Preventing Bone Loss in Renal Transplant Recipients with Vitamin D

AMGAD E. EL-AGROUDY, AMR A. EL-HUSSEINI, MOHARAM EL-SAYED, and MOHAMED A. GHONEIM
Urology and Nephrology Center, Mansoura University, Mansoura, Egypt.

Abstract. Very rapid bone loss, osteopenia, and osteoporosis have been documented in the first 6 to 12 mo after renal transplantation. Investigated was the effect of treatment with active vitamin D on the prevention of posttransplantation bone loss. Forty adult men who were recent renal transplant recipients were enrolled onto the study. Patients were randomized into two groups: group 1 received daily alfacalcidol 0.5 μg by mouth, and group 2 (control) received placebo. Every patient in both groups received daily 500-mg calcium carbonate supplements. Parameters of bone metabolism and bone mineral density measured at three sites were assessed before and after the study period. Bone mineral density was increased by 2.1%, 1.8%, and 3.2% at lumbar spine, femoral neck, and forearm, respectively, in group 1, whereas it decreased by 3.2%, 3.8%, and 1.8% at the same sites in the control group ($P < 0.05$). Serum intact parathyroid hormone level decreased significantly in group 1 compared with the control group ($P = 0.003$). Early bone loss that occurs during the first 1 yr after renal transplantation could be prevented by alfacalcidol. Use of alfacalcidol early after transplantation is safe and well tolerated.

After renal transplantation, an important loss of bone mineral density (BMD) is observed, which contributes to an increased risk for fractures (1). Short-term studies of bone loss after renal transplantation have indicated rapid bone loss within the first 6 to 18 mo after grafting (2,3). However, others have suggested a continuous demineralization process in long-term renal transplant recipients (4). In patients with renal transplants, in addition to known risk factors for bone loss in healthy populations, other abnormalities could negatively affect the bone metabolism, including the pretransplantation renal osteodystrophy, persistent hyperparathyroidism, and use of immunosuppressive drugs (5). The role of glucocorticoids-induced osteoporosis is well documented in many series (6), and there is much evidence that cyclosporin A (CsA) could increase bone resorption as a result of increased osteoclast activity (7,8).

Few data are available regarding potential preventive therapies (9,10). Vitamin D analogs and calcium have beneficial effects in the prevention of glucocorticoids-induced bone loss (11,12). The antiresorptive properties of calcitonin are known in treating high-turnover bone lesions as well as glucocorticoids-induced osteoporosis, but with variable results (13,14). Biphosphonates that inhibit bone resorption have been shown in many studies (15,16) to ameliorate glucocorticoid-induced bone loss. However, there are some concerns, especially for patients with preexisting low bone turnover disease, that biphosphonates could further slow down the rate of turnover and further increase the risk of fracture. The prevention and management of bone loss after renal transplantation have yet to be elucidated. We aimed to investigate the efficacy and safety of active vitamin D (alfacalcidol) for the prevention of bone loss in renal transplant recipients in a prospective controlled, randomized study.

Patients and Methods

Patients

This prospective, placebo-controlled, randomized study assessed 40 patients who underwent live-donor renal transplantation in our institution. All patients provided informed consent. The characteristics of the patients are outlined in Table 1. Inclusion criteria were as follows: men older than 20 yr; no diabetes; no steroids received before transplantation; and hemodialysis for not more than 2 yr. Exclusion criteria included impaired graft function (*i.e.*, serum creatinine >2 mg/dl), presence of previous fractures, and presence of any other endocrine abnormalities.

Immunosuppression

All of the patients received 500 mg methylprednisolone before transplantation and on the day of transplantation, and then oral prednisolone was provided 3.5 mg/kg per d for 2 d; 1.5 mg/kg per d for 5 d; and tapered until it reached 0.15 mg/kg per d from the ninth month thereafter. CsA was given 2 d before transplantation at a dose of 8/mg/kg per day on two divided doses, and then the dosages were adjusted to keep the whole blood trough level between 200 and 300 ng/ml during the first 2 mo and then between 100 and 150 ng/ml thereafter by use of monoclonal antibodies (Abbott Diagnostics, IL).

Study Protocol

Allocation to the different treatment modes was performed by blind randomization. Patients were randomized into two groups; group 1 received alfacalcidol 0.5 mg/d by mouth, and group 2 received served as the control group. All patients received oral 500 mg/d supplemental

Received July 9, 2003. Accepted August 16, 2003.
Correspondence to Dr. Amgad E. El-Agroudy, Urology and Nephrology Center, Mansoura University, Mansoura, Egypt. Phone: +20502262222; Fax: +20502263717; E-mail: amgadelbaz@ahram0505.net
1046-6673/1411-2975
Journal of the American Society of Nephrology

DOI: 10.1097/01.ASN.0000093255.56474.B4

Table 1. Patient characteristics of the two groups

Characteristic	Alfacalcidol ($n = 20$)	Control ($n = 20$)	P Value
Age, yr			
mean ± SD	31.4 ± 10.1	31.6 ± 10.7	0.81
Range	18–50	19–54	
Time on dialysis, mo	10.8 ± 5.4	12.1 ± 6.3	0.85
Acute rejection/patient	1.0 ± 1.1	1.1 ± 1.1	0.54
Cumulative steroid (g) at 1 yr			
mean ± SD	14.2 ± 2.7	13.7 ± 2.3	0.67
Range	10–20	10–17.5	
Cumulative CsA doses (mg/day) at 1 yr	155 ± 27	156 ± 34	0.26
Body weight (kg)	71.5 ± 14.1	69.3 ± 13.6	0.51

calcium carbonate. All patients received treatment drugs within the first week after transplantation. During the observation period, the patients did not receive fluoride, vitamin D, or hormones.

Laboratory Analysis

Routine laboratory tests, including renal function tests, albumin, and CsA trough level, were performed for all patients. Parameters of calcium metabolism included serum calcium and phosphorus, alkaline phosphatase, 24-h urinary excretion of calcium and creatinine, serum intact parathyroid hormone (iPTH) assessed by an immunoradiometric assay (Allergo, Nichole Institute, San Juan Capistrana, CA), serum osteocalcin assessed by RIA (Cis kits, France; interassay coefficient of variation was <3%), and urinary deoxypyridinoline excretion assessed by immunoradiometric assay (ACS Bayer, Germany; with interassay coefficient of variation was <6%). All laboratory tests were performed before treatment and again 1 yr after treatment.

BMD

BMD of lumbar spine, femoral neck, and forearm was determined by dual energy x-ray absorbiometry by use of a scanner (Lunar Corp., Madison, WI). Results were compared with values of an age- and gender-matched reference populations (Z score) and with values of gender-matched peak bone mass of a young control population (T score). The coefficient of variation for femoral neck was 2.1% and 1.6% for L1 and L4, and 1.8% for forearm. All data were evaluated before and 1 yr after treatment. All clinical and laboratory side effects that occurred during the study were recorded, and their causes were evaluated.

Statistical Analyses

Statistical analyses were performed by SPSS version 10 (SPSS, Chicago, IL). Comparison of the basal values was done by the χ^2 test for qualitative variables and by unpaired t test for quantitative variables. Changes within the groups after end of the study period were analyzed by the paired t test. Results are given as means ± SD unless otherwise indicated. $P < 0.05$ was considered significant.

Results

Patient Characteristics

A summary of baseline patient characteristics is shown in Table 1. The median age was 32 and 29 yr in groups 1 and 2, respectively, with no statistically significant difference ($P = 0.54$). The main causes of end-stage renal disease were chronic interstitial nephritis in 12 patients, nephrosclerosis in 6 patients, glomerulonephritis in 3 patients, and hereditary nephritis in 2 patients, with no statistically significant difference between both groups ($P = 0.14$). There were no episodes of acute rejection or delayed graft function in any of the patients before enrolling onto the study. No bony symptoms or fractures were registered in any group during the observation period.

In terms of posttransplantation factors, there were no significant differences in the main cumulative steroids doses after 1 yr of follow-up for all groups ($P = 0.67$). The time of exposure to corticosteroids (date of transplantation) until the starting date of the study was not different between both groups. During the study period, the prednisolone doses were further reduced in all groups, and this reduction was not statistically significant between both groups ($P = 0.32$).

Laboratory Investigations

Detailed biochemical data are summarized in Table 2. Baseline biochemical investigations were performed at the time of entry onto the study. There were no statistically significant differences observed between both groups. High proportions of patients in all groups (85% and 82% in treatment and control groups, respectively) demonstrated elevated iPTH levels at baseline. In fact, more than half of patients demonstrated baseline iPTH values exceeding twice the upper limit of normal values. In contrast, the baseline osteocalcin values were below the lower limits of normal in 25 patients (42.6%), with nonstatistical difference between groups ($P = 0.18$). Values of alkaline phosphates were above upper limits of normal values in more than 70% of patients. Average serum calcium levels were found in most of the patients in both groups; however, the values were near the lower limit of normal in 28 patients (46%). The statistical differences was not different between groups ($P = 0.28$). In the case of serum creatinine values, although there a trend of gradual increase was observed throughout the study period until the end of the study after 1 yr (mean ± SD, 1.4 ± 0.4 mg/dl; range, 1.0 to 2.1 and 1.5 ± 0.4 mg/dl; range, 0.9 to 2.2, respectively), this difference did not reach statistical significance ($P = 0.18$). To simplify data presentation, only the initial and final results of all variables are shown in Table 2.

Table 2. Metabolic parameters at baseline and after 12 mo of treatment

Parameter	Alfacalcidol (n = 20)		Control (n = 20)		*P* Value		Normal Range
	Baseline	12 mo	Baseline	12 mo	P^a	P^b	
Serum creatinine (mg/dl)	1.3 ± 0.5	1.4 ± 0.4	1.3 ± 0.3	1.5 ± 0.4	0.19	0.18	0.6–1.3
Serum calcium (mg/dl)	8.6 ± 0.3	9.9 ± 0.4[c]	8.7 ± 0.5	9.3 ± 0.3[c]	0.29	0.001	8.6–10.6
Serum phosphorus (mg/dl)	4.7 ± 0.9	3.3 ± 0.6[c]	4.8 ± 0.8	3.5 ± 0.4[c]	0.84	0.36	2.4–4.6
Alkaline phosphatase (IU/L)	141 ± 50	90 ± 22[c]	132 ± 33	122 ± 31	0.43	0.007	60–120
Serum albumin (gm/dl)	3.8 ± 0.3	3.9 ± 0.4	3.9 ± 0.4	3.9 ± 0.4	0.24	0.10	3.2–4.2
Serum intact parathyroid hormone (pmol/ml)	5.3 ± 3.2	2.8 ± 1.7[c]	5.2 ± 2.9	4.8 ± 2.7	0.57	0.04	0.1–0.6
Serum osteocalcin (ng/ml)	30.9 ± 14	12.5 ± 6.6[c]	31.9 ± 25	16.5 ± 13[c]	0.18	0.03	<13
Deoxypyridinoline (mg/g urinary creatinine)	74.9 ± 32	61.6 ± 39[c]	74.4 ± 49	63.7 ± 32	0.24	0.06	20–55
Urinary calcium (mmol/day)	6.9 ± 4.0	4.1 ± 2.4[c]	7.7 ± 6.8	4.4 ± 2.5[c]	0.19	0.28	<7.5

[a] Between groups at study entry.
[b] Between groups after 12 mo.
[c] Indicates a significant difference between time 0 and 12 mo (*$P < 0.05$; **$P < 0.01$).

Serum calcium values increased significantly in the control group ($P = 0.05$) and in group 1 ($P = 0.001$). There was a statistically significant difference between both groups at the end of the study ($P = 0.001$). Also, iPTH values decreased significantly in group 1 ($P = 0.003$) compared with the control group ($P = 0.09$). This significant increase in iPTH was matched with the increase in serum osteocalcin levels in group 1, as well as the control groups, as shown in Table 2, with a statistically significant difference between both groups ($P = 0.03$). Urinary deoxypyridinoline decreased, although not significantly, in both groups ($P = 0.06$).

BMD

Table 3 lists the values of BMD observed in renal transplant recipients after 1 yr and before the beginning of the study. At baseline, 35% of patients had BMD-defined osteopenia at the lumbar spine, 30% at the femoral neck of the femur, and 25% at the forearm, whereas a smaller number of patients had BMD-defined osteoporosis at these sites (10%, 15%, and 5% at the same sites, respectively). These differences were not statistically significant ($P = 0.52$).

After 1 yr of follow-up, BMD at lumbar spine increased by 2.1% in the treatment group, whereas it decreased by 3.2% in the control group ($P = 0.05$). At forearm, the improvement in BMD was also statistically significant in the treatment group compared with the control group ($P = 0.03$). At the neck femur, the same trend of improvement was also found in the treatment group compared with the control group ($P = 0.01$) (Table 3). Hypercalcemia occurred in 1 patient (5%) in the treatment group, which necessitated a reduction in the alfacalcidol dose. The drug was well tolerated, and no other clinical side effects were registered.

Discussion

The occurrence of bone loss is independent of the organ transplanted and has been frequently diagnosed after heart, lung, liver, bone marrow, and kidney transplantation (17). Rapid bone loss is primarily confined to the first year after the

Table 3. Bone mineral density (BMD) at baseline and at the end of the study

	Alfacalcidol (n = 20)		Control (n = 20)		*P* Value	
	Baseline	12 mo	Baseline	12 mo	P^a	P^b
BMD at lumber spine (g/cm^2)	1.1 ± 0.12	1.2 ± 0.11	1.1 ± 0.34	1.1 ± 0.30	0.07	0.44
BMD at lumber spine (SD below normal)	−1.3 ± 0.9	−1.1 ± 1.1[c]	−1.3 ± 1.4	−1.3 ± 0.5	0.18	0.003
BMD at lumber spine (% of normal BMD)	85.6 ± 9	87.7 ± 6	81.7 ± 8	78.1 ± 5[c]	0.77	0.05
BMD at femoral neck (g/cm^2)	0.94 ± 0.1	1.0 ± 0.02[c]	0.93 ± 0.1	0.91 ± 0.1	0.48	0.26
BMD at femoral neck (SD below normal)	−1.1 ± 1.0	−0.3 ± 0.2[c]	−0.9 ± 1.0	−1.1 ± 0.7	0.18	0.05
BMD at femoral neck (% of normal BMD)	87.3 ± 6	89.1 ± 2[c]	85.3 ± 9	81.5 ± 7[c]	0.36	0.01
BMD at forearm (g/cm^2)	0.7 ± 0.12	0.9 ± 0.14[c]	0.7 ± 0.12	0.7 ± 0.04	0.69	0.03
BMD at forearm (SD below normal)	−1.4 ± 0.8	−0.7 ± 0.1	−1.3 ± 1.0	−1.3 ± 0.4	0.45	0.001
BMD at forearm (% of normal BMD)	83.6 ± 9	86.8 ± 5	84.8 ± 9	85 ± 7	0.36	0.45

[a] Between groups at study entry.
[b] Between groups after 12 mo.
[c] Indicates a significant difference between time 0 and 12 mo (*$P < 0.05$; **$P < 0.01$).

initial transplantation, but this reduction of bone mass is more evident in the first 6 mo (2,3). The onset of bone loss has been attributed mainly to immunosuppression therapy and in particular to corticosteroids (2,3). However, despite the use of lower maintenance doses of corticosteroids, as a result of the use of CsA, a decrease of early posttransplantation bone loss has not been observe as a result of an imbalance in bone remodeling consistent with the toxic effects of steroids (3). In renal transplant recipients, posttransplantation bone loss is more complex (18). Pretransplantation renal osteodystrophy and persistent hyperparathyroidism are still risk factors. The diagnosis of persistent hyperparathyroidism is based on histomorphological data, but mainly on the presence of elevated serum iPTH levels that are found to be increased until 6 mo after transplantation and may persist in more than 50% of patients at 1 yr after renal transplantation (17).

Because osteoporosis remains a well known risk factor for bony fractures, and because corticosteroids and CsA could not always avoided in immunosuppression protocols, specific antiosteoperosis treatment is necessary to preserve or restore bone mass. Specific treatment modalities include supplemental calcium, antiresorptive drugs, and drugs that exhibit a direct effect on bone formation. However, the use of different methods of prevention has not been well defined.

After 1 yr of treatment with 1-hydroxycholecalciferol (alfacalcidol), there was a consistent improvement in BMD compared with the nontreated control group. These results are in agreement with many other studies that demonstrated substantial prevention of bone loss after transplantation (12,19–25). Moreover, patients in this group clearly had suppression of iPTH accompanied by increase of calcium level, which may be inconsistent with more suppression of persistent hyperparathyroidism, which is a known risk factor for bone loss in renal transplant recipients compared with other organ transplant recipients (25). The transplanted kidney begins to synthesize calcitriol, often within hours of transplantation, and this affects parathyroid activity, reducing parathyroid hormone (PTH) concentration substantially but never enough to reach normal levels, so even transplant recipients with best outcome in terms of renal function fail to manifest full suppression of PTH. Treatment with active vitamin D could result in suppression of PTH, with subsequent improvement of hypophosphatemia (12,26,27).

Patients in the alfacalcidol group tended to show a greater increase of BMD at different sites. In the appendicular skeleton, treatment with active vitamin D had less effect than on the axial skeleton, which, in keeping with the findings of others (21), may be related to fact that trabecular bone (predominantly present in lumbar spine) is more active and responds faster than cortical bone (mainly present in distal radius). Our results confirmed the significant positive effect of active vitamin D on bone loss reported in short-term studies of nontransplant steroid-treated patients (19), nonrenal transplant recipients (20), and in renal transplant recipients (28). De Sevaux *et al.* (28) could reduce bone loss in the lumbar spine and the trochanteric region and almost completely prevent bone loss in the femoral neck by means of treatment with 0.25 μg of 1α-hydroxy vitamin D and calcium for 6 mo for recent renal transplant recipients. The beneficial effects of active vitamin D could be partially explained in renal transplant recipients as a result of the unique presence of secondary hyperparathyroidism and renal osteodystrophy in these patients. A mild vitamin D deficiency might have contributed to the aggravation of secondary hyperparathyroidism (29).

Despite the possible benefits, it is unclear whether antiresorptive therapy reduces the number of fractures because the pathogenesis of bone disease in solid organ recipients differs from that in patients with typical osteoporosis (30). Most fractures in transplant patients occur in the appendicular skeleton, whereas the axial skeleton and trabecular bone sites are most commonly involved in individuals with osteoporosis. Moreover, because renal transplant recipients may suffer from an adynamic or low-turnover bone lesion, the addition of agents that suppress bone remodeling may not improve the mechanical integrity of bone. Thus, it seems unwise to administer these agents to patients after renal transplantation without proper knowledge of bone morphology.

The major limitation of our study is the relatively small number of patients. However, we intended to exclude women in our study to decrease the bias caused by gender distribution, and this exclusion avoided factors such as postmenopausal osteoporosis. Also, an important point in our study is that we initiated treatment as early as the first week after transplantation to exclude the very rapid loss soon after transplantation, so that the initial rapid bone loss after transplantation did not superimpose itself on the preventive treatment effect.

In conclusion, prevention of postrenal transplantation bone loss by early prophylactic treatment might be more effective than treatment of already manifest osteoporosis after kidney grafting as a result of the initial rapid loss of BMD early after transplantation. Use of alfacalcidol seems to be effective and safe in kidney transplant recipients.

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