Nephrol Dial Transplant (2001) 16: 1245–1250

Nephrology Dialysis Transplantation

Original Article

Association of sex hormone status with the bone loss of renal transplant patients

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Abstract

Background. Bone loss is an important problem in renal transplantation recipients. The role of sex hormones in this setting has not been previously addressed. The objective was to investigate whether sex hormone status is associated with bone mass loss in renal transplant recipients.

Methods. Thirty patients (16 men and 14 women, of which eight were post-menopausal) were studied by bone densitometry and bone biopsy. In women, serum oestradiol levels and menopausal status were determined; in men, serum testosterone levels were assessed.

Results. Mean age was 48 ± 11 years. Time on dialysis was 13 ± 17 months, and time since transplantation was 125 ± 67 months. Thirteen patients were on cyclosporine A (CsA) monotherapy, 12 on azathioprine plus prednisolone (PRED) dual therapy, and five on CsA, azathioprine and PRED triple therapy. In men, serum testosterone levels were 19.7 ± 6.8 nmol/l (mean $\pm$ SD). In pre-menopausal women, oestradiol serum levels were 209(128–289) pmol/l (median (percentiles 25–75%)), and in post-menopausal women 93(54–299) pmol/l (non-significant). Univariate analysis in women demonstrated that serum oestradiol levels were positively correlated with Z scores of osteoblast surface ($r = 0.70$, $P = 0.005$), osteoid surface ($r = 0.75$, $P = 0.002$) and trabecular wall thickness ($r = 0.68$, $P = 0.008$). In men, a weak correlation was seen between serum testosterone levels and the cumulative dose of PRED ($r = -0.52$, $P = 0.06$). In the multivariate analysis, two models of multiple regression were employed (one for women and one for men), considering the densitometric and histomorphometric variables (Z scores) as dependent variables. Serum testosterone in men did not predict any of the densitometric nor histomorphometric variables analysed, while serum oestradiol in women was an independent predictor for the osteoblast surface ($r = 0.81$, $P = 0.003$), osteoid surface ($r = 0.82$, $P = 0.009$) and trabecular wall thickness ($r = 0.54$, $P = 0.05$).

Conclusions. In female renal transplant recipients, serum oestradiol levels independently predict the bone status, while in men, factors other than testosterone seem to influence bone loss. Our results give rise to the hypothesis that sex hormone replacement therapy may play a role in prevention and/or treatment of the bone loss in women following renal transplantation.

Keywords: bone densitometry; bone histomorphometry; bone loss; oestradiol; renal transplantation; testosterone

Introduction

Kidney transplantation improves the metabolic environment by restoring glomerular filtration and renal production of 1,25-dihydroxyvitamin D_3. However, this alone may not correct deranged bone metabolism and many graft recipients will develop striking bone loss [1,2]. Several factors may influence this during the post-transplantation period. We have recently shown that male gender, time after transplantation, old age and time on dialysis prior to transplantation are significant predictive factors for a negative effect on bone mass [2].

In normal conditions, both androgens and estrogens are critical for bone formation, mineralization and maturation [3]. It is generally accepted that the sexual dysfunction occurring in end-stage renal disease, and persisting during dialysis, usually improves after successful renal transplantation. However, some authors have reported an incomplete recovery of the

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hypothalamic–hypophyseal–gonadal axis after renal transplantation [4–6]. This hormonal derangement may be secondary to the use of oral glucocorticoid and cyclosporine A (CsA) immunosuppression [7,8]. However, no differences in hormonal status between patients receiving different immunosuppressive drugs have been reported by others [9,10].

The role of sex hormones on the bone loss associated with renal transplantation has not been addressed previously. Therefore, this study was performed to investigate whether sex hormone status in both men and women is associated with the bone loss seen after renal transplantation. We report the results of a cross-sectional, comparative analysis derived from a previous larger study in which the effects of calcitriol and calcium carbonate on renal transplant-associated osteoporosis were examined [11].

Subjects and methods

Patients

Thirty patients were studied: 16 male and 14 female, of which eight were post-menopausal. The cause of renal failure was chronic primary glomerulonephritis in 14, adult polycystic kidney disease in five, reflux nephropathy in four, unknown in two, diabetes mellitus in two, and other causes in three. Patients were randomly selected on the basis of their having a first kidney transplant, stable graft function (serum creatinine <2 mg/dl), and gave informed consent for participation and bone biopsy. Exclusion criteria were any condition (prolonged immobilization, systemic illness or malignancy) or intake of drugs (other than calcium carbonate, calcitriol or immunosuppressives) that could significantly affect bone metabolism. The women were not taking any medications or supplements containing estrogens.

Patients were receiving one of three immunosuppressive regimes currently used in the Renal Unit of the Manchester Royal Infirmary: CsA monotherapy, azathioprine plus prednisolone dual therapy (AZA + PRED), or triple therapy (CsA + AZA + PRED). Rejection episodes were treated with three daily i.v. methylprednisolone boluses (1.0 g each). Intravenous OKT3 or ATG were given in case of steroid-resistant rejection. Cumulative doses of CsA, PRED and AZA were calculated (methylprednisolone and PRED were considered as equipotent).

For the purpose of analysis, patients were classified into groups according to gender, and menopausal status in women. Pre-menopausal women had normal menstruation. In each patient, the following evaluations were performed.

Bone histology evaluation

After double tetracycline labelling, bone biopsies were taken from the anterior iliac crest using an 8-mm Bordier trephine biopsy needle. Specimens were processed as described in detail elsewhere [12], and were analysed by a single pathologist (A.J.F.), who was blinded to patients' details. Bone histomorphometry was performed according to the guidelines of the American Society of Bone and Mineral Research histomorphometry nomenclature committee [13]. Results were compared with age- and sex-matched normal values (Z scores) derived from the database of biopsies from 234 normal subjects (including 84 individuals with double tetracycline labelling) and necropsies currently employed in the Department of Osteopathology, University of Manchester [14].

Bone mineral density (BMD) measurements

BMD of the axial and appendicular skeleton was determined by densitometry. BMD of the forearm (distal radius) was analysed by single energy X-ray absorptiometry (SXA) using a bone densitometer DTX-100 (Osteometer, MediTech, Roedrove, Denmark). The density of the right femoral neck and lumbar spine (L1-L4) was evaluated by dual energy X-ray absorptiometry (DXA) using a DPX scanner (Lunar Corporation, Madison, WI, USA). Results were compared with values of an age- and sex-matched reference population (Z score), and with values of sex-matched peak bone mass of a young control population (T score) currently employed in the Department of Diagnostic Radiology, University of Manchester. Densitometers were calibrated every morning against a commercial phantom. The SXA densitometer is autocalibrated using an integrated computer program. In the case of the DXA densitometer, the coefficient of variability for the femoral neck is 2.07%, and 1.57% for L1-L4.

Laboratory tests

Blood samples were obtained on the day of the bone biopsy. Calcium, phosphate, albumin and creatinine were measured by standard techniques. Total alkaline phosphatase was assayed by colorimetry. Intact PTH (iPTH) was measured by immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), 1,25-dihydroxyvitamin D_3 was evaluated by radioimmunoassay on a 1217 Rackbeta Scintillation Spectrometer (Wallac, Crownhill, UK), and prolactin, oestradiol (in women; in pre-menopausal women, blood samples were taken in the middle of the menstrual cycle) and testosterone (in men) were measured by radioimmunoassay in a 1235 Automatic Immunoassay System Auto Delfia (Wallac, Turku, Finland).

Statistical analysis

Results are shown as mean $\pm$ SD or median (percentiles 25–75%), depending upon whether the data distribution were parametric or non-parametric, respectively. Comparison of dimensional variables between the three groups were performed by analysis of variance (ANOVA) or ANOVA on ranks as appropriate. In the case of a significant model appearing in the latter analyses, all pairwise multiple comparisons were analysed by the Student–Newman–Keuls or the Dunn method. Comparison of nominal variables was performed by Fisher's exact test. Univariate association analysis was by Pearson's or Spearman's correlation coefficients (as appropriate). Multivariate analysis was carried out by Stepwise Multiple Linear Regression. All the univariate and multivariate analyses, in which densitometric and histomorphometric parameters were regarded as dependent variables, were performed using Z scores. We consider these scores to be the best way to control for the influence of age and sex, which are known risk factors for bone loss in the normal population [13,15]. A two-tail $P<0.05$ was considered significant.

Table 1. Clinical characteristics of the patients. Results are shown as mean ± SD or median (percentiles 25–75%) depending upon whether the data distribution were parametric or non-parametric, respectively

	Men	Pre-menopausal women	Post-menopausal women
N	16	6	8
Age (years)	48 (40–54)	38 (37–43)	56 (53–59)*
Type of dialysis before the graft (*n*) Nil/HD/PD	4/5/7	0/2/4	3/2/3
Time on dialysis (months)	11 (0.5–12)	12 (5–36)	7.5 (0–21)
Time since transplantation (months)	120 ± 65	142 ± 73	123 ± 71
Rejection episodes (*n*)	0.7 ± 1.1	0.6 ± 0.7	1.5 ± 1.0
Immunosuppression (*n*)			
CsA monotherapy	8	1	4
AZA + PRED	5	4	3
triple therapy	3	1	1
Cumulative dose of CsA (g)	630 (48−1111)	143 (0−887)	449 (198−772)
Cumulative dose of PRED (g)	9 (0–44)	35 (19–56)	15 (3–37)
Intake of $CaCO_3$ and calcitriol (*n*)	11	1	4

HD, haemodialysis; PD, peritoneal dialysis; CsA, cyclosporine A; AZA, azathioprine; PRED, prednisolone.
*$P < 0.05$ for men *vs* pre-menopausal women.

Table 2. Biochemical and sex hormone results of the patients. Results are shown as mean ± SD or median (percentiles 25–75%) depending upon whether the data distribution were parametric or non-parametric, respectively

	Men	Pre-menopausal women	Post-menopausal women	Reference values
iPTH (pg/ml)	43.1 ± 25.8	41.5 ± 28.7	53.6 ± 33.4	10−60
Corrected calcium (mg/dl)	9.2 ± 0.4	9.2 ± 0.4	9.6 ± 0.4	8.6−10.6
Phosphate (mg/dl)	2.5 ± 0.6	3.4 ± 0.3	2.8 ± 0.6	2.2−4.4
1,25-$(OH)_2$ D_3 (pg/ml)	31.4 ± 14.9	26.2 ± 13.1	31.7 ± 6.3	20−50
Total alkaline phosphatase (U/l)	170 ± 55	132 ± 57	165 ± 58	70−330
Creatinine (mg/dl)	1.7 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	up to 1.2
Oestradiol (pmol/l)		209 (128−2.9)	93 (54−2.9)	120–1650*
Testosterone (nmol/l)	19.7 ± 6.8			8.7−33
Prolactin (mU/l)	357.2 ± 199.3	372.2 ± 363.1	209.1 ± 60.1	83−523

*These values include oestradiol levels from follicular phase (110–183 pmol/l), mid cycle (550–1650 pmol/l) and luteal phase (550–845 pmol/l).

Results

Clinical characteristics are shown in Table 1. The only variable that was significantly different between groups was older age in the post-menopausal women compared with pre-menopausal women and with men. Although a trend towards a different cumulative dose of CsA and PRED in the post-menopausal women was observed, it did not reach statistical significance, probably due to data dispersion. The small sample size in the two female groups resulted from classification according to menopausal status, and probably similarly influenced statistical validity. In fact, when all women are compared with men, those apparent differences notably decrease or disappear.

No biochemical differences were observed between groups (Table 2). Interestingly, all pre-menopausal women showed serum oestradiol levels similar to those observed in the normal follicular phase (even though these patients were in the middle of the menstrual cycle). In fact, there were no significant differences in serum oestradiol between pre- and post-menopausal women. Serum testosterone levels in men were within the normal range. Prolactin values were within the normal range in all groups. No significant hormonal differences were observed between patients on the different immunosuppressive drug schemes.

In the densitometric analysis (Table 3), the only significant observed difference was that the BMD Z score at the distal radius was greater in post-menopausal women than in pre-menopausal women and men. Of note, pre-menopausal women showed a non-significant trend towards lower BMD T scores (compared with young control population) at all anatomical sites than post-menopausal women and men.

Histomorphometric analysis (Table 4) did not show any significant differences between groups. In all groups, there was a notable decrease in parameters of bone structure (bone volume and wall thickness), osteoblast number and function (osteoblast surface and

osteoid surface), osteoclast number (osteoclast surface), and most of all, dynamic parameters (mineralizing surface and appositional rate).

In men, in the univariate association analysis, the only variable correlated (marginally) with the serum testosterone levels was the cumulative dose of prednisolone (Figure 1). In women, serum oestradiol levels were directly correlated with parameters of osteoblast number and function and bone structure (Figure 2).

In the multivariate analysis, serum testosterone levels did not predict BMD at any of the anatomical sites evaluated, nor the histopathological diagnosis in men (Table 5). In women, however, serum oestradiol levels did, importantly, predict the bone structure (wall thickness) and the osteoblast number and function (osteoblast surface and osteoid surface; Table 6).

Table 3. Densitometric results of the patients

	Men	Pre-menopausal women	Post-menopausal women
SXA			
Distal radius			
Z score	−1.2±1.0	−1.1±0.8	0.02±0.7*
T score	−1.4±0.9	−2.2±0.9	−1.6±1.2
DXA			
Femoral neck			
Z score	−0.3±1.2	−0.9±0.8	−0.3±1.2
T score	−0.9±1.0	−1.8±0.7	−1.5±0.9
L1-L4			
Z score	0.8±1.5	−0.4±1.2	0.01±1.6
T score	0.2±1.2	−1.2±0.7	−0.5±1.4

*$P < 0.05$ *vs* men and pre-menopausal women.

Table 4. Histomorphometric results of the patients

	Men	Pre-menopausal women	Post-menopausal women
Z score			
Bone volume	−2.1±1.9	−2.9±1.1	−3.0±1.4
Osteoblast surface	−3.1±4.3	−3.5±8.4	−3.6±2.8
Osteoid surface	−2.8±3.7	−3.5±3.8	−3.6±2.5
Wall thickness	−4.6±5.0	−4.9±1.7	−7.2±4.8
Osteoclast surface	−0.7±4.1	−3.0±2.6	−1.0±3.5
Mineralising surface	−12.6±2.4	−13.0±3.3	−13.5±2.3
Appositional rate	−7.6±2.2	−7.3±2.9	−8.7±2.6

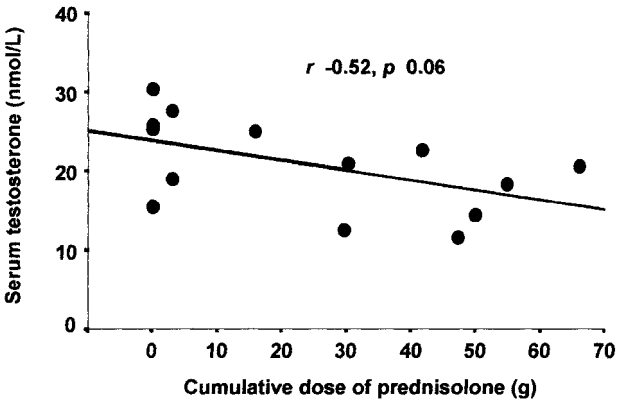

Fig. 1. Negative correlation between serum testosterone levels and cumulative dose of prednisolone.

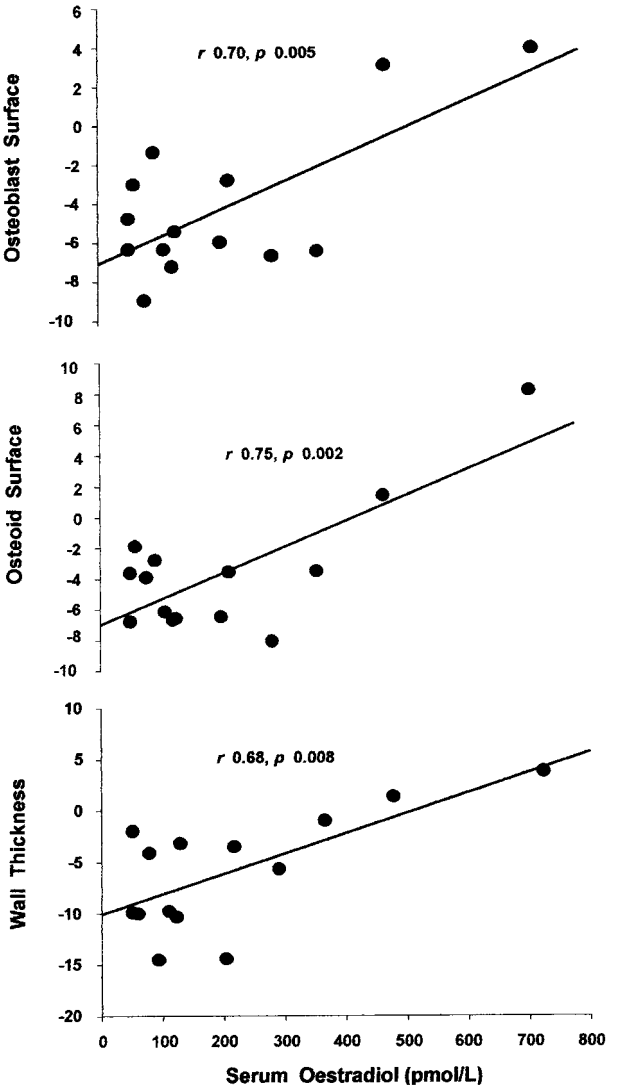

Fig. 2. Positive correlations between serum oestradiol levels and osteoblast surface, osteoid surface and wall thickness.

Discussion

To the best of our knowledge, this is the first study analysing the relationship between sex hormone levels and bone status in the renal transplantation setting. Our results suggest that pre-menopausal women with a stable, long-term functioning renal transplant may have hypothalamic-pituitary-ovary axis alterations and subsequently low serum oestradiol levels. Serum oestradiol levels did not seem to be associated with the dose of CsA or PRED received; however, they correlated significantly with parameters of bone

Table 5. Significant multiple linear regression models predicting histomorphometric and densitometric parameters in men (only significant independent variables are shown)*

Dependent variable	Independent variables	Beta	*P*
Distal radius BMD $r=0.81$, $r^2=0.65$, $P=0.003$	Cumulative dose of PRED	−0.67	0.003
	Serum creatinine	−0.52	0.01
L1-L4 BMD $r=0.75$, $r^2=0.56$, $P=0.01$	Cumulative dose of PRED	−0.64	0.009
	Serum creatinine	−0.44	0.05
Mineralising surface $r=0.58$, $r^2=0.34$, $P=0.02$	Age	0.58	0.03
Appositional rate $r=0.60$, $r^2=0.36$, $P=0.02$	Time on dialysis before the graft	0.60	0.02

*Independent variables: age, serum creatinine, iPTH, rejection episodes, type and time on dialysis before transplantation, time after transplantation, cumulative dose of PRED and CsA, intake of calcitriol and calcium carbonate (yes = 1, no = 0), serum prolactin and serum testosterone were introduced in analysis one at a time until the most significant model was obtained.

Table 6. Significant multiple linear regression models predicting histomorphometric and densitometric parameters in women (only significant independent variables are shown)*

Dependent variable	Independent variables	Beta	*P*
Distal radius BMD $r=0.59$, $r^2=0.34$, $P=0.03$	Age	0.59	0.03
L1-L4 BMD $r=0.56$, $r^2=0.31$, $P=0.04$	Age	0.56	0.04
Wall thickness $r=0.54$, $r^2=0.29$, $P=0.046$	Serum oestradiol	0.54	0.046
Osteoblast surface $r=0.81$, $r^2=0.65$, $P=0.003$	Serum oestradiol	0.75	0.002
	Age	0.58	0.009
Osteoid surface $r=0.82$, $r^2=0.67$, $P=0.009$	Serum oestradiol	0.53	0.019
	Age	0.77	0.003
	Intake of calcium and calcitriol	−0.44	0.04

*Independent variables: age, serum creatinine, iPTH, rejection episodes, type and time on dialysis before transplantation, time after transplantation, cumulative dose of PRED and CsA, intake of calcitriol and calcium carbonate (yes = 1, no = 0), serum prolactin and serum oestradiol were introduced in analysis one at a time until the most significant model was obtained.

structure and osteoblast function, independently of factors such as age, graft function, parathyroid status, type and time on dialysis before transplantation, time since transplantation, and intake of calcitriol and calcium carbonate. Nevertheless, we cannot definitely exclude a type II error (due to the small samples of patients) influencing the results.

The significant association between oestradiol and bone status observed in this study is not surprising as it is well known that oestrogens have a very important effect on metabolism and structural integrity of bone in women [15]. Oestrogen deficiency increases the rate of bone remodelling and leads to an imbalance between bone resorption and formation, resulting in a net bone loss and osteoporosis [16]. The mechanism of action of oestrogens on bone is not completely understood; but it may be related to suppression of cytokine production [15] and better preservation of osteocyte number [17] promoted by these sex hormones.

Few studies have been performed examining the treatment for bone loss associated with renal transplantation [11,18]. In normal subjects, a strong inverse relationship between serum oestradiol concentrations and vertebral and femoral fractures has been reported [19]; therefore, results of the present study would suggest that hormone replacement deserves analysis in women with renal transplantation in an effort to prevent and possibly reverse bone loss.

Despite the negative association between serum testosterone and the cumulative dose of PRED, male renal transplant patients maintained normal testosterone levels. It may be that long-term renal recipients are able to compensate for the well established inhibitory effects of glucocorticoids on testosterone production and secretion [20]. If so, a lack of correlation between testosterone and bone status in male renal transplant recipients is not surprising. This suggests that the association of male gender with worse bone status in renal graft recipients reported previously [2] is more probably related to other non-sex hormone factors in this male population. Such non-sex hormone factors may include time after transplantation, old age, time on dialysis prior to transplantation, renal function and immunosuppressive drugs.

In conclusion, in female renal transplant patients, serum oestradiol levels independently predict the bone status, while in men, factors other than testosterone seem to influence bone loss. Our results give rise to the hypothesis that sex hormone replacement therapy may play a role on prevention and/or treatment of the bone loss in women following renal transplantation. Further clinical studies are needed to evaluate this issue.

Acknowledgements. Dr Cueto-Manzano had an International Society of Nephrology fellowship award during the time when the study was performed (December 1996 to June 1998).

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Received for publication: 22.5.00
Accepted for publication: 8.1.01