Bone alkaline phosphatase in children with chronic renal failure

Barbera Behnke¹, Markus Kemper¹, Hans-P. Kruse² and Dirk E. Müller-Wiefel¹

Departments of ¹Pediatrics and Nephrology and ²Osteology, University Hospital Eppendorf, Hamburg, Germany

Abstract

Background. With the introduction of a new immuno-radiometric assay based on two monoclonal antibodies (Tandem®-Ostase, Hybritech) the determination of bone alkaline phosphatase (BAP) to evaluate bone metabolism in chronic renal failure has become easier and more valid.

Subjects and methods. Using this test we investigated BAP in a total of 90 paediatric patients, 42 (9.2 ± 5.5 years) with chronic renal failure on conservative treatment, 22 (9.5 ± 5.4 years) under chronic dialysis, and 26 (16.2 ± 5.9 years) after renal transplantation, compared to 203 controls (10.1 ± 5.7 years).

Results. The physiological age dependency found in controls including two peaks during infancy and puberty was distinctly disturbed in chronic renal failure. However, in patients BAP significantly correlated with height velocity rather reflecting the last 6 (r = 0.56 P < 0.001) than the last 12 months. Although BAP correlated well with total alkaline phosphatase (TAP; r = 0.95 P < 0.001), a significant correlation with the serum level of the intact parathyroid hormone could only be detected for BAP (r = 0.45 P < 0.001) but not for TAP (r = 0.19 n.s.). Furthermore, BAP positively correlated with trabecular (n = 40; r = 0.40 P < 0.05) and inversely with cortical bone density (n = 19; r = −0.58 P < 0.01) but no relationship was found with conventional X-ray.

Conclusion. BAP determined by the new radio-immunoassay seems to represent an additional diagnostic tool to assess growth and bone turnover in paediatric patients with chronic renal failure that is complementary to the information provided by X-ray and total alkaline phosphatase.

Key words: bone alkaline phosphatase; children; chronic renal failure

Introduction

Up to now, for the clinical monitoring of bone turnover in patients with chronic renal failure (CRF), the determination of total alkaline phosphatase (TAP) has been used. TAP, however, is known to be a mixture of different isoenzyme families coded by at least four genes [1]: (1) the placental, (2) the placental-like, (3) the intestinal, and (4) the tissue non-specific group which consists of liver, bone, and kidney isoenzymes, who differ only by post-translational glycosylation [2].

The bone isoenzyme exclusively derives from osteoblasts where it is expressed during the maturation and early mineralization phases [3] so that it might be suspected as a suitable marker of bone formation, being more specific in indicating disturbances in bone turnover than TAP [4–6].

Previous methods to differentiate and quantitate the bone isofrom, such as heat [7,8,17] and chemical inactivation [9–11], agarose electrophoresis [12,1], high-performance liquid chromatography [14–16], and wheat germ agglutinin (WGA) precipitation [17,18] have been based upon techniques not suitable for routine clinical use.

By the introduction of a new specific immuno-radiometric assay using two monoclonal antibodies (Tandem®-Ostase, Hybritech Europe S. A.) [5,6,13,17,19] for the determination of bone alkaline phosphatase (BAP) we investigated osteoblastic activity in paediatric patients with CRF and tried to evaluate its dependency on growth and development followed by a comparison of the BAP-values with other serological and radiological tools for the diagnosis of renal osteodystrophy.

Subjects and methods

We investigated a total of 90 paediatric patients (54 males, 36 females). The distribution according to the different treatment groups is given in Table 1 showing chronological and skeletal age. The 203 controls (hospital patients; blood sampling indicated for other reasons like preoperative control or exclusion of bacterial infection) were sex and age matched without acute and chronic disease or medication with known influence on bone metabolism nor fracture or immobilization in history (Table 1). Blood chemistry of the patients is given in Table 2.

Twenty-one patients presented radiological changes of osteodystrophy predominantly caused by hyperparathyroidism, such as subperiosteal resorption and cortical thinning (7 in CT, 7 in D, 7 in TP respectively), 43 patients showed...
Table 1. Patients and controls

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>M</th>
<th>F</th>
<th>Chronological age (years)</th>
<th>Skeletal age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>90</td>
<td>54</td>
<td>36</td>
<td>mean ± SD (range)</td>
<td>mean ± SD (range)</td>
</tr>
<tr>
<td>Conservative</td>
<td>42</td>
<td>26</td>
<td>16</td>
<td>9.2 ± 5.5 (1.0–21.8)</td>
<td>8.3 ± 5.5 (0.5–21.8)</td>
</tr>
<tr>
<td>Dialysis</td>
<td>22</td>
<td>13</td>
<td>9</td>
<td>9.5 ± 5.4 (0.5–18.5)</td>
<td>8.1 ± 4.8 (0.5–17.0)</td>
</tr>
<tr>
<td>Transplantation</td>
<td>26</td>
<td>15</td>
<td>11</td>
<td>16.2 ± 5.9 (3.5–25.0)</td>
<td>14.9 ± 6.2 (2.8–25.0)</td>
</tr>
<tr>
<td>Controls</td>
<td>203</td>
<td>91</td>
<td>112</td>
<td>10.4 ± 5.7 (0.5–23.8)</td>
<td>not done</td>
</tr>
</tbody>
</table>

Table 2. Serum parameters of mineral metabolism

<table>
<thead>
<tr>
<th></th>
<th>Conservative treatment</th>
<th>Dialysis</th>
<th>Transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone alkaline phosphatase (μg/l)</td>
<td>62.1 ± 30.3</td>
<td>52.5 ± 34.8</td>
<td>53.3 ± 51.7</td>
</tr>
<tr>
<td>Total alkaline phosphatase (U/l)</td>
<td>405.2 ± 171.1</td>
<td>345.9 ± 256.9</td>
<td>292.1 ± 234.7</td>
</tr>
<tr>
<td>Parathyroid hormone (ng/l)</td>
<td>246.2 ± 134.7</td>
<td>177.5 ± 121.3</td>
<td>185.2 ± 108.3</td>
</tr>
<tr>
<td>Serum calcium (mmol/l)</td>
<td>2.46 ± 0.21</td>
<td>2.59 ± 0.30</td>
<td>2.52 ± 0.20</td>
</tr>
<tr>
<td>Serum phosphate (mmol/l)</td>
<td>1.51 ± 0.42</td>
<td>2.03 ± 0.43</td>
<td>1.26 ± 0.37</td>
</tr>
<tr>
<td>Ca × PO₄²⁻ product (mmol/l × mmol/l)</td>
<td>3.75 ± 1.19</td>
<td>5.06 ± 0.77</td>
<td>3.15 ± 0.92</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>2.2 ± 1.8</td>
<td>8.7 ± 2.1</td>
<td>2.0 ± 1.4</td>
</tr>
</tbody>
</table>

Table 3. Correlations of serum bone alkaline phosphatase with other parameters of mineral metabolism

<table>
<thead>
<tr>
<th></th>
<th>Bone alkaline phosphatase (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total alkaline phosphatase (U/l)</td>
<td>r = 0.95*</td>
</tr>
<tr>
<td>Parathyroid hormone (ng/l)</td>
<td>r = 0.45*</td>
</tr>
<tr>
<td>Serum calcium (mmol/l)</td>
<td>r = -0.15</td>
</tr>
<tr>
<td>Serum phosphate (mmol/l)</td>
<td>r = -0.01</td>
</tr>
<tr>
<td>Ca × PO₄²⁻ product (mmol/l × mmol/l)</td>
<td>r = -0.05</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>r = 0.22</td>
</tr>
</tbody>
</table>

*P < 0.001.

predominantly osteomalacic signs such as demineralization (blurred trabecular bone, ground-glass effect) (18 in CT, 12 in D, 13 in TP respectively), and 26 patients had a normal skeletal X-ray.

Seventy-four patients received calcitriol (37 in CT, 21 in D, 16 in TP) in a dose of 0.25 μg/m² BSA daily or intermittently, 13 patients vitamin D3 (2 in CT, 1 in D, 10 in TP) in a dose of 1000 IU/m² BSA/day, and three patients did not get any vitamin D treatment (CT). Aluminium-containing phosphate binding agents had not been used.

After renal transplantation a combined immunosuppression by prednisone, azathioprine, and cyclosporin A (CsA) was undertaken.

BAP was determined by the Tandem®-Ostase-Test (Hybritech Europe S. A.), a solid-phase, two-site immunoradiometric assay based on two monoclonal antibodies with a coefficient of variation for within-run precision of 3.7–6.7% and for between-run precision of 7.0–8.1% (data from Hybritech) and a minimum detectable concentration of 2 μg/l.

TAP, serum calcium, and phosphate were measured by standardized colorimetric techniques, serum creatinine (SCr) by spectrophotometric methods, and the intact parathyroid hormone (PTH) by means of a monoclonal radioimmunoassay (PTH Kit, Nichols Institutes, Bad Nauheim).

Bone mineral density of the distal radius was measured by peripheral quantitative computed tomography (XCT 900, Stratec, Germany) differentiating between trabecular bone density (TBD; n = 40) distal measured at a distance of 4% and cortical bone density (CBD; n = 19) proximal measured (according to Schönau) at a distance of 20% of the total radius length proximal to the epiphyseal growth plate and expressed in mg/cm² [20–22]. Values are given as means ± SD (medians in case of non-normal distribution). For the calculation of the standard deviation score (SDS) we divided patients and controls in sex-and age-matched groups (males 0–3 years/>3–10 years/>10–15 years/>15–25 years; females 0–3 years/>3–8 years/>8–14 years/>14–25 years). Age-matching of patients was related to bone age.

Statistical evaluation was performed by Wilcoxon’s rank test and by Pearson’s coefficient of correlation. P values lower than 0.05 were considered to be statistically significant.

Skeletal age was determined by X-ray of the wrist according to Greulich Pyle. X-rays were evaluated always by the same radiologist using conventional and special techniques (mammography).

Results

In normal children BAP ranged between 6.0 and 183.5 μg/l with a mean value of 68.7 ± 49.2 μg/l, and two peaks, in infancy and puberty, with an earlier onset in girls than in boys (Figure 1). In children with CRF BAP was in a comparable range (6.5–360.0 μg/l)
corresponding to a mean value of $78.9 \pm 52.7 \mu g/l$. However, the relationship between BAP and chronological age could not be demonstrated in CRF and even showed only a slight tendency to the curve found in controls when calculated for skeletal age (Figure 2). The highest BAP-levels in CRF could be detected in pubertal stage III according to Tanner followed by a steep decline to stage IV and V to levels nearly equivalent to adults (Figure 3). Accordingly, BAP in CRF significantly correlated with height velocity rather reflecting the last 6 than the last 12 months ($r=0.56 P<0.001$, $r=0.49 P<0.001$ respectively) (Figure 4) whereas TAP reflected longitudinal growth to a minor degree ($r=0.49 P<0.001$, $r=0.46 P<0.001$ respectively).

However, BAP positively correlated with TBD ($r=0.40 P<0.05$) and inversely with CBD ($r=-0.58 P<0.01$) whereas there was no association to conventional X-ray. BAP-SDS was either increased or decreased in a high proportion of patients with a predominant hyperparathyroid or osteomalacic component in X-ray or even with missing radiological lesions under all forms of treatment (Figure 5).

Although BAP closely correlated with TAP ($r=0.95 P<0.001$), a significant correlation with PTH only could be detected for BAP ($r=0.45 P<0.001$) but not
reactivity to the liver-related isoenzyme up to 16% is reported [5,13] which has to be considered at least in the case of normal or elevated levels. It has to be proven whether by ELISA technique this cross-reactivity can be decreased [23].

The physiological age dependency of BAP in C (Figure 1) is well in line with previous studies [24–26]. It has got lost in CRF even when corrected for skeletal age (Figure 2) reflecting disturbances of growth and development in uraemic children [27]. However, during puberty the highest levels in CRF were found at stage III which was also reported for healthy girls [26] and is congruent with the peak height velocity in stage II–III.

Concerning the correlation with height velocity (Figure 4) BAP was superior to TAP and reflected the previous 6 months better than the previous 12 months function investigated was found (Table 3).

**Discussion**

The new IRMA for the determination of BAP in serum is based on two monoclonal antibodies derived from an osteosarcoma cell line. Compared to previous methods to differentiate and quantitate BAP [7–18] its main advantage is the technically simple, convenient, and reproducible procedure of determination that does not require any pretreatment of the samples (100 µl) suitable for routine laboratory use. However, a cross-reactivity to the liver-related isoenzyme up to 16% is reported [5,13] which has to be considered at least in the case of normal or elevated levels. It has to be proven whether by ELISA technique this cross-reactivity can be decreased [23].

The physiological age dependency of BAP in C (Figure 1) is well in line with previous studies [24–26]. It has got lost in CRF even when corrected for skeletal age (Figure 2) reflecting disturbances of growth and development in uraemic children [27]. However, during puberty the highest levels in CRF were found at stage III which was also reported for healthy girls [26] and is congruent with the peak height velocity in stage II–III.

Concerning the correlation with height velocity (Figure 4) BAP was superior to TAP and reflected the previous 6 months better than the previous 12 months function investigated was found (Table 3).

BAP also proved to be superior to TAP regarding the relationship to PTH ($r=0.45$ vs $r=0.19$). This finding is all the more important as the level of PTH in serum is reported to be one of the major determinants of cancellous bone formation and turnover in children with chronic renal failure [29].

The superiority of BAP over TAP is in line with the results of Garnero and Delmas [5] studying BAP and TAP levels in adult patients with different metabolic
Bone diseases compared to healthy controls. They also demonstrated a better correlation with PTH for BAP than for TAP. Urena et al. [6] who compared BAP and TAP values of haemodialysed patients with histomorphometric parameters of bone showed on the one hand the stronger relation of BAP to PTH and on the other a higher diagnostic value of BAP than of TAP regarding the histological stage of osteodystrophy.

The positive correlation of BAP with TBD, the metabolically most active part of bone, points to the significance of BAP being a marker of bone formation: It catalyses the hydrolysis of phosphate ester [1] thereby enhancing the mineralization by hydrolysis of pyrophosphate, an important inhibitor of mineralization [30], and by providing a high phosphate concentration at the osteoblastic surface. For CBD which is known to be reduced by hyperparathyroidism much more than is TBD [31] we found an inverse correlation with BAP, which might be mainly the consequence of secondary hyperparathyroidism.

Skeletal X-ray changes in children with chronic renal failure may show resorptive defects in cortical bone and altered amount of cancellous bone (reduced or even increased) as well as epiphyseal slipping and necrosis [32]. Lesions appeared either as the result of the osteomalacic component of osteodystrophy or of hyperparathyroidism or of combined lesions [4]. Regarding these eventual changes there was no clear association of BAP with conventional X-ray (Figure 5). This finding is not unexpected because BAP as a marker of activity is influenced by a variety of normal and pathological metabolic processes [30] indicating even small changes and early disturbances in bone turnover whereas conventional X-ray is known to be altered rather lately in bone disease and only presents a static view irrespective of the activity of bone metabolism. Accordingly, there were 11 patients (12%) with increased or decreased BAP-values and missing radiological lesions.

In conclusion, BAP, as detected by the Tandem® Ostase-Test seems to represent an additional diagnostic tool for both longitudinal growth and bone turnover in paediatric patients with chronic renal failure, especially for the evaluation of their osteoblastic activity.

Acknowledgements. We thank Prof. Dr E. Richter, Director of the Department of Pediatric Radiology, for X-ray examinations of the skeleton and Mrs Meike Kunkel for excellent technical assistance.

References
5. Garnero P, Delmas PD. Assessment of serum levels of bone alkaline phosphatase with a new immunoradiometric assay in
Bone alkaline phosphatase in children with chronic renal failure

patients with metabolic bone disease. J Clin Endocrinol Metab 1993; 77: 1046–1053


Received for publication: 27.3.97
Accepted in revised form: 8.10.97