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RESEARCH ARTICLE

Pre- and post-renal transplant bone mineral metabolism in children and adolescents

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ABSTRACT

Background. Information regarding chronic kidney disease–mineral bone disorder (CKD-MBD) in children who undergo renal transplant is scarce. Despite successful renal transplantation, bone disorders have been described and attributed to immunosuppressive drugs (steroids and calcineurin inhibitors). Therefore, it is important to determine the prevalence and outcome of bone mineral disorders pre- and post-renal transplant. The aim of the study was to describe the prevalence and type of bone mineral disorders in children pre-renal transplant and outcomes.

Methods. The Institutional Review Board and Ethics Committee approved the study. Signed consent/assent was obtained from all participants. Patients <18 years of age and under investigation for a first renal transplant were invited to participate. At transplant and 6 and 12 months after transplantation, anthropometric data were collected and blood samples were collected for serum creatinine, slope levels of tacrolimus, serum calcium, phosphorus, magnesium and alkaline phosphatase. Intact parathyroid hormone (PTH) was measured before transplant.

Results. Thirty-one patients were included with a mean age of 14.6 ± 3.2 years. Females represented 52%, 18 patients had peritoneal dialysis (58%), eight hemodialysis (26%) and five patients were listed as pre-emptive transplantation (16%). All received induction with basiliximab and triple maintenance therapy with prednisone, mycophenolate mofetil and tacrolimus. One patient was changed to cyclosporine at the third post-transplant month due to diabetes mellitus.

Pre-transplant PTH values were <150 pg/ml in 51.6% suggestive of low turnover bone lesions, 38.7% had PTH >300 pg/ml suggestive of high turnover bone lesions and only 9.6% had PTH between 150 and 300 pg/ml.

When pre- and post-transplant studied parameters were compared, serum creatinine was statistically lower during follow-up. No difference was found in serum calcium and alkaline phosphatase, but magnesium and phosphorus values were significantly lower after transplant. Twelve patients (38.7%) had post-transplant hypophosphatemia and required supplementation. Ten patients (32%) had hypomagnesemia, seven of them with concomitant hypophosphatemia. Z-score for weight increased significantly after renal transplant; nevertheless, only patients with no hypophosphatemia during follow-up improved their Z-score for height. Glomerular filtration rate had a positive correlation with serum calcium and a negative correlation with phosphorus and magnesium (p <0.05). Tacrolimus slope levels had a significantly negative correlation with serum magnesium (r = -0.431, p <0.0001).

Conclusions. In our medical center, only 9.6% of patients have PTH between the recommended values of >150 and <300 pg/ml. This situation could be prevented with adequate treatment of vitamin D analogues and phosphorus chelators. During the first post-transplant year, hypophosphatemia is seen in 38.7% and hypomagnesemia in 32% of patients. Glomerular filtration rate had a negative correlation with serum phosphorus at transplant. Tacrolimus slope levels had a negative correlation with magnesium serum values. Patients with no hypophosphatemia during the first year had better growth than those with hypophosphatemia. It is important to monitor and opportunely treat bone mineral disorders in children who undergo transplantation.

Key words: renal transplant, bone mineral metabolism, renal osteodystrophy, chronic kidney disease.

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INTRODUCTION

Chronic renal disease (CRD) has an effect on bones known as renal osteodystrophy. Bone disease has a wide spectrum that appears from initial stages of the renal disease.[1] The severity of the bone disease tends to be associated with the severity and duration of renal disease. In children, if osteodystrophy is not treated properly and in a timely manner, bone deformities and disorders in growth patterns ensue.[2] As kidney function declines, mineral homeostasis deteriorates with changes in blood levels of calcium and phosphorus as well as hormones such as parathyroid hormone (PTH), 25-hydroxyvitamin D (25OHD), 1,25-dihydroxyvitamin D (1.25 OH2D3) and other metabolites of vitamin D, fibroblast growth factor (FGF-23) and growth hormone.[3]

The Kidney Disease Improving Global Outcomes (KDIGO) international guidelines recommend using the term "chronic kidney disease-mineral bone disorder" to describe the broader clinical syndrome comprising mineral alterations (calcium, phosphorus or PTH metabolism), bone (alterations in bone turnover, mineralization, linear growth or strength) and cardiovascular calcifications that develop as complications of chronic renal disease. It is also recommended that the term "renal osteodystrophy" be limited to the description of the bone disorder associated with renal disease. The evaluation and definitive diagnosis of renal osteodystrophy require a bone biopsy using a system of classification based on the parameters of alterations in bone modeling and remodeling.[4]

After successful renal transplant, disorders of calcium metabolism are partially or completely corrected; however, secondary hyperparathyroidism may still persist. Also, bone disease induced by tubular dysfunction or related with immunosuppressive medications may present itself.[5]

Studies in adults have shown that patients with renal transplant have higher levels of PTH, calcium and osteocalcin than control subjects as well as hypophosphatemia, consistent with the persistence of secondary hyperparathyroidism. This occurs even in patients with normal glomerular filtration rate (GFR), which indicates that other factors in addition to renal function are involved in the persistence of hyperparathyroidism. These factors could be the time and severity of uremia, size of the parathyroid glands before transplantation, poor involution of the hypertrophic parathyroid glands caused by an alteration in the clearance of excess cells by apoptosis, type of parathyroid growth because the nodular type is associated with a less uniform distribution of calcitriol receptors than the diffuse hyperplasia type, causing less inhibition with calcitriol.[6] Some patients have a monoclonal growth of the parathyroid tissue; therefore, the involution of the glands may not take place after correction of the factors that caused the secondary hyperparathyroidism.[7] Immunosuppressive drugs used to prevent rejection may affect the bones. In the particular case of glucocorticoids, they are known to cause bone loss, primarily the trabecular type. At modest doses, in the physiological range, they increase the risk of osteoporotic fractures.[8] Among the direct effects of glucocorticoids on bone are increased osteoclastogenesis, changes in the duration of the osteoclast, cell differentiation of the adipocyte to osteoclast, shortening of the life of mature osteoblasts and osteocytes, decrease in osteoblast function and transcription of IGF1. There are also indirect effects affecting mineral metabolism such as calcitriol resistance, decrease in intestinal absorption of calcium, hypercalciuria and hypogonadism.

The effect of calcineurin inhibitors (cyclosporine and tacrolimus) has been extensively studied in animal models, which show a significant loss of cortical and trabecular bone mass after weeks of treatment. This effect is reversible and dose-dependent. High remodeling is found in histomorphometry.[9] Both tacrolimus and cyclosporine induced renal loss of calcium and magnesium and inhibit receptor activation of vitamin D.[10] There are few studies in adults and children that evaluate pre- and post-RT mineral metabolism. Mexico has reported the prevalence of bone adynamia in 16 adult patients with normal renal function according to biopsy 84 months posttransplant.[11]

Koch-Nogueira et al. reported that 41 patients with pre-dialysis transplant had lower levels of PTH than children with dialysis, both pre-transplantation as well as at 3 months post-renal transplant, all with normal graft function. Those with more severe secondary hyperparathyroidism had lower parathyroid involution post-renal transplant.[12] Sanchez et al. carried out a metabolic bone biopsy in 47 children at 3.2 ± 1.7 years post-renal transplant. There were 23% with hyperparathyroidism, 66% normal bone, and 11%, adynamic lesions. Serum PTH levels did not predict bone lesion.[13]

The objective of this study was to describe the prevalence and type of mineral metabolism disorders pre- and post-transplantation and its evolution in children.

SUBJECTS AND METHODS

We performed a prospective descriptive study approved by the Research and Ethics Committee of the hospital (HIM protocol 2010/006). Informed consent was obtained from all participants. Patients with chronic kidney disease <18 years of age were invited to participate in the study as well as those who were being evaluated to receive a first renal transplant.

At the time of transplantation and at 6 and 12 months post-transplantation, anthropometry was performed (weight, height) and blood was collected to determine levels of creatinine, calcium, phosphorus, magnesium and alkaline phosphatase. The levels of intact PTH at the time of the transplant were determined by immunochemiluminescence. The tacrolimus slope levels were measured by chemiluminescence immunoassay (ARCHITECT System, Abbott, Abbott Park, IL).

Hypophosphatemia and hypocalcemia were considered when the numbers of serum phosphorus and calcium were below the recommended range for age (according to the KDOQI guidelines)[14] and hypomagnesemia when the serum magnesium levels were <1.5 mg/dl.[15]

Renal function

Pre-and post-kidney transplantation renal function was assessed by estimating the glomerular filtration rate (eGFR) according to the Schwartz formula:

$$eGFR\ (ml/min/1.73\ m^2) = kL/\ S_{cr}\ (mg/dl)$$

where L = height in centimeters, S_{cr} = serum creatinine, k = value of the constant (0.55 for adolescent boys and girls or 0.7 for male adolescents).[16-18]

Patients who had loss of renal graft before 6 months were eliminated from the study.

Statistical analysis

Descriptive statistics were carried out. Data were expressed as average ± SD for variables with normal distribution and means with ranges for non-normally distributed variables. Pre- and post transplant biochemical changes were analyzed with repeated samples ANOVA. Also analyzed were the pre- and post-renal transplant values with Student t test or with the Wilcoxon range test according to the variable distribution. An analysis of the correlation between the serum levels of tacrolimus, the GFR and the biochemical variables studied was done; $p < 0.05$ was considered to be statistically significant. We used the program Graph Pad Prism v.5.0 for Mac OS X.

RESULTS

We included 31 patients. The average age was 14.5 ± 3.5 years. Female gender predominated (52%). Of the 31 patients, 18 had peritoneal dialysis (58%), eight hemodialysis (25%) and five patients underwent predialysis transplantation (16%) (Table 1). At the time of transplantation all patients received replacement therapy with multivitamins: 26/31 received erythropoietin, 30/31 received calcium carbonate as a phosphate binder and 29/31 were treated with calcitriol. This treatment was suspended the day following renal transplantation. All patients received induction with basiliximab and triple scheme with prednisone, mycophenolate mofetil and tacrolimus. One patient was switched to cyclosporine before 3 months because he presented diabetes mellitus after the transplant.

The biochemical variables studied at the time of the renal transplant as well as at 6 and 12 months of follow-up are shown in Table 2. As expected, serum levels of creatinine and eGFR improved significantly in all patients. Serum calcium increased from 8.6 ± 1.4 mg/dl at transplant to 9.46 ± 0.5 mg/dl at 6 months post-renal transplant and at 9.47 ± 0.6 mg/dl at 12 months post-renal transplant ($p = 0.01$). In contrast, phosphorus and magnesium decreased significantly. At the time of renal transplant, 12 patients had hypocalcemia with total serum calcium <8.8 mg/dl (average 7.5 ± 0.9 mg/dl), and three patients had hypercalcemia with serum calcium >10.3 mg/dl. During follow-up, three patients had less-pronounced hypocalcemia (8.5 ± 0.2 mg/dl). GFR had a positive correlation with serum calcium (Figure 1A) and negative correlation with phosphorus (Figure 1B) and serum magnesium ($p < 0.05$, Figure 1C).

We found a negative correlation between levels of tacrolimus and serum magnesium (Figure 2A) and between serum calcium and phosphorus (Figure 2B) and a positive relationship between serum phosphorus and magnesium (Figure 2C) and magnesium vs. serum alkaline phosphatase (Figure 2D). No relationship was seen between the levels of tacrolimus and other study variables or between the weighted dose of prednisone 6 and 12 months with the studied variables (results not shown).

Prior to renal transplant it was observed that 16 patients had PTH values <150 ng/ml (51.6%), which would suggest bone adynamia, 12 patients (38.8%) had a hyperparathyroid status with values of PTH >300 ng/ml, and only three patients (9.6%) had PTH at the recommended levels (between 150 and 300 ng/mL) (Figure 3). Twelve patients presented hypophosphatemia during the follow-up and ten had hypomagnesemia. They required replacement therapy with magnesium and phosphate salts.

The proportion of patients with hypophosphatemia and hypomagnesia post-transplant is shown in Table 3. Patients with recommended PTH levels did not have hypophosphatemia or hypomagnesemia during follow-up. Patients with low PTH levels tended to have hypophosphatemia, whereas those with PTH levels >300 ng/ml tended to have hypomagnesemia. However, the difference was not statistically significant.

Table 1. Demographics of the 31 patients included in the study

Age (years) (average ± SD)	14.6 ± 3.2
Gender (*n*, %)	
Male	15 (48%)
Female	16 (52%)
Replacement therapy (*n*, %)	
PD	18 (58%)
HD	8 (26%)
Predialysis	5 (16%)
Type of renal transplant	
Living related donor	15 (48%)
Cadaver	16 (52%)

SD, standard deviation; PD, peritoneal dialysis; HD, hemodialysis.

At 12 months post-transplantation, three patients persisted with hypophosphatemia, and five patients with hypomagnesemia (Table 3). In terms of growth, all patients significantly improved the Z-score for weight, although there was no improvement observed in height (Table 4). These results do not change if patients are stratified according to baseline PTH figures.

By classifying the patients among those with post-transplantation hypophosphatemia and those without, children who did not develop hypophosphatemia showed a significant increase in the Z-score for height. In both groups a significant increase in weight was observed (Table 4).

DISCUSSION

CRD causes multiple alterations in mineral metabolism. This is especially important in children. During growth in children, they should gain the bone mass they will have in adulthood; therefore, it is considered to be a critical period for bone health.

In the present study we found that at the time of renal transplantation only 9.6% of patients had PTH values in the recommended range for end-stage renal disease. PTH begins to rise from the time the GFR velocity falls <60 ml/min/1.73 m^2.[1] Hyperphosphatemia contributes to secondary hyperparathyroidism because it decreases both 1,25 dihydroxyvitamin D and the ionized calcium levels as well as being a direct stimulus for PTH secretion.[19] For the treatment of secondary hyperparathyroidism there are the vitamin D analogs. The most widely used for its low cost is calcitriol; however, treatment with overdose of calcitriol and the use of calcium phosphate binders favor the development of disorders of bone remodeling as apparently occurred with these patients because 51.6% had PTH levels <150 pg/ml at the time of transplant.[4]

Table 2. Levels analyzed in serum of the 31 patients at the time of renal transplant and at 6 and 12 months of follow-up

	At the time of transplant	*6 months post-RT*	*12 months post-RT*	*p value**
Creatinine (mg/dl)	10.2 ± 4.3	1.06 ± 0.39	1.08 ± 0.34	< 0.0001
eGFR (ml/min/1.73 m^2)	10.5 ± 5	93 ± 27	88 ± 19	< 0.0001
Calcium (mg/dl)	8.86 ± 1.4	9.46 ± 0.53	9.47 ± 0.6	0.01
Phosphorus (mg/dl)	5.49 ± 1.6	4.55 ± 0.7	4.23 ± 0.75	< 0.0001
Magnesium (mg/dl)	2.48 ± 0.5	1.6 ± 0.2	1.71 ± 0.2	< 0.0001
Alkaline phosphatase (U/l)	245 ± 170	207 ± 101	184 ± 96	0.21

RT, renal transplant; eGFR, estimated glomerular filtration rate.

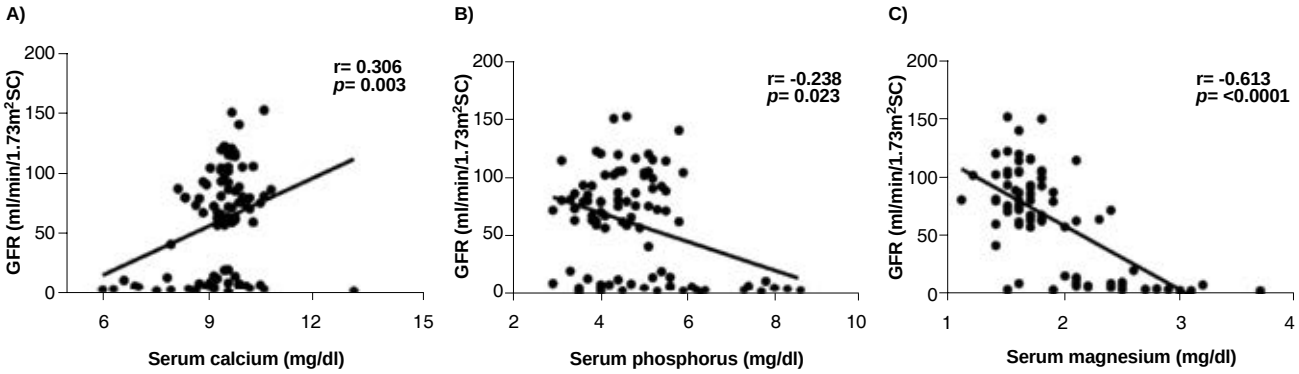

Figure 1. (A) Relationship between the estimated glomerular filtration rate (GFR) by Schwartz formula (eGFR) and serum calcium, **(B)** eGFR and serum phosphorus, **(C)** eGFR and serum magnesium in 31 patients. Included were the samples at the time of renal transplantation and at 6 and 12 months post-transplant. *p* value was obtained using the Spearman test.

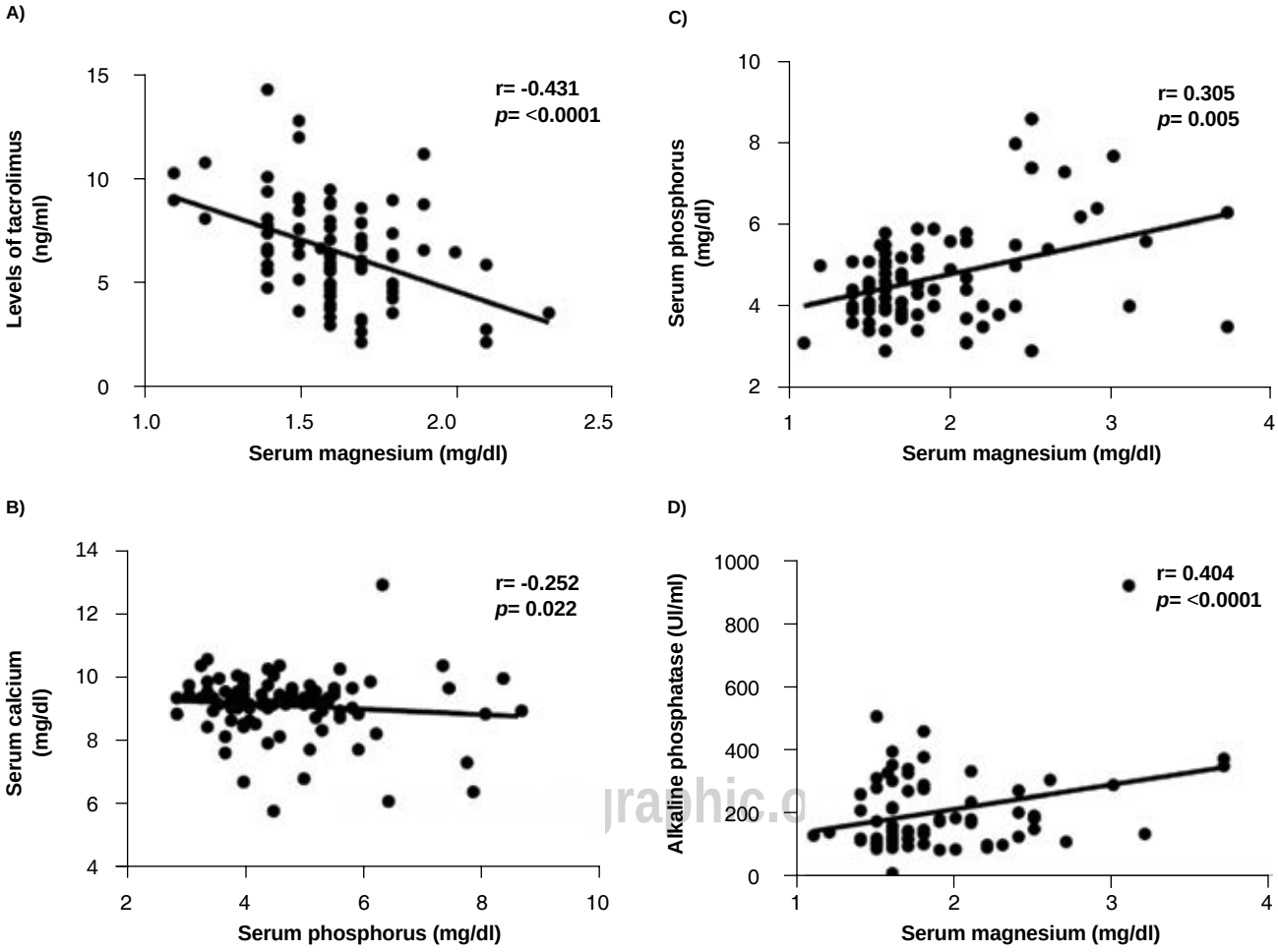

Figure 2. Relationship between magnesium levels and serum tacrolimus **(A)**, serum calcium and phosphorus **(B)**, serum phosphorus and magnesium **(C)** and alkaline phosphatase with serum magnesium **(D)** in 31 patients. *p* values obtained using the Spearman test.

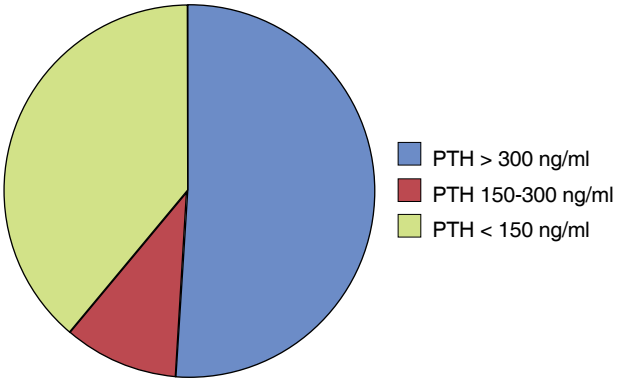

Figure 3. Distribution of pre-transplantation intact parathyroid hormone (PTH) values in 31 children.

Table 3. Proportion of patients with hypomagnesemia and hypophosphatemia during post-transplant follow-up according to parathyroid values at the time of transplant

	PTH < 150 (n =16)	*PTH 150-300 (n =3)*	*PTH > 300 (n =12)*
Hypophosphatemia			
3 months	6 (37.5%)	0	3 (25%)
6 months	3 (18.7%)	0	3 (25%)
12 months	2 (12.5%)	0	1 (8%)
Hypomagnesemia			
3 months	1 (6.25%)	0	3 (25%)
6 months	2 (12.5%)	0	5 (41.6%)
12 months	1 (6.25%)	0	4 (33.3%)

Hyphosphatemia contributes to the development of secondary hyperparathyroidism and has been related with mortality. In children, hypophosphatemia leads to rickets and growth delay.[14] It is evident in the present study that those patients who developed hypophosphatemia post-transplant did not grow adequately after 12 months, regardless of the duration of the hypophosphatemia.

The GFR had a positive correlation with the level of serum calcium and a negative correlation with phosphorus as has been described in other studies that have evaluated bone mineral metabolism in patients with renal disease.[4] It is important to consider that despite the fact that the patients included in the present study had a successful renal transplantation, eGFR at 12 months was 88 + 19 mL/min/1.73 m^2, so that inexorably they will begin to lose renal function in the long term and will continue to be chronically ill.

We also found a negative correlation between levels of tacrolimus and serum magnesium. It should be mentioned that the dosage of tacrolimus was adjusted to reach target slope values between 5 and 10 ng/mL according to post-transplant time. Hypomagnesemia has been linked with the use of calcineurin inhibitors.[20] Both cyclosporine and tacrolimus increase urine secretion of magnesium, apparently by an inhibitory effect on the vitamin D receptor and, independently, of the PTH levels.[21] This effect is more pronounced with cyclosporine.[10] The presence of hypomagnesemia is associated with the development of post-transplant diabetes mellitus.[22] It is believed that it is necessary to monitor and treat mineral disorders in a timely

Table 4. Follow-up of Z-score for weight and height in 31 children with renal transplant, according to the development or not of hypophosphatemia during follow-up

	Hypophosphatemia n =12	*Normal phosphorus n =19*	*All n =31*
Z-score for weight			
At RT	-3.25 ± 2.3	-3.00 ± 2.1	-3.09 ± 2.16
At 12 months	-1.7 ± 1.9*	-1.6 ± 2.0*	-1.64 ± 1.9*
Z-score for height			
At RT	-2.66 ± 1.22	-2.49 ± 1.7	-2.56 ± 1.5
At 12 months	-2.48 ± 0.90^{NS}	-2.16 ± 1.4*	-2.28 ± 1.28^{NS}

Values expressed as mean and standard deviation.
*p <0.05 compared to value at the time of renal transplant (RT) and at 12 months post-transplant.
NS, not statistically significant.

manner in the post-renal transplant period. Some patients may require vitamin D analogs different from calcitriol as well as phosphate binders to avoid low remodeling of lesions. Others may require treatments such as bisphosphonates. It is desirable to have information provided by the bone biopsy with double tetracycline labeling.

Further studies are needed to evaluate potential interventions and their effects on bone, growth, graft function and long-term cardiovascular risk.

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