Relationship between serum alkaline phosphatase and pyridoxal-5'-phosphate levels in hypophosphatasia

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1. Hypophosphatasia is a disorder characterized by low serum levels of alkaline phosphatase (ALP) and a range of skeletal deformities. The levels of a number of phosphorylated metabolites, namely phosphoethanolamine and pyrophosphate, are characteristically raised. Levels of pyridoxal-5'-phosphate (PLP) have also been reported to be raised.

2. Hypophosphatasia is a rare disease and experience of measuring PLP in patients is lacking. We have had the chance to look at PLP levels in four families with hypophosphatasia, specifically to examine the quantitative relationship between ALP and PLP which has not been described before.

3. We confirmed that PLP levels are raised in hypophosphatasia and related to the disease severity. A significant negative linear relationship was found between the log PLP and log ALP (log PLP = 5.99 - 2.76 log ALP; r = -0.85, P < 0.001).

4. Measurement of PLP is simpler than some of the phosphorylated compounds, e.g. pyrophosphate. PLP may be a useful measure in patients with a suspected diagnosis of hypophosphatasia or for screening family members to detect potential heterozygotes and to monitor any response to therapy.

5. There did not appear to be any adverse clinical effects in relation to disturbed vitamin B₆ metabolism in hypophosphatasia.

6. Vitamin B₆ is used therapeutically in a number of conditions with monitoring of PLP levels. In these conditions PLP levels should be interpreted in conjunction with the prevailing serum ALP levels as the metabolism of these compounds is closely interrelated.

INTRODUCTION

The alkaline phosphatases are a group of enzymes that hydrolyse non-specific phosphomonoesters. They are located on cell membranes in tetrameric form and can be released into the circulation in a 'soluble' dimeric form. In humans four separate genes encode for four different isoenzymes of alkaline phosphatase. Three of the genes: the placental, intestinal and the germ cell (placental-like) alkaline phosphatase gene are located on chromosome 2q 34-37. The gene for the tissue non-specific alkaline phosphatase (TNSALP) is located in chromosome 1p 36.1-34. TNSALP has wide tissue distribution and liver, bone and kidney are especially rich in this isoenzyme. Post-translational modification in these tissues produces secondary TNSALP isoenzyme with different physicochemical properties [1].

Hypophosphatasia is a rare inherited disorder characterized by low levels of TNSALP with a spectrum of skeletal defects. Hypophosphatasia has been classified into different clinical types. These are: perinatal, infantile, childhood, adult, pseudo-hypophosphatasia [2]. In its severest form death may occur in utero from skeletal malformation (perinatal hypophosphatasia), while in its mildest form there may be very minor skeletal defects in adults. Among other metabolic abnormalities the levels of phosphorylated metabolites phosphoethanolamine and pyrophosphate have been known to be characteristically raised in this condition for some time [3, 4]. More recently, pyridoxal-5'-phosphate (PLP), another phosphorylated metabolite, has also been reported to be raised in this condition [5].

The exact incidence of hypophosphatasia is not known but this is a rare condition and experience of the value of measuring PLP and confirming the original findings in a significant number of these patients is lacking. We have had the chance to look at serum PLP levels in four families with hypophosphatasia. We also wanted to examine the relationship, if any, between the total serum alkaline phosphatase activity (ALP) and PLP levels as this has not been looked at before. Furthermore, there is some discussion about the best combination of biochemical markers for identifying the heterozygote carrier state for this condition. We report our findings.

Key words: alkaline phosphatase, hypophosphatasia, pyridoxal-5'-phosphate.
Abbreviations: ALP, alkaline phosphatase; PLP, pyridoxal-5'-phosphate; TNSALP, tissue non-specific alkaline phosphatase.
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Table 1. PLP and ALP levels in hypophosphatasia. *Homozygotes. **Heterozygotes. First number indicates generation and the second number family member, e.g. 2:2 is second-generation second family member. Adult reference ranges are 40–130 i.u./l for ALP, and 12–97 nmol/l for PLP.

<table>
<thead>
<tr>
<th>Case identity</th>
<th>Age (years)</th>
<th>Sex</th>
<th>ALP (i.u./l)</th>
<th>PLP (nmol/l)</th>
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<tbody>
<tr>
<td>Family 1</td>
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<td></td>
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<tr>
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<td>27</td>
<td>F</td>
<td>21</td>
<td>134</td>
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<tr>
<td><em>3:6</em>*</td>
<td>44</td>
<td>F</td>
<td>28</td>
<td>55</td>
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<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>1:2</em>*</td>
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<td>M</td>
<td>16</td>
<td>161</td>
</tr>
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<td>F</td>
<td>41</td>
<td>31</td>
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<td>Family 4</td>
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</tr>
<tr>
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<td>31</td>
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<tr>
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<td>F</td>
<td>26</td>
<td>200</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

Patients

All participants gave their informed consent to the study.

Family 1. The index patient was a female (see Table 1, case 2:3) who presented at the age of 47 years with bone pain, bowing of her lower legs and a waddling gait. She had short stature, height 1.47 m <3rd centile, and had lost all her teeth by the age of 20 years. She had a deformed pelvis and had two daughters (Table 1, cases 3:5 and 3:6), both delivered by cesarean section. Her results were as follows (numbers in brackets after the results indicate adult reference ranges): normal full blood count, serum ALP 17 i.u./l. She had raised urinary pyrophosphate was 190 pg/l (17–99). This patient was diagnosed as having adult hypophosphatasia and has previously been reported to have a raised serum acid phosphatase activity [6].

Family 2. The index case was a female who presented at the age of 20 years with bone pain and knee pain due to pyrophosphate arthropathy (Table 1, case 2:1). Her results were as follows: normal full blood count, serum ALP 17 i.u./l. She had raised urinary levels of phosphoethanolamine [21 µmol/mmol creatinine (<10)] and pyrophosphate [16 µmol/mmol creatinine (<5)]. The results for her father (case 1:2) and brother (case 2:2) are also shown (Table 1).

Family 3. The index case was a male who presented at the age of 34 years (Table 1, case 2:2) with essential hypertension. He was noted to have marked kyphoscoliosis and had a height of 1.60 m. He had bone pain and developed pyrophosphate arthropathy with chondrocalcinosis. He had moderat-ely severe rickets in infancy. His sister also had severe rickets and died from this at the age of 1–2 years. His results were: normal full blood count, serum ALP 12 i.u./l. He had raised urinary pyrophosphate, 29 µmol/mmol creatinine, and phosphoethanolamine, 47 µmol/mmol creatinine.

Family 4. At 16/17 weeks of a pregnancy the foetus was discovered on ultrasound to have gross skeletal abnormalities. This pregnancy was terminated. X-rays of the foetus suggested the possibility of hypophosphatasia. No blood samples had been taken from the foetus. Both the parents were investigated for the possibility of hypophosphatasia. The father had no clinical skeletal problems. His serum ALP levels were 31 and 26 i.u./l. His urine results were: pyrophosphate, 30 µmol/mmol creatinine; phosphoethanolamine, 6.0 µmol/mmol creatinine. The mother had no skeletal problems and had serum ALP levels of 22 and 30 i.u./l. Her urinary pyrophosphate was 22 µmol/mmol creatinine and phosphoethanolamine 12 µmol/mmol creatinine. Both the parents had normal full blood counts.

Routine biochemistry

This was measured on the SMAC I/SMAC II autoanalyser (Technicon Ltd, Tarrytown, U.S.A.). For total serum ALP activity (which includes all circulating isofoms) this method uses para-nitrophenylphosphate as a substrate with 2-amino-2-methyl-1-propanol buffer, pH 10.5, measured at 30°C. The quoted adult reference range is 40–130 i.u./l.

The PLP method has been described in detail elsewhere [7]. The method employed HPLC instrumentation using a C18 analytical column with fluorometric detection (325 nm excitation, 400 nm emission after post-column derivatization with pH 7.5 phosphate buffer containing 1 g/l sodium sulphide). The mobile phase is 0.067 mol/l KH2PO4, pH 2.5, adjusted with concentrated orthophosphoric acid and containing 215 µmol/l sodium hexane sulfonate as an ion-pairing reagent. PLP was used as an internal standard.

RESULTS

These are tabulated (see Table 1 and Fig. 1). Many people have reported the normal reference range for PLP on the basis of mean standard deviation [8, 9]. In his study Reynolds (unpublished work) found a non-Gaussian distribution with the method described here. When the data were transformed to log10 a Gaussian distribution was obtained. The reference range he quotes for the method used here is 2.5–97.5 percentile range.

To look at the relationship between TNSALP and PLP we analysed the data by Kendall’s rank correlation ( = −0.69, 0.003 <P<0.01), and to define the linear relationship we used linear regression
from childhood. The index patient from family 1 had a moderate level of skeletal deformity with troublesome pyrophosphate arthropathy. The index case from family 2 had troublesome symptoms and pyrophosphate arthropathy with a minimal degree of skeletal deformities. In the fourth family we only measured levels in the two heterozygote carriers. Our observations show that those patients with the lowest levels of ALP tend to have the highest levels of PLP and often the most severe skeletal deformity.

Low levels of PLP have been associated with a number of clinical complications. These include convulsions, oral mucous abnormalities, disturbance of oxalate metabolism [11], sideroblastic anaemia [15] and polyneuropathy [16]. Vitamin B6 toxicity is also described and is associated with a polyneuropathy [17]. In spite of the disturbed PLP metabolism we did not observe any such clinical problems in our patients.

Molecular diagnostic methods for DNA analysis are beginning to be used in the diagnosis of hypophosphatasia [18]. However, looking for mutations in the DNA can be labour intensive and may perhaps only be available in specialized centres. Some preliminary biochemical work for diagnosis may still be required and PLP levels may be helpful towards this. PLP can be measured relatively easily, unlike pyrophosphate which has also been suggested as a marker for the disease [19]. Levels of PLP may be diagnostically high in homozygotes but in heterozygotes, PLP levels after oral vitamin B6 load may be more discriminatory [20]. The diagnostic criteria for this test, however, are yet to be defined. PLP levels may also be of value in monitoring any response to therapy. It is also worth noting that since vitamin B6 is used therapeutically in a wide variety of metabolic conditions PLP levels should be interpreted in conjunction with the prevailing ALP levels as the metabolism of these compounds is strongly interrelated.

**REFERENCES**

10. Reference deleted.


