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Original Paper

Bone Metabolism and Arterial Stiffness After Renal Transplantation

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Key Words
Chronic kidney disease • Transplantation • Bone turnover • Metabolic bone disease • Arterial stiffness

Abstract
Background/Aims: To assess the relationship between bone and vascular disease and its changes over time after renal transplantation. Metabolic bone disease (MBD) is common in chronic kidney disease (CKD) and is associated with cardiovascular (CV) disease. Following transplantation (Tx), improvement in CV disease has been reported; however, data regarding changes in bone disease remain controversial. ***Methods:*** Bone turnover and arterial stiffness (pulse wave velocity (PWV)) were assessed in 47 Tx patients (38 (3-191) months after Tx). ***Results:*** Bone alkaline phosphatase (BALP), osteocalcin (OC) and beta-crosslaps were significantly higher in Tx patients, and decreased significantly after one year. There was a negative correlation between BALP, OC and steroid administered (r=-0.35;r=-0.36 respectively). PWV increased in the Tx group (1.15 SD). In patients with a follow up of <24 months, PWV was correlated with BALP and beta–crosslaps (r=0.53; r=0.69 respectively) while in the ≥24 months group, PWV was correlated with cholesterol (r=0.38). ***Conclusions:*** Increased bone turnover and arterial stiffness are present following kidney transplantation. While bone turnover decreases with time, arterial stiffness correlates initially with bone turnover, after which the influence of cholesterol becomes significant. Non-invasive estimation of bone metabolism and arterial stiffness may help to assess CKD-MBD following renal transplantation.

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Introduction

Abnormalities in bone and mineral metabolism are common complications in chronic kidney disease (CKD) patients [1]. These abnormalities are associated with cardiovascular (CV) disease according to recent evidence [2-4]. Chronic kidney disease-mineral bone disorder (CKD-MBD) has been suggested as a non-traditional risk factor in explaining the high rates of CVD in CKD patients [5, 6].

Renal transplantation is the optimal treatment in patients with end stage renal disease as it may correct most uremic metabolic alterations. Accordingly, improvement in uremic CV disease following renal transplantation has been reported; however, data on improvement of bone disease are somewhat controversial [7-9].

Assessing bone turnover is an important diagnostic tool in the management of renal bone disease. Given the invasive nature of the "gold standard" bone biopsy procedure, biomarkers are used to evaluate and monitor bone turnover [10]. There is however little information regarding bone metabolism and its relation to arterial elasticity in children following renal transplantation (Tx).

In a previous study, we had shown an increased bone turnover in patients following renal transplantation even with relatively well-preserved bone mineral density [11]. On the other hand, in another study evaluating arterial stiffness in children with Tx, we were able to show that following successful Tx, pulse wave velocity (PWV), a marker of arterial stiffness [12], was lower than in dialyzed patients, which could imply that vascular changes in children are reversible [13].

Post-transplant bone metabolism reflects both the effects of previous CKD-MBD persisting after transplantation and remodelling after correction of the metabolic effects of CKD [14]. During the first years post-transplantation, catch-up growth and bone remodelling predominates, with normalization of bone turnover later on. Thus the contribution of each component to the overall presentation changes over time.

The aim of the present study was to evaluate the relationship between bone and vascular disease and its change over time after transplantation onward. Using non-invasive parameters, we assessed bone turnover and arterial stiffness in renal transplant children with stable graft function (CKD 1-3).

Patients and Methods

Forty-seven transplanted patients (aged 15.5 (4.6) years, 32 males) were examined. The time spent on dialysis prior to Tx was 11 (0-61) months while the elapsed time since transplantation was 38 (3-191) months [median (range)].

Anthropometric, blood pressure and PWV data of the patients are shown in Table 1.

The diagnoses leading to end-stage renal disease (ESRD) were (number of patients in parentheses): renal hypoplasia, obstructive uropathy (16), focal segmental glomerulosclerosis (8), cystic renal disease (6), Alport syndrome (3), cystinosis (2), systemic lupus erythemotosus (2), acute tubular necrosis (1), Prune Belly syndrome (1), nephrocalcinosis (1), other syndromes (VACTERL, Joubert, Barakat, acrorenal) (4), unknown origin (3).

Table 1. Anthropometric and clinical characteristics of renal transplant patients

	Renal transplant patients
Age (years)	15.5 (4.6)
Height (cm)	153 (18)
Weight (kg)	52.5 (16.8)
BMI (kg/m^2)	21.8 (4.5)
Systolic BP SDS	0.96 (0.94)
Diastolic BP SDS	0.52 (0.80)
Heart rate (1/min)	75 (14)
PWV	5.69 (0.95)
PWV SDS	1.15 (1.65)

BMI: body mass index; BP: blood pressure; SDS: tandard deviation score; WV: pulse wave velocity

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The diagnosis of hypertension was based on 24 h ambulatory blood pressure measurement (ABPM); Z scores of systolic and diastolic BP (SBP and DBP) were used according to Soergel et al [15]. Hypertension was defined as SBP and/or DBP equal or exceeding the 95th percentile for height.

Table 2. Distribution of patients with a follow-up <24 months and ≥24 months

	Transplant patients <24 months after Tx (n=13)	Transplant patients ≥24 months after Tx (n=34)
Age (years)	12.9 (4.5)	16.4 (4.4)*
Weight (kg)	44.1 (19.9)	55.7 (14.5)*
Height (cm)	143 (25)	157 (14)*
Time after Tx (months)	12.2 (5.5)	63.2 (43.3)*

*p<0.05; Tx transplantation

Eight children were normotensive while thirty nine patients received antihypertensive treatment. Thirteen patients received calcium channel blocker (amlodipine) or angiotensin converting enzyme inhibitor (enalapril or ramipril). Twenty six patients received combination therapy (ACE inhibitor and beta-blocker (metoprolol) and/or α1 blocker (prazosin).

With regard to immunosuppressive treatment, basic immunosuppressive therapy included tacrolimus (40 patients) or cyclosporine (7 patients) and mycophenolate mofetil (47 patients); twenty-two patients received steroid therapy at the time of PWV measurement.

Native Vitamin D3 supplementation (1000 IU/day) was administered routinely to all patients.

As patients suffered from mild dyslipidemia only we did not use specific lipid lowering treamtment (statins) other than diet in the study population.

PWV measurement

Aortic PWV was measured by applanation tonometry (PulsePen device, DiaTecne, Milan, Italy) [16] using sequential recordings of the arterial pressure wave at the carotid and femoral arteries, and by measuring the distance between the carotid sampling site to the suprasternal notch and from the suprasternal notch to the femoral sampling site, as described previously [13]. All measurements were performed twice to confirm reproducibility. Recordings with >10% systolic or diastolic variability or <80 mV amplitude of the pulse wave signal were discarded. All measurements were performed by OC and EK.

PWV was expressed as PWV-Z score according to Reusz et al [17].

Laboratory data

Blood samples were collected at the timed control evaluations. Serum Ca, phosphate and creatinine were measured by routine laboratory methods. Jaffe's kinetic method was used for creatinine measurements. Creatinine clearance (CCl) was calculated according to Schwartz et al [18].

Intact parathyroid hormone (PTH) (1-84) was determined by an immunochemiluminometric two-site assay (CIBA-CORNING, Frenwald, Germany).

Serum level of bone alkaline phosphatase (BALP) was measured by HYDRAGEL 15 ISO-PAL reagent, using the HYDRASIS (Issy-les-Moulineaux, France) system. Serum beta-crosslap level was determined by electrochemiluminescence immunoassay (ECLIA) on a Roche Elecsys 2010 immunoassay analyser (Mannheim, Germany).

Control group for bone markers: leftover serum samples of 47 patients matched for age, gender and pubertal stage (according to Tanner) and assessed for minor surgical interventions were used anonymously as controls for bone markers.

Effect of time since transplantation

To evaluate the effect of time since transplantation, patients were divided into two groups: those with a follow-up of less than 24 months, and those with a follow up of 24 months or longer. Thirteen out of the 47 patients were followed for <24 months (Table 2.)

Fourteen patients participating in this study had already been assessed in a previous study evaluating renal bone disease [19]. These patients had one-year follow-up data on bone markers (BALP, osteocalcin (OC), beta-crosslaps).

To overcome the problem of the change in bone markers with age in the individual patient, paired data was normalized using the individual patient's values with those from matched controls according to the following formula:

normalised bone marker level = bone marker level / age- and gender-matched control bone marker level

Table 3. Creatinine clearance, lipid levels and bone metabolism markers in renal transplant and control groups

	Transplant patients (n=47)	Control (n=47)
Creatinine clearance (ml/min/1.73 m^2)	92.9 (24.8)	>90#
Ca (mmol/l)	2.37 (0.22)	2.3-2.6#
P (mmol/l)	1.36 (0.29)	1.16-1.67#
Cholesterol (mmol/l)	4.22 (1.45)	3.0-5.0#
Triglycerides (mmol/l)	1.96 (1.41)	0.5-2.0#
iPTH (pg/ml)	49 (8-190)	10-65#
ln iPTH	3.95 (0.56)	2.3-4.17#
Bone alkaline phosphatase -BALP (U/l)	251 (51-1460)*	213 (68-488)
ln BALP	5.56 (0.73)*	5.23 (0.61)
Osteocalcin - OC (ng/ml)	110 (27-1843)*	49 (12-1470)
ln OC	4.71 (0.85)*	4.11 (0.84)
beta-crosslaps (pg/ml)	1354 (79-3283)*	816 (48-2345)
ln beta-crosslaps	7.11 (0.74)*	6.59 (0.72)
PWV (m/s)	5. 69 (0.95)	5.11 (0.71)##
PWV - Z	1.15 (1.65)##	NA

Paediatric laboratory reference values; ##PWV reference values from Reusz et al.[17]; NA: not applicable; P: phosphates; iPTH: intact parathyroid hormone; PWV: pulse wave velocity; ln: natural logarithm; *p <0.05 Transplant vs. control

Statistical analysis

Database analysis was performed using STATISTICA 8.0 software (Stat Soft., Inc. USA). Data are presented as mean ± standard deviation (SD) unless indicated otherwise. In case of data with non-normal distribution, data are expressed as median (range). As the distribution of the bone markers was skewed, logarithmic transformation was used to normalise the data for further statistical analysis. Laboratory data were compared by Student's t test or analysis of variance (ANOVA), where appropriate; the Mann-Whitney U test was used to compare data with non-normal distribution. Univariate regression analysis was applied to assess associations between laboratory data. The factors influencing PWV were assessed by standard and forward multiple regression analysis. A p value of <0.05 was considered statistically significant.

Ethics

The study was in accordance with the Helsinki declaration and was approved by the local ethics committee. Parental informed consent was obtained for all subjects participating in the study.

Results

Data on creatinine clearance, lipids and bone metabolism markers are summarized in Table 3.

Renal transplant children could be classified according to their CCl (numbers of patients in parentheses) CKD 1 (22), CKD 2 (23), CKD 3 (2).

Ca and P values of transplant patients were in the normal range. PTH values were under 60 pg/ml in 30 patients and under 120 pg/ml in 42 patients. BALP, OC and beta-crosslaps were significantly higher in transplanted children. As the distribution of the bone markers was skewed, logarithmic transformation was used for further statistical evaluation. The correlations between PTH and studied bone markers are shown in Table 4.

There was a negative correlation between BALP, OC and the (daily) steroid dose administered during the last year prior to the present evaluation (average of doses taken during the regular visits/year, expressed in mg/day) (Figure 1.). (r=-0.35 and r=-0.36, both p <0.01).

Effect of time since transplantation

The transplant patient group was divided into those with a follow-up of less than 24 months, and those with a follow-up of 24 months or longer (Table 2.).

The patients with the shorter follow-up were younger and had a shorter time since transplantation. The only significant metabolic difference between the two groups was a higher cholesterol level in patients with a longer interval time since transplantation (3.44 (1.45) versus 4.52 (1.35) mmol/l, p <0.05). There was no significant difference between the groups in any of the other parameters studied (Ca, P, creatinine, CCl, iPTH, and BALP, OC, beta-crosslaps corrected for age)

Pulse wave velocity in the renal transplant group

PWV was elevated by 1.15 SD in the Tx group. There was no correlation between PWV and bone markers and cholesterol for the transplanted group as a whole. However, in patients with a follow up of <24 months, PWV correlated with BALP and beta-crosslaps (BALP: r=0.53; p<0.05 and beta-crosslaps: r=0.69; p=0.01). In multiple regression analysis, ß-crosslaps was the determinant factor of PWV (beta=0.57, p<0.03).

In patients who were transplanted ≥24 months before, PWV was correlated with cholesterol (r=0.38; p <0.05). (Figure 2). There was no correlation between PWV and any of the bone markers in this subgroup.

Table 4. Correlations between PTH and studied bone markers (BALP, OC, beta-crosslaps)

	ln PTH	ln BALP	ln OC	ln beta-crosslaps
ln PTH	1	0.49*	0.45*	0.29*
ln BALP	0.49*	1	0.65*	0.59*
ln OC	0.45*	0.65*	1	0.35*
ln beta-crosslaps	0.29*	0.59*	0.35*	1

*p <0.05; PTH: parathyroid hormone, BALP: bone alkaline phosphatase; OC: osteocalcin

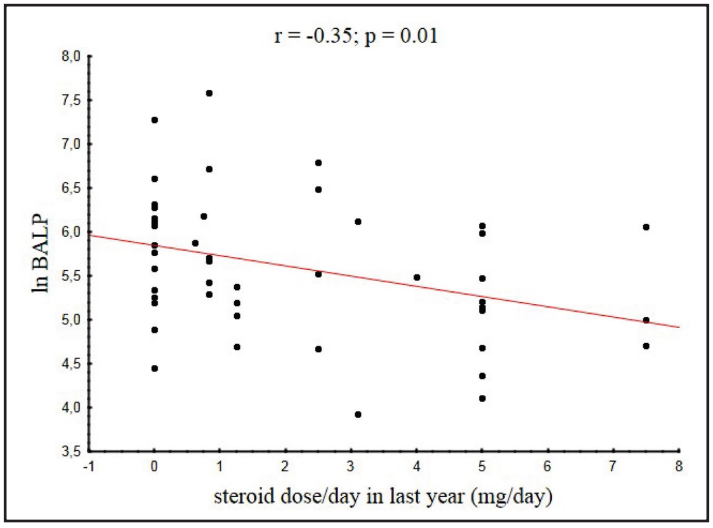

Fig. 1. Correlation between ln BALP and the daily steroid dose administered (y=–0.11*x+5.79).

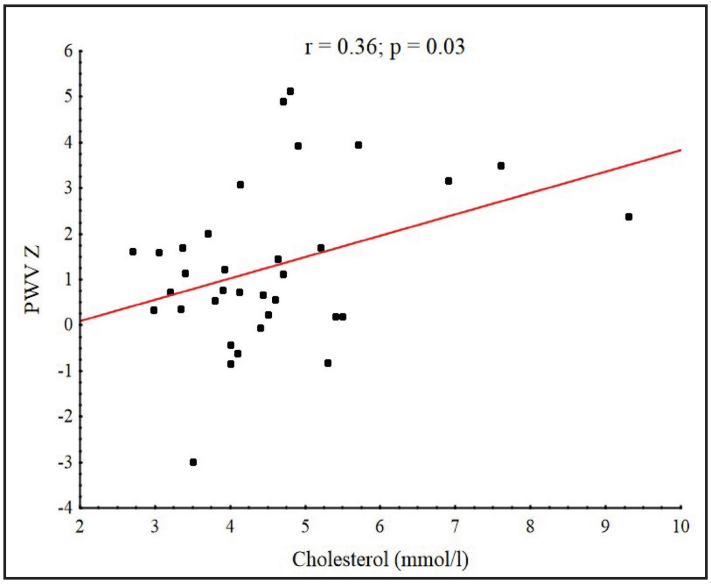

Fig. 2. Correlation between cholesterol and PWV-Z score in children transplanted ≥24 months before(y=0.45*x-0.87).

Table 5. Follow-up of bone markers in 14 Tx children (between baseline and at 12 months)

	initial data	12-month follow-up
Age (years)	14.8 (4.3)	16.1 (4.3)
PTH	3.91 (0.40)	3.57 (0.58)
ln BALP/control	1.34 (0.38)	0.44 (0.69)*
ln OC/control	1.39 (0.36)	0.45 (0.80)*
ln beta-crosslaps/control	1.27 (0.31)	0.56 (0.67)*

* $p < 0.05$; PTH: parathyroid hormone; BALP: bone alkaline phosphatase; OC: osteocalcin; ln: natural logarithm

The follow-up data of the fourteen children at 12-months follow-up are presented in Table 5. As can be seen, a significant decrease in BALP, OC and beta-crosslaps activity occurred over time.

Discussion

The present study used a series of biochemical markers and the measurement of pulse wave velocity in order to explore the relationship between bone metabolism and arterial stiffness in renal-transplanted children.

Since histological analysis of the iliac bone biopsy is not routinely performed, considerable efforts have been undertaken to develop reliable non-invasive methods to characterize bone turnover. As a result, a number of non-invasive markers are now available for clinical use representing different steps of bone metabolism [11, 20, 21].

In the present study, measurement of bone formation by BALP and OC was used as indicators of osteoblast activity [20]. Bone resorption is characterized by beta-crosslaps that have been shown to be more specific to bone resorption than any other currently available test [22]. Herein, the markers of bone formation and degradation were all closely interrelated, although only signifying that formation and degradation are indeed coupled processes.

The main results of the study are that a significantly increased activity of both bone formation and resorption was found, together with an increased arterial stiffness in patients after renal transplantation. Furthermore, BALP and OC were negatively correlated with mean steroid dose administered.

In the subgroup of patients with a one year follow-up, bone turnover decreased significantly, reflected by markers of both formation and degradation.

In parallel, during the two first years following transplantation, arterial stiffness was related to BALP and beta-crosslaps, whereas in patients transplanted longer than 2 years prior, serum cholesterol was the variable influencing PWV.

Previously, it was generally accepted that most patients who had received a renal graft during childhood had moderate to severe bone resorption and osteopenia even after many years following transplantation [23, 24]. However, more recent data indicate that following successful transplantation, the initial increase in remodelling helps to correct the consequences of uremic vintage on the bone and after the second year following transplantation, bone mineral density (BMD) actually increases [11, 25-28]. Accordingly, to evaluate the effect of time since transplantation, patients were divided into two groups: those with a follow-up of less than 24 months, and those with a follow up of 24 months or longer.

Both bone remodelling and CV can be influenced by current kidney function, PTH levels and the effects of immunosuppressive drugs [28, 29]. Indeed, cyclosporine and tacrolimus have been reported to increase both bone formation and resorption in the rat and to reduce bone volume [30, 31]. Similar changes have also been described in adult and paediatric renal transplant recipients [32-34].

In the present study, immunosuppressive therapy was based on tacrolimus (40 patients) or cyclosporine (7 patients) and mycophenolate mofetil (all patients); twenty two patients

received steroid therapy at the time of PWV measurement. Thus, calcineurine inhibitors likely account for the higher rates of bone formation in patients with normal serum iPTH levels, since prednisolone and other corticosteroids are usually associated with a decrease rather than an increase in bone formation and turnover [35]. During glucocorticoid therapy, levels of bone formation markers are generally low while those of bone resorption markers are either normal or low [36-38]. Presumably, the reduction in bone resorption is not sufficient to overcome the reduction in bone formation. This appears to be confirmed by the present finding showing a negative correlation between steroid treatment dose and BALP and OC, both markers of bone formation.

As expected, PWV was increased in the transplant group. As shown earlier, this increase is mainly the consequence of pre-transplantation uremic vintage, correlating with pre-transplant Ca and phosphate values, the time spent on dialysis and the dose of vitamin D administered prior to transplantation [13, 19]. PWV may be influenced by hypertension. As the blood pressure of our patients was in normal range for age and height, we could not find any influence of blood pressure on PWV. In the present study, PWV was associated with the actual markers of bone turnover up to two years following transplantation, indicating a relationship between bone metabolism and the pathomechanism of arterial stiffening. Interestingly, in patients assessed more than two years following transplantation, this relationship was no longer present and the level of cholesterol was the independent determinant of PWV.

Conclusion

In summary, increased bone turnover and arterial stiffness can be observed in children following kidney tranplantation. While bone turnover decreases with time; arterial stiffness correlates initially with bone turnover whereas the influence of cholesterol becomes significant later on. Steroid administration is negatively correlated with arterial stiffness.

In conclusion, non-invasive estimation of bone metabolism and arterial stiffness may help to assess CKD-MBD in children following renal transplantation.

Limitations

This study has several limitations. The relatively low number of patients involved decreases the power of the conclusions. The predominantly cross-sectional nature of the study allows only to describe correlations but not causality. There are important factors influencing bone metabolism such as vitamin administration and FGF-23 levels that were not recorded or measured. In addition, evaluation of bone mineral density could have provided further information regarding bone status.

Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

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