Effect of hemodialysis and renal failure on serum biochemical markers of bone turnover

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**Abstract**

The aim of the present study was to evaluate the effect of hemodialysis and renal failure on serum bone markers. Serum total alkaline phosphatase (TAP), procollagen type I aminoterminal propeptide (PINP), and β-carboxyterminal telopeptide of type I collagen (β-CTX), as well as intact parathyroid hormone (iPTH), creatinine, and total protein were measured in 14 patients with endstage renal disease (ESRD) before and at 1, 2, and 4 h during a hemodialysis session, and at the same sampling interval in 6 renal transplant recipients. The results were compared to those obtained in 20 healthy adults. All patients showed increased baseline mean values of PINP, β-CTX, and iPTH. β-CTX differed significantly between hemodialysis patients and renal transplant recipients. TAP and β-CTX were the only markers which correlated with iPTH (P < 0.05) and creatinine values (P < 0.001), respectively. Renal transplant recipients did not show significant variations in the evolution of mean values of bone markers throughout the study, whereas, during the dialysis period, all the bone markers analyzed in the study showed a significant change. The change differed depending on the marker considered: β-CTX showed a significant decrease at the end of the session, TAP increased at this time and, although PINP showed an initial increase during hemodialysis, no significant changes were observed at the end of the session. We conclude that bone markers are significantly influenced by hemodialysis, especially serum TAP and β-CTX. ESRD is associated with an increase in these bone markers, in some cases related to iPTH values and in others to glomerular function. These findings should be taken into account when evaluating bone markers in these patients.

**Key words**

β-carboxyterminal telopeptide of type I collagen · intact parathyroid hormone · procollagen type I aminoterminal propeptide · total alkaline phosphatase

**Introduction**

Endstage renal disease (ESRD) is often associated with various metabolic bone disorders, mainly secondary hyperparathyroidism, which can persist after kidney transplantation [1]. The gold standard for the precise diagnosis of renal osteodystrophy is obtained by bone histology. However, this is an invasive and time-consuming method not suitable for routine clinical measurement. Consequently, the determination of serum intact parathyroid hormone (iPTH) has been used for many years as an indirect predictor of bone disease in renal osteodystrophy [2,3]. In recent years a great number of biochemical markers have been developed which allow us to determine more precisely the evolution of bone turnover. In addition, they have proven to be of value in assessing bone metabolism, and some of them have shown good predictive values for the evaluation of bone turnover, especially in women with postmenopausal osteoporosis. Studies on the usefulness of bone markers in patients with renal disease are scarce, and only include a small number of markers. These studies have shown a moderate correlation between some markers, such as serum bone alkaline phosphatase, osteocalcin, pyridinoline (Pyr), and deoxypyridinoline (Dpyr), and histomorphometric parameters of bone turnover [4–7]. However, it should be taken into account that renal function can modify the serum and urinary levels of all these markers. It is well known that in healthy people some markers, such as Pyr and Dpyr, and carboxy- and amino-terminal telopeptides of type I collagen and osteocalcin are cleared by the kidneys, and some of them (osteocalcin, telopeptides) are also metabolized in the kidneys [8,9]. Therefore, in renal failure these markers may not accurately reflect bone turnover. In addition, little data are available on the effect of hemodialysis on the serum levels of bone markers and, theoretically, the dialysis procedure could modify the serum concentrations of these markers.
All of these considerations make it necessary to study the effect of renal failure and hemodialysis on the serum concentrations of the new available serum markers of bone turnover.

Therefore, the aim of this study was to evaluate the effect of hemodialysis and renal failure on serum concentrations of new bone markers such as procollagen type I aminoterminal propeptide (PINP) and β-carboxyterminal telopeptide of type I collagen (β-CTX).

**Subjects and methods**

**Subjects**

The study included 14 patients on hemodialysis, 10 men and 4 women, aged 18–73 years (mean ± SD; 39 ± 4.3 years). These patients had been undergoing hemodialysis for an average of 6 ± 2.3 years and all were receiving calcium carbonate as a phosphorus chelant.

In order to compare the variability of bone markers, an additional group, of 6 renal transplant recipients (3 men and 3 women, aged between 32 and 65 years) with correct renal function (creatinine clearance >60 ml/min per 1.73 m²) was included in the study. All recipients are currently being treated with the same immunosuppression protocol, including cyclosporine A and 10 mg/day of prednisone. No subject has had any other treatment known to influence bone metabolism. At the time of the study, the mean time since transplantation was 3 ± 0.3 months, and the mean ± SD serum creatinine level was 167 ± 41 µM/l.

Reference values were obtained from 20 healthy subjects of similar age (control group). All subjects provided informed consent for participation, and the Ethics Committee of the Hospital approved the study.

**Study design**

The study was prospective. Hemodialysis was performed using a low-permeability dialyser (cellulose triacetate). Laboratory assessment was carried out on each subject at baseline (between 8:30 and 9:00 a.m.) and at 1, 2, and 4 h during dialysis. The same sampling interval was used in the kidney transplant group.

**Biochemical analysis**

Blood samples were drawn after an overnight fast. Tubes containing the blood samples for determination of iPTH were immediately placed on ice for a maximum period of 2 h until centrifugation. The serum was then stored at −20°C until analysis.

Creatinine, total protein, and alkaline phosphatase measurements were performed shortly afterwards on the same day and serum aliquots for measurement of bone markers were stored frozen at −20°C until analysis.

The following bone formation markers were determined: serum total alkaline phosphatase (TAP) activity, which was measured by a spectrophotometric kinetic assay according to the recommendations of the Scandinavian Committee for Clinical Chemistry and Clinical Physiology, using DEA buffer in a DAX 72 analyzer (Bayer Diagnostics, Tarrytown, NY, USA) and serum PINP, which was determined by radioimmunoassay, using a kit from Orion Diagnostica (Espoo, Finland). As a marker of bone resorption, we determined serum β-CTX, which was analyzed by electrochemoluminescence in an Elecsys 2010 automated analyzer (Roche Diagnostics, Mannheim, Germany), using β-Crosslaps serum reagents. iPTH was measured by an immunoradiometric assay (Allegro; Nichols Institute Diagnosis, San Juan Capistrano, CA, USA). Creatinine was measured using a modified Jaffe method, and total protein was measured by the Biuret method, both being done in the DAX 72 analyzer with the reagents supplied by the manufacturer (Bayer Diagnostics).

The intraassay coefficients of variation for bone markers and iPTH, at both normal and pathological levels, were as follows: TAP, 0.75% and 0.65%; PINP, 4.0% and 3.1%; β-CTX, 3.1% and 0.83%; and iPTH, 3% and 4%, respectively. The interassay coefficients of variation for each of these assays, at both normal and pathological levels, were as follows: TAP, 3.1% and 1.5%; PINP, 5.6% and 7.4%; β-CTX, 3.6% and 0.88%; and iPTH, 10% and 5%, respectively. The intraindividual variabilities (coefficients of variation) of bone markers obtained from healthy women were as follows: TAP, 4.5%; PINP, 18.4%; and β-CTX, 38.9%. The reference ranges for controls were as follows: TAP, 77–238 U/l; PINP, 16–53 ng/ml; β-CTX, 0.154–0.483 ng/ml; and iPTH, 10–65 pg/ml.

**Statistical analysis**

Values for results are expressed as means ± SEM. Differences between groups were assessed by means of the Mann-Whitney U-test. Comparisons of the variations of bone markers between and within groups were made by multivariate analysis of variance (MANOVA) for repeated measures. MANOVA was also used to analyze separately variations in both study populations with time. The Spearman’s rank correlation test was used for correlation studies. Values of $P < 0.05$ were considered significant. Statistical analyses were performed using SPSS software (Chicago, IL, USA) for Windows (version 10.0).