Determinants of Reduced Bone Mineral Density and Increased Bone Turnover after Kidney Transplantation: Cross-sectional Study

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Aim. To analyze bone metabolism and the risk factors of bone loss in kidney transplant recipients.

Methods. The bone mineral density (BMD) of the lumbar spine, femoral neck, and radius was determined by dual-energy X-ray absorptiometry in 52 patients 8 days to 228 months after kidney transplantation. Total and bone alkaline phosphatase (BAP), osteocalcin, procollagen, type I collagen telopeptide, collagen cross links, calcium, intact parathyroid hormone (iPTH), and creatinine were measured in all patients.

Results. The BMD of the spine and femoral neck was reduced in 57%, and of the radius in 72% of the patients. Reduced BMD was associated with significantly increased levels of iPTH, osteocalcin, and procollagen. Dialysis duration negatively correlated with the radius BMD in all patients and the femoral neck BMD in women. No relationship between BMD and length of post-transplantation time, age, cumulative steroid dose, or serum creatinine level was established. All biochemical parameters negatively correlated with the spine BMD, but not with the BMD of the femoral neck and radius. The correlation between BAP and telopeptide and length of post-transplantation time was also negative. No difference in the incidence of osteopenia was found between genders.

Conclusion. Osteopenia/osteoporosis and increased bone turnover were present in more than a half of the kidney transplant recipients. Reduced BMD was associated with enhanced bone remodeling, primarily mediated by PTH hypersecretion. The length of post-transplantation period, cumulative steroid dose, gender, and age could not be identified as risk factors of reduced BMD.

Key words: absorptiometry; bone density; creatinine; gender role; kidney transplantation; osteocalcin; osteoporosis; parathyroid hormones; photon; transplantation, renal

End-stage renal failure and long-term dialysis treatment are well-recognized risk factors of developing mineral metabolism disorder and skeletal impairment, i.e., renal osteodystrophy (1,2). Major contributors to the development of this complex disorder are increased parathyroid secretion and decreased calcitriol synthesis (2-5). Successful kidney transplantation corrects many of the disturbances in calcium and phosphorus metabolism, and restores the production of biologically active vitamin D. Nonetheless, bone disorder may persist from the pre-transplantation period. Reduced BMD was associated with enhanced bone remodeling, primarily mediated by PTH hypersecretion. The length of post-transplantation period, cumulative steroid dose, gender, and age could not be identified as risk factors of reduced BMD.

Many studies have been conducted with the aim of better understanding of different factors influencing bone mass and mineral metabolism. So far, steroids, both genders, dialysis duration, age, and hyperparathyroidism (15,18,19) have been recognized as having the most adverse effects upon the skeleton. Follow-up studies reported short-term variations of biochemical markers of bone metabolism within the first two post-transplantation years (20). However, this follow-up period may have been too short, as a resolution of hyperparathyroidism can take many years (2) and high bone turnover rate is directly related to the...
increased secretion of parathyroid hormone. The cross-sectional studies on patients at different times after transplantation and/or dialysis offer grounds for assumptions on the dynamics of bone metabolism. Both longitudinal and cross-sectional studies on transplant recipients have shown advantages and disadvantages, which should be taken into account when evaluating the results.

We analyzed bone metabolism in 52 kidney transplant recipients using dual-energy X-ray absorptiometry and biochemical markers of bone metabolism. The relevance of recognized risk factors was evaluated according to the bone status.

Methods

Patients

Fifty-two patients (29 men, 23 women) with stable kidney graft function were included in this cross-sectional study. Average age (mean±SD) was 45.3±10.0 years, range 21-64 years. Twelve women had been in postmenopause for one year or more. Dialysis duration before transplantation was 62.6±49.7 months (range 2-216 months) and the length of post-transplantation period was 47.0±46.7 months (range 8 days to 228 months). No difference was found between men and women (t-test) in age, the length of post-transplantation period, or duration of dialysis treatment. Mean serum creatinine at the time of the study was 124.3±31.9 mmol/L (range 68-199 mmol/L), which indicated a satisfactory graft function. For patients who had a rejection crisis, at least 3 months had lapsed before they were included in the study. Immunosuppressive therapy consisted of cyclosporine A, azathioprine, and prednisone in 37 patients, and of cyclosporine A and prednisone in 15 patients. Cumulative steroid dose was calculated and included in the statistical analysis (14,276±9,474 mg; range 470-34,962 mg). None of the patients received calcitriol or other treatment for osteopenia.

Biochemical Measurements

The serum for measuring biochemical parameters was obtained from the blood of the patients, after overnight fasting. If assays were not performed on the same day, serum was frozen (-20°C), stored, and analyzed later. Markers of bone formation, i.e., products of osteoblast activity, such as bone alkaline phosphatase, osteocalcin, and procollagen (C-terminal propeptide type I procollagen) were measured, as well as telopeptid (C-terminal telopeptide type I collagen) and collagen cross-links (free deoxypyridinoline, free pyridinium cross-links), as products of the bone collagen breakdown. Serum levels of the following biochemical parameters were also determined (reference range): total calcium (2.14-2.53 mmol/L) by standard method, intact parathyroid hormone (iPTH), osteocalcin, and telopeptide levels were measured, as above the reference range in approximately 60% of the patients (Table 1). Other parameters were increased to a lesser extent.

Analysis of biochemical parameters in patients with kidney transplant showed that patients with reduced bone mineral density had significantly higher iPTH, osteocalcin, and procollagen than those with normal bone mineral density (Table 2). No difference between patients with reduced or normal bone mineral density was found with respect to age, post-transplantation period, dialysis duration, serum creatinine, or received cumulative dose of steroids.

There was no correlation between the length of post-transplantation period and bone mineral density or T-scores at any of the three densitometry sites. Moreover, the number of patients with reduced bone mineral density was the same before and after 12 post-transplantation months, as shown by chi-square test (Table 3). This is in agreement with the result that showed the lack of correlation between bone mineral density and the length of post-transplantation period.
There was a significant negative correlation between the dialysis duration and the radius bone mineral density and T-score in all patients (r=-0.58, p<0.01, n=29, and r=-0.56, p<0.01, n=29, respectively), but only in women the dialysis duration correlated with the femoral neck bone mineral density (r=-0.44, p<0.05, n=23). There was no statistically significant correlation between bone mineral density and age, cumulative steroid dose, or serum creatinine.

Correlations between biochemical parameters and bone mineral density were found negative (Table 4). All parameters were statistically significant for the L1-L4 site, but not for the femoral neck and the radius site.

Correlations were statistically significant and negative between post-transplantation period and both bone alkaline phosphatase (r=-0.39, p<0.01, n=41) and telopeptide (r=-0.39, p<0.05, n=23).

The expected difference in bone mineral density between genders, as tested by t-test, was statistically significant at all three sites (Table 5). But, as no overall difference in T-scores existed, women did not have significantly lower T-scores. However, the effect of the menopause was observed. Half of the women were postmenopausal and had significantly lower bone mineral density and T-score at the L1-L4 and the femoral neck than premenopausal women (Table 6). There was no difference in the radius bone mineral density and T-score.

Table 2. Biochemical parameters (mean±SD) in kidney transplant recipients with normal and reduced bone mineral density (BMD)

<table>
<thead>
<tr>
<th>Variable (unit)</th>
<th>Finding in patients with normal BMD (T&gt;-1)b</th>
<th>Finding in patients with reduced BMD (T&lt;-1)a</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact PTH</td>
<td>7.2±6.3 (21)b</td>
<td>17.2±20.7 (27)</td>
<td>0.039</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>16.5±9.9 (18)</td>
<td>31.9±21.5 (16)</td>
<td>0.010</td>
</tr>
<tr>
<td>Procollagen</td>
<td>89.9±22.7 (18)</td>
<td>125.6±43.3 (25)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

aPercent of patients whose findings were above the reference range.
bNumber of patients.

Discussion

In this study, reduced bone mineral density was found in more than half of the patients, i.e., in 57% at the spine and the femoral neck, and in 72% at the distal radius. Our results are in agreement with other reports that also stressed the cortical bone loss in the radius as a predominant one (4,8,21,22). Parry et al (25) reported higher incidence of osteopenia/osteoporosis in the femoral neck, which contains more cortical bone than the spine. Excess of PTH, which often occurs during dialysis treatment of chronic kidney failure and in a considerable number of patients after kidney transplantation, is responsible for this salient cortical bone loss (3,4,19).

Bone turnover was increased in 60% of patients included in this study, as assessed indirectly by biochemical markers of bone metabolism: intact parathyroid hormone, osteocalcin, and telopeptide. These three parameters are indicators of bone tissue activity (remodeling, formation, and resorption) and an evidence of high bone turnover rates. Increased levels of osteocalcin were also reported in 45% of pancreas-kidney transplant recipients, in whom they again correlated with iPTH (8). In our previous study on bone markers in kidney transplant recipients, we reported positive relationships between iPTH and biochemical markers of bone formation and resorption (24,25).

Patients with reduced bone mineral density had significantly higher levels of iPTH, osteocalcin, and procollagen when compared with those with normal bone mineral density. Other factors, such as age, the length of post-transplantation period, dialysis duration, serum creatinine, or the cumulative dose of received steroids, were ruled out as possible causes. Also, the lower the bone mineral density, the more active was bone remodeling. This calls attention to a subgroup of kidney transplant recipients who lose bone at high rate and are at particular risk of fracture (19). Parathyroid hormone most probably played a significant role in the accelerated bone loss (19,21),
since its level was increased in the osteopenic/osteoporotic patients and negatively correlated with bone density at all three bone sites. Carlini et al (26) also reported on an inverse relationship between parathyroid hormone level and the spinal bone mineral density.

The fact that all measured biochemical parameters were negatively correlated with the spinal bone mineral density, only some of them with the femoral neck bone mineral density, and iPTH and bone alkaline phosphatase with the radius, indicated that the observed bone mineral density variance in the skeletal parts containing more trabecular bone was better accounted for by biochemical parameters, i.e., the increased bone turnover. This means that factors other than parathyroid hormone influenced the bone turnover and bone mineral density. The cumulative steroid dose could not be identified as one of them in this particular study group, as was also shown by Smets (8) for kidney-pancreas transplantation patients.

Despite ample evidence that bone loss continues after transplantation, most rapidly in the first post-transplant year and thereafter it slows down (4,12,13), the decline in bone mineral density over post-transplantation time could not be proven by all investigators (8). We found no change in bone mineral density, T-scores, or incidence of reduced bone mineral density over post-transplantation time. This could have been a consequence and one of the drawbacks of a cross-sectional study, but employing T-scores and frequencies of osteopenia/osteoporosis in the analysis might have partly counteracted this if there had been a genuine trend of deterioration of bone mineral density over post-transplantation time.

Slowing down of the bone turnover rate after transplantation was indicated by negative correlations of bone alkaline phosphatase and telopeptide over post-transplantation time. Return of iPTH to normal levels or healing of renal osteodystrophy was reported to take many months, even years (4 and more) (2,15,26). Normalization of parathyroid secretion and, consequently, of bone remodeling could be responsible for prevention of further deterioration of the bone density (bone mineral density and T-scores) over time post-transplantation.

Among factors influencing the skeletal status is the duration of dialysis treatment prior to transplantation (10,25). Its deleterious effect was associated with the radius bone mineral density and T-score in both genders and the femoral neck bone mineral density in women only. It can be assumed that the “dialysis factor” comprises the function of time, i.e., duration of chronic kidney failure, and parathyroid hypersecretion with its consequences upon bone tissue.

Women had lower bone mineral density than men at all the three measured sites but were not more affected by bone loss, since the genders did not differ regarding T-scores. Julian et al (12) found no difference between men and women in bone loss at the spine and radius site, but Nisbeth et al (9) reported more fractures in women. In contrast, Cueto-Manzano et al (10) reported that male gender

### Table 4. Correlations between the bone mineral density (spine, femoral neck, and radius) and biochemical parameters in kidney transplant recipients

<table>
<thead>
<tr>
<th>Variable</th>
<th>L1-L4</th>
<th>Femoral neck</th>
<th>Radius</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>n</td>
</tr>
<tr>
<td>Intact parathyroid hormone</td>
<td>-0.34</td>
<td>0.013</td>
<td>48</td>
</tr>
<tr>
<td>Total alkaline phosphatase</td>
<td>-0.30</td>
<td>0.037</td>
<td>50</td>
</tr>
<tr>
<td>Bone alkaline phosphatase</td>
<td>-0.32</td>
<td>0.037</td>
<td>42</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>-0.40</td>
<td>0.019</td>
<td>34</td>
</tr>
<tr>
<td>Procollagen</td>
<td>-0.40</td>
<td>0.009</td>
<td>43</td>
</tr>
<tr>
<td>Telopeptide</td>
<td>-0.42</td>
<td>0.030</td>
<td>26</td>
</tr>
<tr>
<td>Deoxypyridinoline</td>
<td>-0.41</td>
<td>0.031</td>
<td>27</td>
</tr>
<tr>
<td>Pyridinium cross-links</td>
<td>-0.44</td>
<td>0.021</td>
<td>27</td>
</tr>
<tr>
<td>Total calcium</td>
<td>-0.32</td>
<td>0.021</td>
<td>51</td>
</tr>
</tbody>
</table>

*aOnly statistically significant correlations between pairs of variables are presented.

### Table 5. Bone mineral density (BMD) and corresponding T-scores for the spine, femoral neck, and radius in men and women with a functional kidney graft (mean±SD, t-test)

<table>
<thead>
<tr>
<th>Measured site</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1-L4 BMD</td>
<td>1.06±0.20</td>
<td>0.93±0.19</td>
</tr>
<tr>
<td>L1-L4 T-score</td>
<td>-0.99±1.15</td>
<td>-1.64±1.86</td>
</tr>
<tr>
<td>Femoral neck BMD</td>
<td>0.92±0.15</td>
<td>0.76±0.19</td>
</tr>
<tr>
<td>Femoral neck T-score</td>
<td>-0.92±1.56</td>
<td>-1.66±1.58</td>
</tr>
<tr>
<td>Radius BMD</td>
<td>0.66±0.08</td>
<td>0.56±0.09</td>
</tr>
<tr>
<td>Radius T-score</td>
<td>-1.80±1.02</td>
<td>-1.76±1.65</td>
</tr>
</tbody>
</table>

*aMen – n=29 for femoral and L1-L4 values, n=19 for radius values; women – n=23 for femoral and L1-L4 values, n=10 for radius values.

### Table 6. Bone mineral density (BMD) and corresponding T-scores for the spine, femoral neck, and radius in premenopausal (n=11) and postmenopausal (n=12) women with a functional kidney graft (mean±SD, t-test)

<table>
<thead>
<tr>
<th>Measured site</th>
<th>Findings in premenopause</th>
<th>Findings in postmenopause</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1-L4 BMD</td>
<td>41.02±0.17</td>
<td>0.84±0.17</td>
</tr>
<tr>
<td>L1-L4 T-score</td>
<td>-0.94±1.80</td>
<td>-2.31±1.00</td>
</tr>
<tr>
<td>Femoral neck BMD</td>
<td>0.86±0.17</td>
<td>0.67±0.17</td>
</tr>
<tr>
<td>Femoral neck T-score</td>
<td>-0.71±1.62</td>
<td>-2.49±1.60</td>
</tr>
</tbody>
</table>

*aMen – n=29 for femoral and L1-L4 values, n=19 for radius values; women – n=23 for femoral and L1-L4 values, n=10 for radius values.
was among the strongest predictive factors for low bone mass. Menopause also showed an adverse influence upon the female skeleton, with lower bone mineral density and T-scores for the spine and the femoral neck in postmenopausal women.

In conclusion, the length of post-transplantation period, cumulative steroid dose, gender, and age could not be identified as risk factors for reduced bone mineral density in kidney transplant recipients. But the lower bone mineral density was associated with enhanced bone remodeling, primarily mediated by PTH hypersecretion.

References
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