Osteoporosis Int (1996) 6:213–218

Osteoporosis International

Original Article

Bone Loss after Liver Transplantation is not Prevented by Cyclical Etidronate, Calcium and Alphacalcidol

S. C. Riemens[1], A. Oostdijk[2], J. J. van Doormaal[3], C. J. P. Thijn[4], G. Drent[1], D. A. Piers[2], E. W. J. Groen[3], L. Meerman[1], M. J. H. Slooff[5] and E. B. Haagsma[1] on behalf of the Liver Transplant Group, Groningen

Departments of [1]Gastroenterology and Hepatology, [2]Nuclear Medicine, [3]Endocrinology, [4]Radiology and [5]Surgery, University Hospital. Groningen, The Netherlands

Abstract. After orthotopic liver transplantation (OLT) bone mass rapidly declines and vertebral fracture rate increases. We studied bone loss and parameters of bone turnover in 53 consecutive patients. In an attempt to reduce bone loss the patients were prophylactically treated with cyclical etidronate in addition to daily 1α-hydroxyvitamin D_3 and calcium. During the first 3 months after transplantation median lumbar spinal bone mineral density (BMD) decreased 4.5%; subsequently no significant changes occurred. Median hip BMD continued to fall during the first post-transplantation year and deteriorated 7% over the whole study period. New vertebral fractures were seen in 25% of the patients, which is not lower than previously reported rates in patients not receiving cyclical etidronate. Parathyroid hormone levels increased after OLT ($p = 0.01$), but remained within normal ranges. Urinary hydroxyproline levels were increased and normalized in the second half-year after OLT. Elevated fasting calciuria increased further after OLT. 1,25-Dihydroxyvitamin D_3 levels were lowered pre-OLT (25 vs 66 pmol/l, $p<0.001$) and normalized at 3 months after OLT. Serum osteocalcin concentrations remained unchanged and were reduced compared with levels in healthy controls. In summary, increased bone resorption occurs after OLT with persistent decreased bone formation, leading to vertebral fracture in 25% of patients. Etidronate, 1α-calcidol and calcium treatment did not prevent bone loss.

Correspondence and offprint requests to: S. C. Riemens, MD, Department of Gastroenterology and Hepatology, University Hospital, Hanzeplein 1, NL-9700 RB Groningen, The Netherlands. Fax: +31–50–613474.

Keywords: Bisphosphonates; Bone density; Liver transplantation; Osteoporosis

Introduction

Chronic liver disease predisposes to bone disease. A high prevalence of low-turnover osteoporosis has been reported in liver patients by dynamic skeletal histomorphometry, while osteomalacia is rarely found [1–6]. Recently Hodgson et al. [7] reported in patients with primary biliary cirrhosis that bone turnover tends to increase as the liver disease progressively worsens. After liver transplantation bone mass rapidly declines, despite preventive use of calcium and vitamin D supplements, leading to vertebral compression fractures in one third of patients, mainly in the first post-transplantation year [8–11].

To gain greater insight into the mechanisms of bone loss in these patients we studied bone density, fracture rate and markers of bone turnover in a consecutive series of 53 adult liver transplant patients. In an attempt to reduce bone loss we added cyclical etidronate to the preventive regimen of calcium and vitamin D.

Patients and Methods

Patients and Study Design

Between July 1990 and May 1993 64 consecutive patients with various chronic liver diseases scheduled for orthotopic liver transplantation (OLT) were

Table 1. Patient characteristics before liver transplantation (n=53)

Age (years) at transplantation: median (range)	46 (23–61)	
Females/Males	33/20	
Liver diseases		
Cholestatic liver diseases	28 (53%)	
Primary biliary cirrhosis		13
Primary sclerosing cholangitis		11
Secondary biliary cirrhosis		1
Caroli's disease		2
Familial biliary cirrhosis		1
Chronic active hepatitis	4 (8%)	
Miscellaneous liver diseases	21 (39%)	
Cryptogenic cirrhosis		5
Cirrhosis due to chronic hepatitis B		6
Alcoholic liver disease		4
Alpha-1 antitrypsin deficiency		2
Cirrhosis due to chronic hepatitis C		2
Other		2

enrolled. Bone mineral density measurements, radiographs and biochemical measurements of markers of bone turnover were obtained shortly before listing for transplantation (<1 month) and at 3, 6 and 12 months after transplantation. After exclusion of 10 patients who died within 3 months after transplantation and 1 patient who refused the protocol 6 months after OLT, the data on 53 patients with a survival time of more than 12 months could be analyzed.

Preoperative patient characteristics are listed in Table 1. Median age was 46 years (range 23–61 years). Median interval between listing for transplantation and OLT was 4 months (range 0–20 months). Median hospital stay after OLT was 8 weeks (range 4–20 weeks). Retransplantation was necessary in 7 patients: in 3 because of hepatic artery thrombosis in the first weeks after OLT and in 4 because of either chronic rejection (3 patients) or recurrent bacterial cholangitis (1 patient) in the second half of the first post-transplantation year. Data on the patients who needed a retransplantation were included in the final analyses.

Drug Therapy

Immunosuppressive therapy consisted of prednisolone, azathioprine and cyclosporine A. Prednisolone was started at 200 mg for 3 days, followed by 100 mg for the next 5 days and gradually reduced. The median daily doses of prednisolone after OLT (with ranges in parentheses) at 3, 6, 9 and 12 months were 20 mg (15–40 mg), 18 mg (14–30 mg), 16 mg (10–40 mg) and 14 mg (10–24 mg) respectively. The dose of azathioprine was 125–150 mg/day from day 1 onward. Cyclosporine A was started intravenously from day 2, or later, when the creatinine clearance was over 50 ml/min. Cyclosporine A trough blood levels in the first 4 weeks, as measured by a high-pressure liquid chromatography method, were 200–250 ng/ml and 100–150 ng/ml thereafter. Episodes of biopsy-proven rejection with clinical symptoms were treated in 9 patients (17%). Treatment consisted of methylprednisolone 1 g daily for 3 days and subsequently OKT3 monoclonal antibody therapy in 1 patient or ATG therapy in 1 patient if success was not achieved.

In an attempt to prevent bone loss all patients received 1 μg 1α-hydroxycholecalciferol daily, 1 g calcium daily and cyclical etidronate (400 mg daily for 14 days, followed by 13 weeks without etidronate) from the day they were listed for OLT.

Bone Mass Measurements

Bone mineral density (BMD) of lumbar spine (L1–4) and of proximal femur was measured by dual-energy X-ray absorptiometry using a Hologic QDR 1000 apparatus. The coefficient of variation in our laboratory is 1.0% for the spine and 1.6% for the hip. BMD's of the lumbar spine and proximal femur (femoral neck, greater trochanter and Ward's triangle) of the patients were compared with mean BMDs in a large population database from age- and sex-matched controls (Hologic Corp.) and a Z-score (standard deviations from the mean) was calculated.

Radiographic Assessment

Anteroposterior and lateral radigraphs of the thoracic and lumbar spine and radiographs of the hips were obtained. Spine radiographs were assessed for the presence of either impression of the upper and lower bone plate of the vertebral body (fish vertebra) or in addition wedge-shaped deformity of the vertebral body by compression fracture. Furthermore, the radiological presence of avascular necrosis of the hips was noted. The radiographic assessments were done by an experienced radiologist (C.J.P.T) who was unaware of the patient's status.

Biochemical Studies

Osteocalcin was employed as a marker of bone formation. Parathyroid hormone, urinary hydroxyproline/creatinine ratio and urinary calcium/creatinine were used as parameters of bone resorption.

Blood drawn for osteocalcin and parathyroid hormone measurements was immediately stored on ice, until centrifuged and stored at −20 °C. Serum osteocalcin was measured by radioimmunoassay (RIA; Incstar Corp., reference range 1.9–7.1 μg/l) within 1 week after samples were taken. Serum concentrations of intact parathyroid hormone were measured by an immunoradiometric assay (IRMA; Incstar Corp., reference range 0–5.8 pmol/l). Serum 1,25-dihydroxyvitamin D_3 was measured by a competitive protein binding assay (CPB; Incstar Corp., reference range 0.020–0.112 nmol/l). After an overnight fast a sample of urine was collected. According to protocol there had been no

calcium intake the night before. Urinary calcium, measured by standard procedure, and hydroxyproline levels measured by high-performance liquid chromatography as described by Hughes et al. [12], were expressed relative to urinary creatinine levels (reference range 0.008–0.302 mmol calcium/mmol creatinine [13] and 2.8–29.6 μmol hydroxyproline/mmol creatinine).

Reference ranges (mean ± 2 SD) were obtained from healthy controls (men and women aged 20–60 years) with the exception of the fasting calciuria range, which was obtained from the literature.

Statistical Analysis

Because of skewed data, medians are presented; the Wilcoxon rank-sum test was used for paired comparisons and Friedman's two-way analysis of variance for multiple comparisons. Multiple regression analysis was performed to evaluate the dependency of the amount of bone loss at the lumbar spine and hip in the first 3 months after OLT on the duration of the hospital stay and the time elapsed between the first bone density measurements and OLT. Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS Inc.). A two-tailed p value of less than 0.05 was considered to indicate statistical significance.

Results

Bone Densitometry

Z-scores of the lumbar spine and hips were negative before transplantation in 79% and 77% of the patients respectively. Median lumbar spinal BMD decreased sharply at a rate of 18% per year during the first 3 months after transplantation ($p = 0.01$); subsequently no significant changes occurred (Fig. 1). Median BMD of the lumbar spine deteriorated by 6% over the first year after transplantation. Median BMD of the hip decreased in the first 3 months after transplantation ($p = 0.006$) and continued to fall subsequently (Fig. 2).

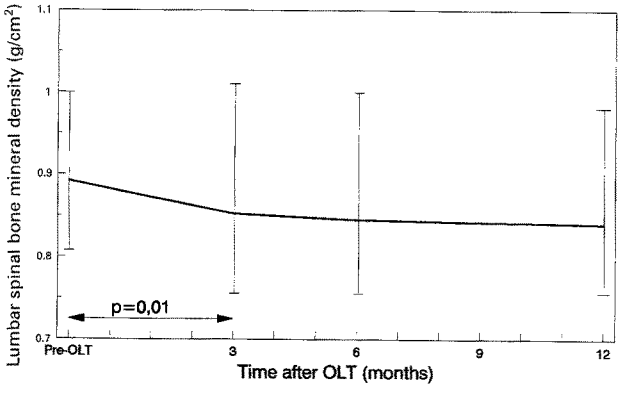

Fig. 1. Median ± interquartile (50%) range of lumbar spinal BMD before and during the first year after transplantation in patients treated with etidronate, 1α-hydroxyvitamin D_3 and calcium.

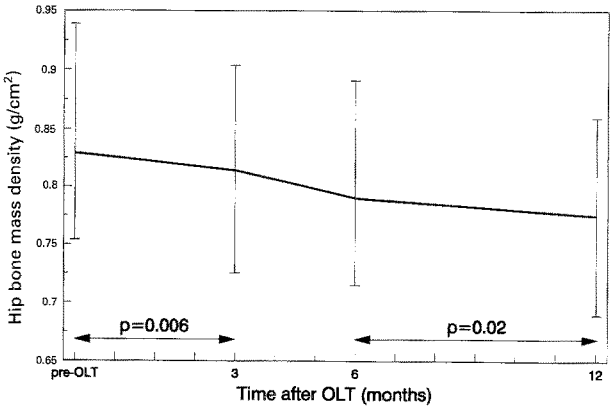

Fig. 2. Median ± interquartile range of hip BMD before and during the first year after transplantation in patients treated with etidronate, 1α-hydroxyvitamin D_3 and calcium.

Though median BMD of the hip fell by 7% in the first post-transplantation year, hip fractures did not occur.

The extent of BMD loss at the lumbar spine and hip during the first 3 months after OLT did not show any relationship with the duration of hospitalization after OLT or with the time elapsed between the first bone density measurements and OLT, but correlated significantly with parathyroid hormone levels at 3 months ($r = 0.38$ and $r = 0.41$, $p = 0.01$). There was no significant correlation with calciuria, urinary hydroxyproline excretion or osteocalcin levels.

Radiology

Prior to transplantation vertebral fractures were present in 3 patients and atraumatic rib fractures were detected on chest radiographs in 4 patients. Thirteen of the 53 patients sustained new vertebral fractures in the first year, representing an incidence of 25%. Pretransplantation risk factors for fractures were male sex [9 of 20 males (45%) sustained fractures compared with 4 of 33 females (12%)] and increased age, with concomitantly lower Z-scores of the spine.

In 6 of the 13 patients the fractures occurred in the first 6 months after OLT. In 4 patients fractures were detected in the first 3 months after transplantation and in 2 patients between the third and six months.

In patients requiring retransplantation, 3 of 7 (43%) developed vertebral fractures. Two of these 3 patients had chronic rejection and 1 suffered from recurrent bacterial cholangitis. In contrast early retransplantation with subsequently good liver function was not associated with vertebral fractures.

The fracture threshold is defined as the BMD below which 90% of osteoporotic fractures occur [14]. When vertebral fractures were detected on the radiographs, BMD was below the fracture threshold of 0.965 g/cm^2 in 10 of the 13 patients (79%).

None of our patients developed vascular necrosis of one or both hips during the study period.

Biochemistry

Parathyroid hormone concentrations increased during the study period ($p = 0.01$) but remained within normal ranges for most patients both before and after OLT.

Before transplantation and the first 3 months after OLT urinary hydroxyproline excretion was significantly elevated (Fig. 3a). Excretion decreased to normal values in the second half-year after OLT ($p<0.001$).

Median fasting calciuria was elevated before OLT and increased significantly after OLT (pre-OLT 0.28, at 3 months after OLT 0.39 mmol/mmol creatinine; $p = 0.03$). In the second half of the first post-transplantation year fasting calciuria diminished significantly but remained elevated (at 6 months after OLT 0.38, at 12 months after OLT 0.33 mmol/mmol creatinine; $p = 0.02$) (Fig. 3b).

The pretransplantation total 1,25-dihydroxyvitamin D_3 concentrations were significantly lower than in healthy controls (25 pM vs 66 pM; $p<0.001$). Median concentrations in 18 patient who were already using vitamin D supplementation prior to the start of the etidronate protocol were not higher than in those without supplementation (21 pmol/l vs 29 pmol/1; NS). Three months after OLT concentrations of 1,25-dihydroxyvitamin D_3 were normalized and did not change during the rest of the study period (Fig. 3c).

Pretransplantation mean osteocalcin levels were significantly ($p<0.01$) reduced compared with levels in healthy controls (95% confidence interval for the mean difference: 0.38–1.64 μmol/l). Osteocalcin levels did not change significantly during the study period ($p = 0.64$) and remained reduced compared with levels in healthy controls.

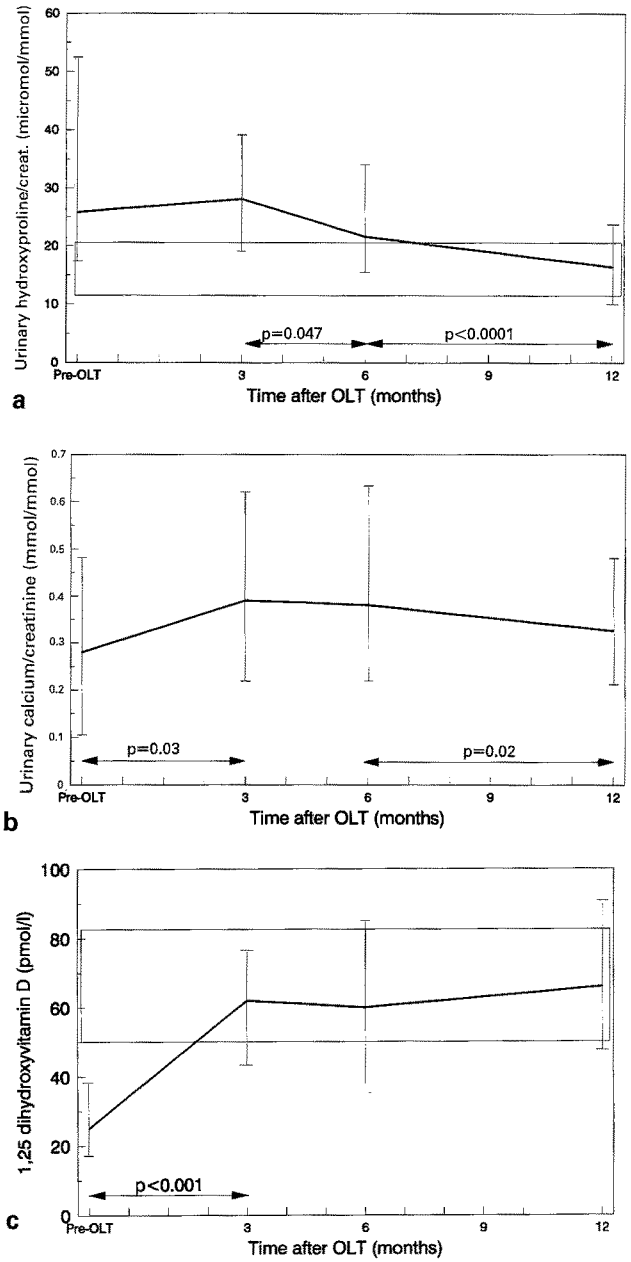

Fig. 3. a Median ± interquartile range of urinary hydroxyproline/creatinine excretion before and during the first year after transplantation in patients treated with etidronate, 1α-hydroxyvitamin D_3 and calcium. The *rectangle* indicates the interquartile range of urinary hydroxyproline/creatinine excretion in healthy controls. **b** Median ± interquartile range of calciuria before and during the first year after transplantation in patients treated with etidronate, 1α-hydroxyvitamin D_3 and calcium. **c** Median ± interquartile range of 1,25-dihydroxyvitamin D_3 before and during the first year after transplantation in patients treated with etidronate, 1α-hydroxyvitamin D_3 and calcium. The *rectangle* indicates the interquartile range of 1.23-dihydroxyvitamin D_3 in healthy controls.

Discussions

Hepatic osteodystrophy is a complication of chronic liver disease. After OLT bone disease may deteriorate leading to the occurrence of vertebral fractures. A 15- to 30-fold increase in the rate of bone mineral loss per year in the early post-transplant period has been described [9,11,15].

We found that median vertebral BMD decreased significantly at a rate of 18% per year during the first 3 months after transplantation. Subsequently no significant changes occurred (Fig. 1). Median hip BMD decreased more slowly but continued to fall during the first post-transplantation year and deteriorated 7% (Fig. 2). In the first year after transplantation 25% of patients sustained vertebral fractures. After the initial reports stating that intermittent cyclical therapy with etidronate given to postmenopausal females with osteoporosis significantly increased spinal bone mass and reduced the incidence of new vertebral fractures [16,17], we treated our patients with a regimen of cyclical etidronate, vitamin D and calcium. However, this regimen does not seem more effective than previous regimens with only calcium and vitamin D supplements, since BMD loss and fracture rate were the same as reported in the literature [8–11,15].

Changes in bone resorption and bone formation were studied non-invasively by measuring biochemical parameters of bone turnover. Pretransplantation parathyroid hormone concentrations were normal as

described by Diamond et al. [6], and after OLT parathyroid hormone concentrations increased slightly ($p = 0.01$) but remained within normal ranges. These data agree with the findings of Arnold et al. [10]. The reasons for this increase may be a glucocorticosteroid-induced decrease in intestinal calcium absorption and/or glucocorticosteroid-induced calciuria.

Urinary hydroxyproline excretion in the fasting state, reflecting bone resorption, was increased before transplantation as has also been described by Diamond et al. [6]. Hydroxyproline excretion decreased significantly only at 6 months after transplantation.

Post-transplantation fasting calciuria was significantly higher than pretransplantation values ($p = 0.03$). This may be due to increased skeletal mobilization of calcium by increased osteoclastic activity and glucocorticosteroid use, which can increase urinary calcium excretion [18,19].

Pretransplantation total 1,25-dihydroxyvitamin D_3 concentrations were reduced. This may be due to a reduction in carrier proteins, such as albumin and vitamin D binding protein, and possibly a reduction in hepatic 25-hydroxylase. Free 1,25-dihydroxyvitamin D_3 has been reported to be normal in subjects with liver disease, although inexplicably low values may be found [20,21]. After administration of 1α-calcidol normalization of 1,25-dihydroxyvitamin D_3 was measured at 3 months after OLT. Rabinovitz et al. [22] reported a statistically significant reduction in 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 30 days following liver transplantation and suggested that the reduction was probably a result of the glucocorticosteroid therapy used postopertively. We cannot confirm this observation because 1,25-dihydroxyvitamin D_3 levels were measured by us only at 3 months.

Osteocalcin, or bone Gla-protein, is a non-collagenous bone protein synthesized by osteoblasts. Osteocalcin levels were measured within 1 week after blood samples had been taken, since osteocalcin probably requires long-term storage at −70 °C to prevent degradation and we stored it at −20 °C. Serum osteocalcin levels reflect bone formation rates [5,23]; they were significantly reduced before OLT, consistent with earlier studies in patients with chronic liver diseases [6,24], and did not change over the study period. This is in contrast to the findings of Watson et al. [24] and McDonald et al. [15] who reported an increase in the first year after transplantation. Differences in dosages of immunosuppressive drug regimens may be responsible for these conflicting findings; we used lower dosages of cyclosporin and higher dosages of corticosteroids. The role of osteocalcin as a reliable marker of bone formation in liver transplant recipients, at least in the early postoperative period, has been questioned recently [22] due to the opposite pharmacological effects of prednisolone and cyclosporin on bone remodelling. Cyclosporin may protect bone formation in the presence of lower doses of prednisolone, but at higher doses the intrinsic suppressive effect of glucocorticoids on bone formation becomes dominant [25]. Furthermore, calcitriol has a stimulating effect on osteocalcin gene expression and synthesis in osteoblasts and opposes the suppressive effects of glucocorticosteroids [26]. Despite normalizing 1,25-dihydroxyvitamin D_3 concentrations after OLT in the presence of declining glucocorticosteroid dosages and low dosages of cyclosporin, osteocalcin levels did not rise.

We conclude that increased bone resorption occurs after liver transplantation with persistent decreased bone formation, leading to a deterioration in both spinal and hip bone mineral density and spinal fractures in 25% of our patients. Cyclical etidronate in addition to daily 1α-hydroxyvitamin D_3 and calcium did not prevent post-transplantation osteopathy. Prevention of bone disease before OLT and immunosuppressive regimens with reduced glucocorticosteroid dosages and more powerful pharmacological interventions are needed to preserve bone and minimize fractures.

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Received for publication 10 June 1995
Accepted in revised form 4 December 1995