Could biochemistry lab alert for low alkaline phosphatase prompt diagnosis of hypophosphatasia?

Asma Deeb¹ & Abubaker Elfatih²

¹) Dr Asma Deeb, MD (corresponding author)
adeeb@mafraqhospital.ae
Paediatric Endocrinology Department
P O Box 2951
Mafraq Hospital, Abu Dhabi, United Arab Emirates
Tel; +971-508350568
Fax; + 971-2-5012199

²) Dr Abubaker Elfatih, MD
aelfatih@skmc.ae
Biochemistry Department
Shaikh Khalifa Medical City
Abu Dhabi, United Arab Emirates

Key Words
Alkaline phosphatase, hypophosphatasia, inborn error, laboratory, biochemistry

Short title
Low ALP lab alert to diagnose HPP

Received: Feb 26, 2017
Accepted: Jul 29, 2017

Abstract

Objectives: Hypophosphatasia (HPP) is an inborn error of metabolism with significant morbidity and mortality. Its presentation is non-specific leading to delay or missed diagnosis. Low ALP is a diagnostic test. Unlike high ALP, low level is commonly un-flagged by laboratories as abnormal. A new treatment has proved to be effective in HPP. We aim to study frequency of flagging of low ALP level by laboratory and the clinical manifestations of patients presenting with low ALP for a possible diagnosis of HPP.

Methods: Patients under 18 with low ALP were identified from biochemistry records over a period of 6 months. Reference ranges were used as per the ARUP directory. Electronic results for patients with low level were checked for flagging as abnormal/low ALP results. Charts of identified patients were reviewed. Presenting features were categorized under groups of disorders.

Results: 2890 patients had ALP test. 702 had values less than 160 U/L. 226 had age/gender specific low ALP. None of the low ALP results was flagged as low. 205 patients were excluded. Charts of 21 patients were studied further. 4 patients under the neuromuscular and 4 in the miscellaneous group presented with features suspected of HPP and had no specific diagnoses. Such patients could be potentially HPP patients.

Conclusion: Low levels of ALP are not alerted for by lab. Persistently low level in patients with unspecified diagnoses could be a key to diagnose HPP. Implementing lab-specific ranges and alerting for low levels could be a way to detect undiagnosed HPP.
What is already known on this topic:

- Hypophosphatasia is a rare disorder with significant morbidity and mortality
- High level of Alkaline Phosphatase is commonly alerted for by biochemistry labs
- Asfotase alpha is a new and effective medication for hypophosphatasia treatment

What this study adds:

- Unlike high alkaline phosphatase, low alkaline phosphatase is not always alerted for by biochemistry labs
- Identification of children presenting with unspecific clinical features and have more than one reading of low alkaline phosphatase could help diagnosing children with hypophosphatasia.
- Devising lab specific reference ranges for alkaline phosphatase is important to avoid missing of abnormally low levels.

Introduction

Hypophosphatasia (HPP) is an inborn error of metabolism characterized by low serum alkaline-phosphatase (ALP) due to a defect in the gene encoding the tissue-nonspecific isozyme of alkaline phosphatase (TNSALP) [1]. Inheritance can be autosomal recessive or dominant. As many as 260 genetic mutations in the TNSALP gene have been associated with HPP [2]. Penetrance is variable which results in a wide range of clinical features, with a spectrum ranging from stillbirth with no bone mineralization to early loss of teeth without bone symptoms. Clinically, there are six clinical forms of the disease based on the age at presentation. These are: perinatal (lethal), perinatal benign, infantile, childhood, adult and odontohypophosphatasia [1, 3]. Severe forms of HPP (perinatal and infantile) are inherited as autosomal recessive traits and in milder forms (adult and odontohypophosphatasia), autosomal recessive and autosomal dominant inheritance coexist [4]. Genotype is known to be associated with specific outcomes in the perinatal lethal type, whereas genotype phenotype correlation is less pronounced in other less severe forms [5].

HPP causes major morbidity to patients with a substantial bone disease, myopathy and weakness. Hypercalcemia associated with nephrocalcinosis are known features of HPP [1]. Craniosynostosis and skull dysmorphology occur in around 40% of infants [6]. HPP is, almost, always fatal when severe skeletal disease is obvious at birth [1, 7]. Skeletal deterioration typically results in death from respiratory insufficiency [7]. Bone fragility and recurrent fractures can be presenting features of HPP in childhood [8]. The perinatal form might present with intractable seizures caused by secondary pyridoxine deficiency encephalopathy. This is due the deficiency of alkaline phosphatase that is required for the metabolism of pyridoxal-5'-phosphate neurotransmitters [9]. Accordingly, HPP should be considered in neonates presenting with convulsions responding to pyridoxine.

Although some of the above features might point out to the diagnosis of HPP, other presenting features of HPP can be more non-specific and include various symptoms and signs for other more common diseases [10]. This fact has been a major cause for the disease to be
underdiagnosed and misdiagnosed [11]. A low ALP is a key for differentiating the diagnosis of HPP from many other more common paediatric disorders [2]. Alerts by biochemistry labs on abnormal levels are useful to point attention to specific diagnoses. Although high level of ALP is usually flagged up by biochemistry labs, low levels are not equally alerted for. Alerting for low ALP level could be an opportunity to diagnose HPP in patients presenting with un-specific manifestations. Detecting patients with HPP (previously undiagnosed) will offer them the opportunity to benefit from the enzyme replacement treatment that is proven recently to be an effective modality to treat this potentially fatal disease [12-13].

Aim

We aim to check if biochemistry lab flag up low ALP levels to alert physicians and examine the clinical features in those patients who had persistently low ALP un-flagged.

Patients & Method

Biochemistry electronic records at Mafraq Hospital were searched for patients 18 years or under who had low ALP readings (phase I). Records were obtained for a study period of 6 months (between July 2014 and Dec 2014). Low ALP of 160 U/L is taken as the lower limit of the reference range to be searched. This value is selected as per the ARUP laboratories online test directory being the highest level of the low range of ALP [14] (www.aruplab.com). The list of patients of ALP obtained was filtered based on age and gender in accordance to ARUP lab reference range (phase II). Biochemistry records were looked at for flagging low levels as abnormal. Patients who had at least 2 readings of ALP lower than normal value per age and gender and no normal value had their charts reviewed (phase III).

Three groups of patients were excluded:
- Patients who had 2 low values of ALP but had one or more normal value detected.
- Those with a single low value of ALP with normal value after or before.
- Borderline normal value on one presentation of acute illness with no further medical presentation.

Following the remaining chart reviews, a list of the main diagnoses patients presented with was drawn and stratified into subcategories (phase IV). Details of patients’ presentations, working diagnoses and suspected features linked to HPP were drawn. The categories of diseases linked with a possible diagnosis of HPP included musculo-skeletal, rheumatological, neurological, renal, respiratory-related and fractures. Approximate number of patients with suspected cases with HPP is estimated and presenting features under particular disease categories are highlighted for further studies. The study is approved by the Research & Ethics Committee at Mafraq hospital (MAF-REC-12/2015_06).

ALP Assay

ALP level is measured on a fully automated Roche Cobas® 8000 modular analyzer series c701 system (Roche Diagnostics GmbH, Mannheim, Germany, 2010). The assay is a basic standardized colorimetric assay traceable to the International Federation of clinical chemistry (IFCC) Reference Gen2 method as an optimized assay. ALP is measured in a reaction where by ALP catalyzes the cleavage of phosphate from 4 nitrophenyl phosphate (4 NPP) to form 4 nitrophenoxide (benzenoid form), which undergoes spontaneous rearrangement at alkaline pH to the quinonoid form (yellow color). The reaction is followed by measuring absorbance of the reactant color at 405 nm on the automated analyzer detection system. The ALP assay performance specifications include an
analytical measuring range of 5-1200 U/L with a lower detection limit (analytical sensitivity) of 5.00 U/L which represents the lowest measurable analyte level that can be distinguished from zero. The assay has a clinically Reportable Range of 5.00 – 6000 U/L. The ALP assay has a within-run precision coefficient of variation CV% of 0.7 % at an ALP mean of 84.3 U/L and a 2.4% at a mean of 92.8 U/L, while the assay demonstrates a coefficient of variation of 0.5 % at an ALP mean of 222 and a CV% of 1.7 % at a mean of 224 U/L. The interindividual CV is 6.7%, with an intra-individual CV of 25.4% and a critical significant difference of 37%.

Results

During the 6 months study period, there were 2890 tests for ALP performed in subjects 18 years of age or younger. In phase I of the study, this number was reduced to 702 patients who had readings below 160 U/L. None of the low levels of ALP was flagged as abnormal by the biochemistry lab. Age stratifications for normal reference values was done, 349 patients were selected (phase II). Further filtering was done to the selected group to match reference range per gender. Patients’ number went down to 226 at this stage (phase III). Of those, 1 (male) was between the age bracket of 16-18, 24 (20 males) between 14-15 years, 24 (14 males) between 12-13, 19 between 10-11 years, 74 between 4-6 years, 48 between 1-3 years and 1 between 1-11 months (Table 1). Charts for all patients identified in phase III were reviewed. 205 patients were excluded as per the exclusion criteria and 21 patients were studied further (figure 1).

The 21 patients were classified under disease categories based on presentation and working diagnoses. These were Rheumatology disorders (5 patients), Fractures (5 patients), Neuromuscular diseases (4 patients), Immobility and repeated fractures (3 patients) and a miscellaneous group (4 patients) (Table 2). The 5 patients under the rheumatology category had a confirmed diagnosis of systemic lupus erythematosus in 3 and Juvenile rheumatoid arthritis in 2. 5 patients had a single fracture of a long bone. Of those, one had a dislocated shoulder with a humerus fracture and another had orthodontic treatment for teeth malposition and crowding. 3 patients were diagnosed with cerebral palsy and were immobile with repeated fractures. The neuromuscular category included 4 children who did not have definite diagnoses. One had arthrogryposis, and another was diagnosed with arthrogryposis and repeated fractures. One presented with multiple skeletal deformities and the fourth patients had neuromuscular deformities with fractures. The miscellaneous group included a child with Down syndrome who was diagnosed with short limbs antenatally and has been admitted to intensive care unit repeatedly with recurrent chest infections. One child was diagnosed with Nemaline myopathy and had kidney stones and another was diagnosed with severe demyelinating sensory and motor neuropathy. The fourth patient had repeatedly low ALP readings and suffered from recurrent infections.
Discussion

Hypophosphatasia is a disease that is associated with major co-morbidity and poor prognosis. A complicating factor is its diagnosis as it has a wide range of presenting features which are non-specific [11]. In the past, various treatment approaches have been tried to treat the severe form of the disease with poor results. Treatment modalities included transplantation therapy using bone fragments and cultured osteoblasts [7], infusion of enriched plasma with ALP from patients with Paget disease [15], bone marrow transplant [16] and conservative treatment using low calcium milk and pamidronates [17]. Calcitonin and chlorthiazide have been used to reduce calcium level, which can reach very high levels [18]. Bisphophonates are pyrophosphate analogs and can precipitate the disease progression. Patients with undiagnosed HPP presenting with fractures and osteoporosis, and treated with bisphophonates are reported to go into renal failure [19] and using bisphophonate to treat HPP is currently contraindicated.

Asfotase Alfa is a recombinant, fusion protein comprising the TNSALP ectodomain and a terminal deca-aspartate motif for bone targeting [20]. It has been used in clinical trials and was shown to enhance healing of skeletal abnormalities and improve respiratory and motor dysfunction [12]. Asfotase Alfa has, now, been approved by the European Medicines Agency for use in patients with HPP [13].

In our cohort, we detected a group of patients who had low ALP and do not have a specific diagnosis (Table 2). Despite the low ALP level in more than one occasion of testing, there was no alert by biochemistry lab indicating the low value. In 2 groups; rheumatology and fractures with immobility, the ALP abnormality can be possibly attributed to the underlying disease. The 3rd group with single fractures, patients are healthy otherwise and unlikely to have an undiagnosed HPP. However, patients in the neuromuscular disorder group (4 patients) and the miscellaneous group (4 patients) are worth examining further to rule out the possibility of HPP. 2 of the miscellaneous group, in particular, had repeated chest infection and ICU admissions and one had a kidney stone. The 4 patients under the neuromuscular group had skeletal deformities and fractures and they do not have specific diagnosis. They, too, qualify for further investigations to exclude HPP.

High level of ALP is seen in variety of bone disorders, however low level is not as frequently seen in clinical practice. High level of ALP is routinely flagged up by biochemistry lab but this is not the case for low ALP levels. Biochemistry labs need to have reference ranges for the low level of ALP to highlight possible abnormalities particularly in case of associated hypercalcemia. ALP essay is widely done and is a fairly inexpensive test. It is a key to diagnose HPP and makes a good screening test to diagnose HPP for which a treatment is now available.

Conclusion and further plans

We conclude that using persistently low ALP level in patients presenting with unspecific signs and symptoms could be used as a guide to further investigate and exclude HPP. This is particularly important because medication is now available and is proven to be effective to ameliorate morbidity and improve quality of life in this disease. Accordingly, alerting physicians to low level of ALP by biochemistry labs can be very useful. We highlight the importance of having age and gender adjusted ALP reference ranges specific for local labs or populations to avoid missing the diagnosis of HPP. A clear plan of action needs to be drawn on how to proceed with suspected patients detected based on low ALP and unspecific presentation.
Competing Interest Statement
Authors confirm that there is no conflict of interest to declare.

Authors Contribution
Asma Deeb designed the project, obtained ethical approval, studied patients’ notes, collected and collated data and wrote the manuscript.

Abubaker Elfatih obtained the biochemistry records, filtered the data based on the required analyte range level, wrote the biochemical assay part and reviewed the manuscript.

References

14) ARUP Laboratories online test directory. www.aruplab.com

- **Table & Figure Legends**

**Table 1:** Number of patients with lower Alkaline phosphatase than reference per age and gender (total 226).

**Table 2:** Categorization of system involvement of 21 patients with persistent alkaline phosphatase and repeated medical presentation

**Figure 1:** Flow chart showing filtering of Alkaline phosphatase according to lowest normal value by age and gender
Table 1: Number of patients with lower Alkaline phosphatase than reference per age and gender (total 226)

<table>
<thead>
<tr>
<th>Age Range (yr)</th>
<th>16-19</th>
<th>14-15</th>
<th>12-13</th>
<th>10-11</th>
<th>7-9</th>
<th>4-6</th>
<th>1-3</th>
<th>1-11 (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest Normal Value U/L</td>
<td>40</td>
<td>60</td>
<td>55</td>
<td>130</td>
<td>110</td>
<td>160</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Number of Patients</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>20</td>
<td>10</td>
<td>14</td>
<td>19</td>
<td>35</td>
</tr>
</tbody>
</table>

No Gender difference in Reference Range
Table 2: Categorization of system involvement of 21 patients with persistent alkaline phosphatase and repeated medical presentation

<table>
<thead>
<tr>
<th>Category</th>
<th>Neuromuscular</th>
<th>Rheumatology</th>
<th>Repeated Fractures &amp; Immobility</th>
<th>Single Fracture</th>
<th>Other Conditions/associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

**Clinical Description**

- **Arthrogryposis**
  - SLE, Lupus Nephritis
  - Cerebral Palsy, single fracture
  - One fracture (wrist)
  - Nemaline Myopathy & kidney stones

- **Multiple Skeletal Deformities**
  - Juvenile Rheumatoid Arthritis
  - Cerebral Palsy, repeated fracture
  - One fracture (radius & ulna)
  - Demyelining sensory and motor neuropathy

- **Arthrogryposis & Repeated Fractures**
  - SLE
  - Cerebral Palsy, single fracture
  - One fracture, distal ulna
  - Recurrent infections

- **Neuromuscular Deformities & Fractures**
  - Juvenile Rheumatoid Arthritis
  - One humerus fracture and shoulder dislocation
  - Down syndrome, repeated ICU admission for respiratory infections. Short femur/humerus

- **Juvenile Rheumatoid Arthritis**
  - One forearm fracture and teeth crowding
Figure 1: Flow chart showing filtering of Alkaline phosphatase according to lowest normal value by age and gender

Alkaline phosphatase analyses for patients under 18 years of age (July-Dec 2014)
No. 2890

- Alkaline phosphatase less than 160 U/L
  No. 702
  - Low values per age stratification
    No. 349
    - Low values per age and gender
      No. 226
      - A minimum of 2 low readings
        No. 21