Tartrate-resistant acid phosphatase 5b and its correlations with other markers of bone metabolism in kidney transplant recipients and dialyzed patients

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Abstract

Purpose: Renal osteodystrophy is a common complication of chronic renal failure and renal replacement therapy. Successful kidney transplantation reverses many of these abnormalities, however, the improvement is often incomplete. The osteoclast specific 5b isoform of tartrate-resistant acid phosphatase (TRAP) 5b has recently been proposed a specific and sensitive marker of bone resorption. The aim of the study was to assess correlations of TRAP 5b with markers of bone resorption and formation in kidney transplant recipients, hemodialyzed and peritoneally dialyzed patients and healthy volunteers.

Material and methods: We assessed PTH, markers of bone formation-alkaline phosphatase and its bone isoform, osteocalcin, markers of bone resorption – procollagen type I carboxy-terminal extension peptide, procollagen type I cross-linked carboxy-terminal telopeptide, serum CrossLaps-Ctx, β2--microglobulin and urinary deoxypyridynoline (DPD), expressed as DPD/creatinine ratio. (BMD) bone mineral density measurements were determined for femoral neck and lumbar spine (L2-L4) using DEXA.

Results: In dialyzed patients markers of bone formation and resorption were significantly higher than in healthy volunteers, whereas in kidney transplant recipients these disturbances were less pronounced. TRAP 5b correlated positively with age and mainly with markers of bone resorption in kidney transplant recipients, dialyzed patients and healthy volunteers. TRAP 5b did not correlate with BMD in any groups studied.

Conclusion: Since TRAP 5b correlated mainly with markers of bone resorption, it may serve as a new additional marker of bone resorption in the assessment of renal osteodystrophy.

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Introduction

Renal osteodystrophy is a common complication of chronic renal failure and renal replacement therapy. Unfortunately, monitoring for the presence and progression of renal osteodystrophy remains problematic. Bone biopsy, a gold standard for diagnosis of renal osteodystrophy, is rarely performed mainly due to patients refusal. Thus, considerable efforts have been devoted to the development of reliable non-invasive methods to assess bone metabolism in uremic patients [1,2]. Successful kidney transplantation reverses many of these abnormalities, however, the improvement is often incomplete. Over the last several years various biochemical markers of bone metabolism have been proposed [3].

Bone metabolic status can be determined by measuring the amount of various biochemical markers of bone resorption and formation in urine and serum samples. The degree of bone formation rate is assessed by measuring plasma levels of bone-specific alkaline phosphatase (bALP), osteocalcin or procollagen type I carboxy-terminal extension peptide (PICP) [3]. On the other hand, osteoclasts resorb bone by secreting acid and lysosomal proteases to the space between cell membrane and bone matrix. Acid dissolves hydroxyapatite and proteases remove and degrade organic components (type I collagen and other matrix proteins) from the matrix. Therefore, pyridinoline (PYD), deoxypyridinoline (DPD), cross-linked N-terminal telopeptides of type I collagen and C-terminal telopeptides of type I collagen (ICTP), degradation products of C-terminal telopeptides of type I collagen (CrossLaps) and tartrate-resistant acid phosphatase (TRAP) have been proposed as markers of bone resorption [3]. Osteoclasts secrete TRAP into the circulation during bone resorption. Human serum contains two forms of TRAP, 5a and 5b, of which 5b is derived from osteoclasts, and 5a from some other, yet unindentified source [4]. Newly developed

immunoassay, specific for TRAP 5b, may be a specific method for the determination of bone resorption rate from serum samples [4,5]. So far there are no data about TRAP 5b in kidney allograft recipients and their correlation with other markers of bone resorption and formation. The majority of studies on renal osteodystrophy dealt with hemodialyzed patients, while a relatively low number have studied kidney allograft recipients and patients on (CAPD) chronic ambulatory peritoneal dialysis; extremely rarely these conditions have been studied comparatively.

Taking all this into consideration the aim of the study was to assess a new marker of bone resorption – TRAP 5b and its correlation with other markers of bone metabolism and BMD in kidney transplant recipients and dialyzed patients.

Materials and methods

47 kidney allograft recipients (age range 26-63 years), with stable graft function (serum creatinine 1.6±0.7 mg/dl), no infection (C-reactive protein within the normal ranges), and liver dysfunction (normal prothrombin time and normal activities of alanine and asparaginine aminotransferases) were enrolled in the study. The immunosuppressive therapy consisted of cyclosporine (the average concentration was 151±52 ng/ml), azathioprine and prednisone. Patients were engrafted for a period of 1 to 10 years (mean time 48±28 months). The average time on dialyses before renal transplantation was 38±19 months.

Two groups of clinically stable dialyzed patients were also included in the study: 45 chronically hemodialyzed patients and 25 peritoneal dialysis patients maintained on CAPD. The causes of renal failure among HD patients varied between chronic glomerulonephritis (n=26), chronic interstitial nephritis (n=12), polycystic kidney disease (n=3) and other or unknown causes (n=4). In CAPD patients renal failure was due to glomerulonephritis (n=13), chronic interstitial nephritis (n=7), polycystic kidney disease (n=2) and other or unknown causes (n=3). All the patients were offered a choice as to the prefered dialysis modality after demonstration of both methods. Dialyzed patients were included in the study on the basis of having residual renal function (necessary to assessed urine DPD).

In HD patients blood was drawn in the morning between 8.00 and 9.00 am. to avoid circadian variations before the onset of dialysis session (and heparin administration) and after hemodialysis from the arterial line of hemodialysis system immediately before discontinuation of the extracorporeal circulation. Ultrafiltrate samples were also taken. All the patients had required regular hemodialyses for 4-5 h a day 3 times a week. Blood flow was usually 150-200 ml/min with a dialysate flow rate of 500 ml/min. Ultrafiltration was varied according to patient's actual weight. Among all HD patients, 41 subjects were dialyzed on polysulphone membranes (Fresenius, Bad Homburg, Germany) and 4 on cuprophane membranes (Gambro, Nipro, Braun or Terumo dialyzers). All the patients were dialyzed with bicarbonate dialysates.

In CAPD subjects blood samples were drawn in the morning when subjects, receiving their normal diet, appeared for routine office assessment of dialysis therapy after an overnight oral fast. The same applied for kidney transplant recipients. All the CAPD patients were performing four 2 l exchanges. They were using Baxter Twin Bag system or Fresenius Andy Plus system with low dialysate calcium concentration (1.25 mmol/l or 1.00 mmol/l, respectively). Dwell times were generally 4-6 h during the day and 8 h overnight. The osmotic pressure of CAPD fluid was adjusted in accordance with the extent of ultrafiltration in each patient. The patients height and weight were recorded for all groups.

At the time of the study, all the dialyzed patients were also receiving concomitant drugs as calcium carbonate (dose ranged from 3 to 11 g/d) and alphacalcidol (0.25 μg/d). None of the patients had received aluminium hydroxide or other drugs known to affect bone metabolism. None of the patients had any history of fractures. All the patients were Caucasians. None of female patients were postmenopausal. None of them were receiving estrogen therapy.

All the patients were informed about the aim of the study and gave their consent. The study was approved by Local Ethical Committee. Healthy volunteers served as a control group to establish the normal ranges for the markers of bone metabolism. Regional BMD were measured by a Lunar DPX bone densitometer according to the principle of DEXA. BMD measurements were determined for femoral neck and lumbar spine (L2-L4).

Intact PTH, osteocalcin concentrations were studied by radioimmunoassay using kits from Cis, France. Levels of PICP (procollagen type I carboxy-terminal extension peptide) and ICTP (procollagen type I cross-linked carboxy-terminal telopeptide) were estimated by radioimmunoassay kits from Orion Diagnostica, Finland. Serum CrossLaps-Ctx were assayed by ELISA using commercially kit from Osteometer, Denmark. Concentration of β2-microglobulin was measured using kits from AlphaDialab, Vienna, Austria. Bone-specifc alkaline phosphatase was estimated using commercially available kit from Metra Biosystem, USA. Creatinine concentration in serum and urine was assayed by means of a standard laboratory method. DPD in urine was assayed by immunochemiluminescence (ACS 180 Bayer) and expressed as DPD/creatinine ratios. Serum TRAP 5b was estimated using commercially available kit from Suomen Bioanalytika Oy, Finland. Data were analyzed using Statistica 6.0. computer software (Pearson or Spearman correlations).

Results

All the clinical and biochemical data are presented in the *Tab. 1*. In kidney transplant recipients concentrations of PTH, osteocalcin, ICTP, serum CrossLaps, PICP, β2-microglobulin were significantly higher than in healthy volunteers, whereas TRAP 5b, DPD, ALP and bALP were similar to those values obtained in the healthy subjects. In both groups of dialyzed patients PTH, osteocalcin, TRAP 5b, PICP, DPD, Ctx and β2-microglobulin were significantly higher, but concentrations of calcidiol and calcitriol were significantly lower than in the healthy volunteers. Dialyzed patients have higher DPD (only HD), ICTP, Ctx, β2-microglobulin and lower calcidiol and calcitriol than kidney transplant recipients. HD patients have higher DPD, ICTP and lower cholesterol than CAPD patients.

Table 1. **Markers of bone metabolism in the hemodialyzed patients (HD), peritoneally dialyzed patients (CAPD), kidney transplant recipients (Tx), and healthy volunteers (CG)**

	HD n=45	CAPD n=25	Tx n=47	CG n=25
age (years)	51±16	47±15	44±13	43 ±14
BMI (kg/m^2)	23.1±4.2	25.1±3.1	24.8±2.97	24.9±3.5
duration of dialyses (months)/time after Tx	38±29	33 ±24	38±19	NA
cholesterol (mg/dl)	176±46°	214±42**	219±41**	168±34
triglycerides (mg/dl)	119±62*	161±70***	221±43***	89±25
total protein (g/l)	6.40±0.60*	6.21±0.68**	6.82±0.68	6.99±0.65
albumin (g/l)	3.90 ±0.51*	3.52±0.59**	4.17±0.46	4.29±0.45
urea before HD (mg/dl) urea in CAPD, Tx,	119.3±27.2***	120.4±28.5***	99.7±32.2***	19.6±6.2
total Ca (mmol/l)	2.14±0.30##	2.09±0.30##	2.29±0.36	ND
P (mg/dl)	5.59±1.88##	4.83±1.86	4.54±1.59	ND
vit D_3 (ng/ml)	11.3±8.0***###	9.9±6.9***###	56.0±17.1	55.9±14.7
$1,25(OH)_2D_3$ (ng/ml)	12.8±5.1***###	12.7±5.8***###	27.9±8.8***	64.0±11.8
PTH (pg/ml)	319±300***	287±202***	129±102***	33±11
Osteocalcin (ng/ml)	171±102***##	172±90***##	99±61**	23±5
ALP (U/l)	152±116	101±68	100±51	ND
bALP (ng/ml)	45.0±31.5	49.0±31.0	31.6±21.2	18.1±5.5
PICP (μg/L)	238.9±114.8*	265.8±132.1*	213.4±105.4*	147.9±42.1
TRAP (U/L)	1.79±1.12*	1.94±1.63*	1.54±1.40	1.08±0.80
DPD (nM/mmol)	11.9±8.0***##°	7.1±4.1**	5.5±4.0	4.9±1.8
ICTP (μg/L)	42.6±18.0***#°	37.1±17.0***#	11.8±8.0***	2.6±08
Ctx (pmol/L)	14737±8694**#	15297±9984**#	10535±6945*	3079±1293
β2-microglobulin (μg/mL)	14.9±6.2***#	12.5±3.8***#	6.5±3.8*	2.2±0.7
BMD – lumbar spine – score (kg/m^2)	1.01±0.18	1.15±0.20*#	1.03± 0.17	ND
BMD – femur neck T-score (kg/m^2)	0.84±0.26	0.85±0.38	0.89± 0.14	ND

Values given are means ± SD; * p<0.05, ** p<0.01, *** p<0.001 vs control group; # p<0.05, ## p<0.01 ### p<0.001 vs Tx; ° p<0.05; HD vs CAPD; ND – not done, NA – not applicable

In our study in kidney transplant recipients TRAP 5b correlated significantly both with a marker of bone formation – ALP (r=0.34, p=0.01) and resorption (ICTP r=0.41, p=0.01, β2-microglobulin r=0.36, p=0.01) as well as with PTH (r=0.42, p=0.005). Moreover, TRAP 5b correlated with current concentration of cyclosporine (r=-0.30, p=0.04), current dosage of prednisone (r=-0.41, p=0.009), azathioprine (r=-0.33, p=0.01). In HD patients TRAP 5b correlated significantly with age (r=-0.33, p=0.028), time on dialyses (r=0.55, p=0.0001), serum albumin (r=0.30, p=0.046), Kt/V (r=0.45, p=0.002), PTH (r=0.40, p=0.008). Correlations between TRAP and total protein (r=0.27, p=0.077), phosphorus (r=0.27, p=0.077), alkaline phosphatase (r=0.29, p=0.054), bone-specific alkaline phosphatase (r=0.29, p=0.06), pH (r=-0.28, p=0.071) almost reach statistical significance. In CAPD patients TRAP 5b correlated with age (r=-0.43, p=0.029), albumin (r=0.38, p=0.047), ICTP (r=0.44, p=0.33). In the healthy volunteers TRAP 5b correlated with age (r=0.55, p=0.02), ICTP (r=0.47, p=0.045), β2-microglobulin (r=0.63, p=0.012). TRAP 5b did not correlate with BMD in any studied groups.

Discussion

So far, data about bone metabolism and its assessment in kidney transplant recipients are limited, particularly in comparison with dialyzed patients, both HD and CAPD. In the recent study, Durieux et al. [6] reported that serum $25(OH)D_3$, PTH and urinary N-telopeptides excretion were normal in kidney transplant recipients. Cruz et al. [7] reported that in kidney allograft recipients either high-bone turnover or normal bone turnover was observed. Patients with high-bone turnover exhibited lower BMD at the lumbar spine, and the hip and higher PYD or DPD and/or osteocalcin. In our study we assessed also a new marker of bone resorption – TRAP 5b. The osteoclast specific 5b isoform of TRAP has recently been proposed as a sensitive and specific marker of bone resorption. Recently, a new immunoassay was developed to estimate only TRAP 5b. Halleen et al. [4] have shown that serum TRAP 5b measure using this assay is a much more specific and sensitive marker of bone resorption than total serum TRAP [4]. In a recent paper, Halleen et al. [5] reported that changes in serum TRAP 5b after 6 months of hormone replacement therapy, determined by this immunoassay, correlated significantly with the changes of all markers of bone turnover determined, including serum N- and C-terminal propeptides of type I collagen and urinary-free deoxypyridinoline. They also reported an elevated TRAP 5b in patients with osteoporosis [5]. Moreover, in their study, performed on early postmenopausal women TRAP 5b showed a significant negative correlation with BMD. In our study in neither kidney transplant recipients nor dialyzed patients (both HD and CAPD, evaluated together or separately) TRAP 5b did not correlate with BMD.

Up to date, there are no data about TRAP 5b in kidney transplant recipients and its correlations with other markers

of bone metabolism. In patients on peritoneal dialyses TRAP 5b highly correlated with bALP, PTH and osteocalcin in the preliminary report of Poege et al. [8]. Nowak et al. [9] also described a statistically significant correlation between TRAP 5b and PTH in dialysed patients (both hemodialyzed and peritoneally dialyzed). In our study TRAP 5b correlated with PTH only in kidney transplant recipients and HD patients. In our study TRAP 5b correlated significantly with markers of resorption as well as with PTH. Moreover, TRAP 5b correlated negatively with current concentration of cyclosporine, current dosage of prednisone, azathioprine in kidney transplant recipients. According to Minisola et al. [10] serum CrossLaps (betaCtx) seems to be characterized by a superior sensitivity relative to TRAP 5b measurement, at least in the disorders studied (chronic renal failure, established osteoporosis, primary hyperparathyroidism, glucocorticoid excess etc.). However, we have reported previously that serum CrossLaps correlated with urine DPD and serum ICTP in CAPD subjects [11], such correlations were not observed in hemodialyzed patients. We have also observed that serum CrossLaps correlated positively with PTH, markers of bone formation – bALP, osteocalcin, PICP, and positively with ICTP – a marker of bone resorption [12]. Due to this dual correlations – with markers of bone formation and resorption, value of Ctx in assessment of renal osteodystrophy remains to be established.

In the study of Poege et al. [8] TRAP 5b did not differ between hemodialyzed and peritoneally dialysed patients. In our study, TRAP 5b in CAPD and HD patients was significantly higher when compared to the healthy volunteers. TRAP 5b in kidney transplant recipients was within normal ranges.

A reported by Minisola et al. [10] and Nakasato et al. [13] TRAP 5b activity in patients with chronic renal failure and maintenance hemodialyses was not significantly increased, but TRAP 5b concentration in HD patients were significantly higher than in the healthy volunteers. Nakasato et al. [13] suggested the presence of subpopulations of HD patients with increased TRAP 5b acitivity. As reported previously [8,9]. TRAP 5b was not affected by age, gender and time on dialysis in kidney transplant recipients, whereas in dialyzed patients (both HD and CAPD) and in the healthy volunteers TRAP 5b correlated positively with age as previously reported Minisola et al. [10] but only for healthy women. In the study of Lopez Gavilanes et al. [14], on patients with chronic renal failure (without hepatopathy) and 9 hemodialysis patients (with hepatopathy), good relation between PICP, total TRAP (not TRAP 5b) and the biochemical indexes of bone activity and PTH suggested the clinical values of these markers in the follow-up of renal osteodystrophy. There were any differences in levels of markers of bone metabolism between groups with and without liver damage, in spite of the fact that PICP and TRAP were cleared mainly by the liver. In our study patients with liver dysfunction were excluded from the study. Previously, only total TRAP was assayed as in the study of Lopez Gavilanes et al. [14]. Since it is a thermolabile enzyme, the results of its determination were sometimes difficult to interpret.

Concluding, since TRAP 5b correlated mainly with markers of bone resorption, it may serve as a new additional marker of bone resorption in the assessment of renal osteodystrophy.

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