reference material measurements yields the final parameter of bone mineral strength index (BMSi) as previously described [9].

The results were compared with those found in a control group of healthy individuals, selected from our reference data for microindentation. They were healthy people without history of fragility fracture, bone disease, rheumatoid arthritis, metabolic or endocrine diseases, concurrent or prior treatment with bisphosphonates, oral corticosteroids, or any other bone-active drug. DXA, TBS, and microindentation were performed following the same protocol than ESRD patients, and a lateral radiograph of the spine was obtained if there is any history of trauma or spinal symptoms to rule out vertebral fractures.

Statistical analyses were performed with SPSS v 22.0 (IBM®) by using a Student's *t* test for parametric variables and Mann-Whitney *U* test for non-parametric variables. Multivariable linear regression models were fitted to analyze the association between chronic kidney disease (CKD) status and BMD, TBS, and BMSi after adjustment for age, gender, and body mass index (BMI). We also use it to analyze the association between being fractured at the time of transplantation and BMD, TBS, and BMSi.

Results

Patients

Fifty-three patients were included in the study. Mean age was 55.8 ± 12.1 years, with 77.8% of Caucasians and 53.7% female. Mean BMI was 27.5 ± 5.7 kg/m^2 with 25.9% of diabetic patients. It was the first transplant for 90.74% of them with a median time on dialysis of 21 months [IQR 11–36].

We compared patients with 94 healthy controls with a mean age of 50.2 ± 16 years, 78.7% female, and BMI 24.8 ± 4 kg/m^2 (supplementary Table S1).

Laboratory measurements

Just before KT, the patients had serum calcium levels of 9.6 ± 0.7 mg/dl (corrected by serum albumin), phosphorus 4.4 ± 1.2 mg/dl, 25[OH]D 7.9 ng/ml [3.1–16.7], and iPTH 214 pg/ml [102–390]. To achieve this HPT control, 76% were receiving phosphorus binders, 28% native vitamin D, 17% active vitamin D, 22% analogs of vitamin D, and 32% calcimimetics.

Bone tests

Bone health assessments were taken at a median time of 8 days [IQR 6–16] after transplant surgery. Fourteen patients (26.4%) had prevalent asymptomatic fractures in spinal X-ray at the time of KT. Mean baseline values of BMD were 0.966 ± 0.15 (lumbar), 0.852 ± 0.15 (total hip), and 0.733 ± 0.15 (femoral neck). Median TBS was 1.20 [IQR 1.11–1.3]. RPI was performed in 35 of 53 ESRD-KT candidates with median BMSi value of 79 [IQR 71.8–84.2].

The results for patients and controls are shown in Table 1. DXA was performed in 77 controls. Controls had better BMD (g/cm^2) at the lumbar spine, total hip, and femoral neck when we adjusted the model by age, BMI and sex, and better Z-score adjusting the model by BMI. They also had higher values of BMSi and TBS in the univariate analysis and after the adjustment.

Given the known higher risk of fracture and markedly lower absolute BMD values between women compared to men, we have performed the analysis in men and women separately. We found a similar behavior regarding BMD at hip and BMSi values despite gender in ESRD patients (supplementary Tables S2 and S3).

We compared bone parameters between patients who had preexisting asymptomatic fractures at the time of transplantation and those without fractures. BMD, TBS, and BMSi were similar between both subgroups (supplementary Table S4). They also remained unmodified after excluding diabetic patients (supplementary Table S5). On the other hand, longer time on dialysis and higher PTH levels seem to have a negative impact on BMD, with no alteration in TBS or BMSi values (supplementary Tables S6 and S7). Finally, we did not find any difference in bone parameters after adjustment for age, sex, and BMI according to previous management with vitamin D, paricalcitol, or cinacalcet (data not shown).

Discussion

We have performed a cross-sectional study in a cohort of ESRD patients undergoing KT, in whom we assessed bone and mineral status using three different techniques that measure bone density, trabecular microarchitecture, and tissue-level quality. We describe an important deterioration in all these markers of bone strength providing a comprehensive picture of the skeletal impact of ESRD. Despite all this, calcium/phosphate and iPTH levels were relatively controlled, stressing the value of preoperative specific bone health assessments (DXA or similar) for the identification of patients at high risk of post-transplantation fractures.

Bone and mineral disease related to CKD represents a constellation of disorders that may affect vascular system and skeleton of renal patients. The analytical values recommended by the guidelines [17] are not always easy to achieve in this population. In comparison with other studies [4, 8], our cohort of ESRD patients presented with better levels of phosphorus and PTH according to guidelines but with a significant 25[OH]D deficiency.

Low bone mass is frequently seen in patients in predialysis CKD [18] and ESRD [4, 8]. Measurements of BMD by DXA have classically perceived as the minimal workup for bone assessment. However, real-life practice suggests that they are not a common practice in the management of CKD patients, as their capability to predict fractures in this population is not fully established [17]. Although recent reports show a higher risk of fractures among CKD patients with lower BMD values [18], other aspects such as bone quality [5] might substantially contribute to the 4.4-fold higher risk of fractures in ESRD population [19]. In addition, the limitations of DXA in CKD go further because measurements can be affected by extra-skeletal calcifications (common in this population), osteomalacia, and osteosclerosis [5]. Our patients had lower values of BMD at lumbar spine, total hip, and femoral neck and a high prevalence of asymptomatic fractures, confirming a fragile bone status that may worsen early after transplantation. Previous studies in ESRD population have reported low BMD values predominantly in total hip and femoral neck [20]. We do not have a clear explanation for this discrepancy, and we could speculate that some uncontrolled characteristic of our series might account for this finding.

Stepping forward, TBS has been reported to be below normal ranges in dialysis patients [8]. We have recently showed

Table 1 Bone study comparison between end-stage renal disease patients and healthy controls

	Patients (n = 53)	Controls (n = 94)	p value (crude)	B coefficient [95% CI] (age, sex, and BMI adjusted)	p value (adjusted)
Dorso-lumbar X-ray	14 with fracture	–			
Dual-energy X-ray absorptiometry					
BMD lumbar (g/cm^2)	0.966 ± 0.15	0.982 ± 0.15	0.558	−0.05 [−0.1 to (−0.003)]	0.037
Z-score[a]	0.1 [−0.95–0.9]	0.05 [−0.5–1]	0.370	−0.189 [−1.03 to (−0.004)]	0.032
BMD total hip (g/cm^2)	0.852 ± 0.15	0.902 ± 0.13	0.061	−0.1 [−0.14 to (−0.05)]	<0.001
Z-score[a]	−0.4 [−1.15–0.5]	0.05 [−0.6–0.8]	0.013	−3.55 [−1.05 to (−0.413)]	<0.001
BMD femoral neck (g/cm^2)	0.733 ± 0.15	0.775 ± 0.12	0.070	−0.8 [−0.12 to (−0.04)]	<0.001
Z-score[a]	−0.4 [−1.1–0.6]	0.2 [−0.5–0.7]	0.062	−0.282 [−0.859 to (−0.227)]	<0.001
Trabecular bone score[b]					
	1.20 [1.11–1.3]	1.31 [1.19–1.43]	<0.001	−1.13 [−0.165 to (−0.06)]	<0.001
Reference point indentation[c]					
BMSi (units)	79 [71.8–84.2]	82.6 [77.5–88.9]	0.004	−4.7 [−8 to (−1.5)]	0.005

Values are expressed by mean ± standard deviation or median [interquartile range]

BMD bone mineral density, *BMSi* bone mineral strength index

[a] Z-score is BMI adjusted

[b] Performed in all patients and 77 controls

[c] Performed in 35 patients and all controls

similar TBS values between a cohort of long-term KT recipients and controls, demonstrating an almost complete recovery in their values more than 10 years after transplantation [14]. However, our ESRD patients had significantly lower TBS values than controls, indicating previous microarchitectural damage and potentially a higher fracture risk following transplantation, particularly when exposed to agents such as corticosteroids.

The development of minimally invasive techniques that may improve the estimation of the bone strength in populations at risk of fracture has been achieved by RPI, a novel technique that directly measures the mechanical properties of bone at a tissue level [9, 10]. In previous studies, RPI has demonstrated potential value in estimating bone strength in several clinical scenarios where BMD does not fully account for the increased risk of fracture [9, 12, 13, 21]. In KT patients, RPI has been tested in a pilot study [14], assessing the feasibility of the technique. In our cohort, BMSi values are also lower than controls, confirming poor bone mechanical properties of the tissue among ESRD patients undergoing KT.

In addition, this bone deteriorated status remained when we performed sensitivity analysis in order to discriminate by other risk factors for fractures (fractured patients, women, and diabetics). Longer time on dialysis and higher levels of PTH were also related to worse BMD.

Our study has some limitations. Other bone biomarkers apart from PTH were not available. The relatively limited number of study subjects prevents from carrying out subgroup analyses that might provide a deeper insight on the mechanisms of bone deterioration. However, fractures imply an important morbidity and mortality. Therefore, a comprehensive evaluation in the advanced CKD population of bone resistance to fracture, including microarchitectural and tissue mechanical strength with feasible and convenient techniques, is warranted, especially in those undergoing KT. Obviously, longitudinal analyses will be necessary to confirm the usefulness of the applied bone assessment techniques in ESRD patients.

In summary, ESRD patients undergoing KT present altered bone properties (in terms of bone density, trabecular microarchitecture, and tissue-level strength) despite well-controlled analytical HPT. Bone health assessments should be considered for the identification of high-risk patients regardless of pretransplantation lab findings.

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Compliance with ethical standards Written informed consent was obtained, and the Ethics Review Board in our institution approved the study protocol

Conflict of interest Maria José Pérez-Sáez, Sabina Herrera, Laia Vilaplana, Xavier Nogués, Dolores Redondo-Pachón, Marisa Mir, Roberto Güerri, Marta Crespo, and Julio Pascual declare that they have no conflict of interest.

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