

## Biochemical markers of bone turnover in the diagnosis of renal osteodystrophy in dialyzed children

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**SUMMARY:** Baskın E, Beşbaş N, Saatçi Ü, Hasçelik G, Topaloğlu R, Özen S, Bakkaloğlu A. Biochemical markers of bone turnover in the diagnosis of renal osteodystrophy in dialyzed children. Turk J Pediatr 2004; 46: 28-31.

In this study we investigated the value of biochemical markers of bone turnover in the diagnosis of renal osteodystrophy in dialysis patients. The study was carried out in 22 chronic renal failure patients (mean age: 16.1±4.5) being treated with chronic dialysis. There were three groups according to intact parathormone (iPTH) levels: Group I (n: 6): iPTH levels were less than 200 pg/ml; Group II (n: 9): iPTH levels were between 201 and 500 pg/ml; and Group III (n: 7). iPTH levels were higher than 501 pg/ml. We investigated iPTH, bone alkaline phosphatase, total serum alkaline phosphatase, osteocalcin, serum type I procollagen peptide (PICP) and insulin-like growth factor-1 (IGF-1) levels in all patients.

In group III mean bone alkaline phosphatase level (126.0±10.95) was significantly higher than in both group I and group II (52.16±22.8, 57.35±16.21) (p<0.001). Mean osteocalcin level (35.13±2.93) in group I was significantly lower than in group III (40.52±2.83) (p<0.05). Serum alkaline phosphatase, PICP and IGF-1 levels were not different between the groups (p>0.05). There was a significant positive correlation between bone alkaline phosphatase and iPTH (r=0.80, p<0.0001). Serum osteocalcin correlated with both bone alkaline phosphatase and iPTH (correlation) coefficients were r=0.44 and r=0.51 respectively, p<0.05). It is concluded that bone alkaline phosphatase and osteocalcin combined with iPTH level seem to be useful noninvasive markers of bone metabolism in dialysis patients.

**Key words:** renal osteodystrophy, biochemical markers, bone alkaline phosphatase, osteocalcin, chronic dialysis.

It has been known for over 100 years that bone disease accompanies renal failure. Bone histology remains the gold standard for the diagnosis of renal osteodystrophy and the distinction between high and low bone turnover disease. However, bone biopsy is an invasive procedure accompanied by technical difficulties in the processing and studying of the specimens. For that reason, specific and sensitive serum biochemical markers are required for monitoring bone turnover in uremia. The ideal biochemical marker of bone turnover should be unique to bone and reflect total skeletal activity and be well correlated with histomorphometric and radiocalcium kinetics results<sup>1</sup>. Serum alkaline phosphatase has been used as a biochemical marker of bone disease for many years. But total alkaline phosphatase

originates from different organs (liver, bone, intestine, placenta etc.) and sometimes lacks specificity. In the last decade it has been shown that measurement of intact parathormone (iPTH) is a useful predictor of bone histology and a noninvasive tool in distinguishing between high turnover, normal and low turnover bone disease in large patient groups<sup>2</sup>. However, in an individual patient serum iPTH alone is frequently unable to distinguish adynamic bone from hyperparathyroid bone disease<sup>3</sup>. Combined with other biochemical markers it may be useful in solving this problem.

In this study we investigated the value of biochemical markers of bone turnover in the diagnosis of renal osteodystrophy in dialysis patients.

## Material and Methods

The study was carried out in 22 chronic renal failure patients being treated with chronic dialysis. The study group included 11 girls and 11 boys, ages between 8 and 18 years (mean age  $15.1 \pm 3.5$ ). Dialysis therapy was hemodialysis for three to four hours thrice weekly in 17 patients and continuous ambulatory peritoneal dialysis in five patients. There were three groups according to iPTH levels. Group I included 6 patients (2 girls, 4 boys), and iPTH levels were less than 200 pg/ml. Group II included 9 patients (5 girls, 4 boys) and iPTH levels were between 201 and 500 pg/ml. Group III consisted of 7 patients (4 girls, 3 boys), and iPTH levels were higher than 501 pg/ml.

Phosphate binder therapy consisted of either calcium acetate or calcium carbonate. Aluminum hydroxide was not used unless hypercalcemia developed. None of the patients had clinical or biochemical evidence of liver disease.

500 pg/ml (group III), parathyroid glands were imaged with ultrasonography and technetium-99m-sestamibi ( $^{99m}\text{Tc-MIBI}$ ) for the detection of parathyroid hyperplasia or adenoma.

Statistical analysis included the evaluation of correlation matrix, one-way ANOVA, and Mann-Whitney U test. A value of  $p < 0.05$  was considered significant. All the results are expressed as the mean  $\pm$  SD.

## Results

Mean ages of groups I, II and III were  $14.7 \pm 4.1$ ,  $16.2 \pm 4.8$  and  $15.3 \pm 3.9$ , respectively ( $p > 0.05$ ). Mean bone alkaline phosphatase, osteocalcin, PICP, IGF-1 and total alkaline phosphatase levels are shown in Table I.

In group III patients mean bone alkaline phosphatase level ( $126.0 \pm 10.95 \mu\text{t}$ ) was significantly higher than in both group I ( $52.16 \pm 22.8 \text{ u/L}$ ) and group II ( $57.35 \pm 16.21 \text{ u/L}$ ) ( $p < 0.001$ ) (Table I). Mean osteocalcin level

**Table I.** Biochemical Markers in Dialysis Patients

	Group I* (n=6)	Group II* (n=9)	Group III* (n=7)	
BAP (U/L)	$52.16 \pm 22.8^{\dagger}$	$57.33 \pm 16.21$	$126 \pm 10.95^{\ddagger}$	$^{\dagger}(p: 0.0000)$
Osteocalcin (ng/ml)	$35.13 \pm 2.93^{\S}$	$39.11 \pm 3.96$	$40.52 \pm 2.83^{\S}$	$^{\S}(p: 0.027)$
PICP (ng/ml)	$249.80 \pm 180.12$	$347.31 \pm 174.71$	$423.20 \pm 208.63$	$(p: 0.27)$
IGF-1 (ng/ml)	$192.00 \pm 136.97$	$297.45 \pm 229.59$	$445.21 \pm 279.60$	$(p: 0.15)$

\* Mean  $\pm$  SD.

Immediately before a dialysis session, blood samples were drawn from the arterial port for the assay of iPTH, bone alkaline phosphatase, total serum alkaline phosphatase, osteocalcin and serum type 1 procollagen peptide (PICP). iPTH was determined by chemiluminescent enzyme immunoassay (Immulate 2000, Bio DPC, USA); the normal range for this assay is 12-62 pg/ml. Serum osteocalcin was measured by ELISA (Novocalcin, Metra Biosystems, Inc., USA): the normal range is 3.40-9.10 for males and 3.70-10 for females. Total alkaline phosphatase, calcium and phosphate levels were determined by autoanalyzer (Hitachi 747). Bone alkaline phosphatase level was measured by ELISA (Alk phase-B, Metra Biosystems, Inc., USA). Serum PICP was evaluated by ELISA (Prolage-C Metra Biosystems, Inc., USA). Insulin-like growth factor-1 (IGF-1) level was measured by two-site immunoradiometric assay (DSL-5600 ACTIVE IGF-1 Coated-Tube IRMA Kit, Diagnostic System Laboratories, Inc, USA). In patients with PTH levels higher than

( $35.13 \pm 2.93 \text{ ng/ml}$ ) in group I (iPTH levels less than 200 pg/ml) was significantly lower than in group III ( $40.52 \pm 2.83 \text{ ng/ml}$ ) ( $p < 0.05$ ). However, there was no significant difference between group II and group III ( $p > 0.05$ ). Group I patients had lower mean serum PICP and IGF-1 levels than group II and group III, but the difference was not significant ( $p > 0.05$ ). There was no significant correlation between PICP and bone alkaline phosphatase ( $r = 0.39$ ,  $p > 0.05$ ). Although total alkaline phosphatase level was higher in group III than in the others, there was no significant correlation between total alkaline phosphatase and iPTH ( $r = 0.33$ ,  $p > 0.05$ ) (Table II).

There was a significant positive correlation between bone alkaline phosphatase and iPTH ( $r = 0.80$ ,  $p < 0.0001$ ) (Fig. 1). Bone alkaline phosphatase also correlated with total alkaline phosphatase ( $r = 0.47$ ,  $p < 0.05$ ). Serum osteocalcin correlated with both bone alkaline phosphatase and iPTH (correlation coefficients were  $r = 0.44$  and  $r = 0.51$ , respectively,  $p < 0.05$ ).

**Table II.** Biochemical Markers and Correlation Matrix

	iPTH		BAP	
TAP	0.33	(P: 0.13)	0.47*	(P: 0.031)
Osteocalcin	0.51*	(p: 0.014)	0.44*	(p: 0.035)
PICP	0.28	(p: 0.19)	0.39	(p: 0.06)
IGF-1	0.37	(p: 0.08)	0.38	(p: 0.07)
BAP	0.80*	(p: 0.0000)		

\* Significantly correlated.

PICP: type 1 procollagen peptide, BAP: bone alkaline phosphatase, TAP: total alkaline phosphatase, IGF-1: insulin-like growth factor-1, iPTH: intact parathormone.

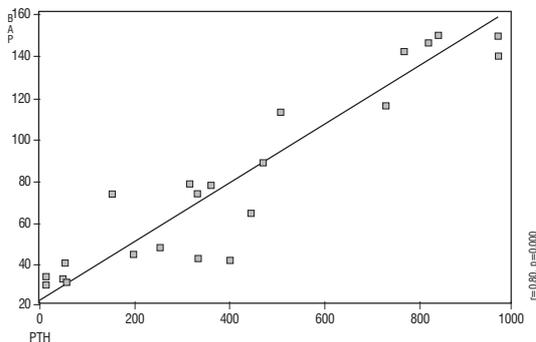


Fig. 1. Relationship between intact parathormone (iPTH) and bone specific alkaline phosphatase (BAP).

## Discussion

In the last years, several enzymes and matrix proteins synthesized by osteoblasts and protein fragments released after bone matrix breakdown have been proposed as serum biochemical markers of bone formation and bone resorption. Markers of bone formation are alkaline phosphatase, bone-specific-alkaline phosphatase, osteocalcin, PICP, and IGF-1. Markers of bone resorption are tartrate-resistant acid phosphatase, PICP cross-linked telopeptide, pyridinoline and deoxypyridinoline, beta-2 microglobulin, bone sialoproteins, cytokines and growth factors<sup>1,4,5</sup>.

In our study bone alkaline phosphatase showed significant correlation with iPTH and correlation coefficients (r) values better than those of total alkaline phosphatase and osteocalcin. Serum bone alkaline phosphatase is considered superior in determining total alkaline phosphatase activity for assessing bone metabolism, as has been previously reported<sup>6-8</sup>. Although optimal iPTH cut-off levels have been uncertain, iPTH of less than 100 pg/ml may strongly suggest adynamic bone disease<sup>9</sup>. Recently it was found that adynamic bone disease should be suspected when plasma iPTH

levels were less than 150 pg/ml and bone alkaline phosphatase (BAP) levels were lower than 27 pg/ml<sup>10</sup>. In our patients with PTH levels less than 200 pg/ml, mean osteocalcin and BAP level were significantly lower than in group III patients. This may be associated with decreased bone formation. Therefore, low iPTH combined with low osteocalcin and BAP levels may reflect low turnover bone disease.

In our patients we did not observe a significant correlation between serum PICP and other parameters as has been previously reported<sup>11,12</sup>. It is suggested that PICP is not a sensitive marker of bone metabolism in uremia. Recently it has been reported that pyridinoline seems to be currently the most sensitive and specific marker for evaluation of bone resorption in renal osteodystrophy<sup>11,13</sup>. Besides these biochemical markers, serum beta-2 microglobulin, cytokines and growth factors might have a role in the noninvasive diagnosis of renal osteodystrophy<sup>14-16</sup>. Ferreira et al.<sup>14</sup> found serum beta-2 microglobulin correlated with osteocalcin, BAP and pyridinoline, and in patients with high turnover bone disease, serum beta-2 microglobulin was higher than in patients with normal and low turnover bone disease. IGF-1 might also prove to be of potential interest in evaluating bone turnover. In addition to being an indicator of nutritional status, it has been suggested that IGF-1 could serve as a bone remodeling marker as well<sup>17</sup>. Beşbaş et al.<sup>17</sup> reported that determination of IGF-1 levels in childhood hemodialysis patients in conjunction with anthropometric measurements is useful for identification of nutrition. However, other clinical studies have not confirmed a correlation between IGF-1 and serological or histological parameters of renal osteodystrophy<sup>18</sup>. In our patients we did not observe a significant correlation between IGF-1 and other parameters.

The evaluation of bone turnover should include a combination of different markers so that the balance between bone formation and bone resorption can be evaluated. Bone alkaline phosphatase and osteocalcin seem to be good markers for bone formation but measurement of other markers such as pyridinoline are needed to determine bone resorption<sup>1,13</sup>.

It is concluded that BAP and osteocalcin combined with iPTH seem to be useful noninvasive markers of bone metabolism in chronic dialysis patients both with high turnover and low turnover bone disease.

Future studies are clearly needed to better understand the value of these markers in uremic patients.

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