A Diagnostic Algorithm for Children with Low Alkaline Phosphatase Activities: Lessons Learned from Laboratory Screening for Hypophosphatasia

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Objectives To explore the role of laboratory screening for hypophosphatasia and propose a diagnostic pathway for children with low serum alkaline phosphatase (ALP) activities.

Study design A retrospective hospital-based study over an 8-year period was conducted to identify children younger than 16 years of age with low ALP activity (<100 U/L). Study-positive patients were contacted for repeat sampling, and those with persistently low ALP had plasma pyridoxal-5′-phosphate and urinary phosphoethanolamine measured.

Results Of 323 064 analyzed samples, 1526 had ALP activities <100 U/L. Most patients had transient hypophosphatasemia. Of 50 patients with last-recorded ALP <100 U/L, 32 were excluded given previous ALP >100 U/L. Eighteen were identified as study-positive. Of the 15 surviving children, 13 were traceable. Four had persistently low ALP activity on retesting, of whom 2 had raised pyridoxal-5′-phosphate and phosphoethanolamine concentrations and were subsequently tested for *ALPL* gene mutations; a 4-year-old asymptomatic girl with a novel homozygous *ALPL* missense mutation and a 23-year-old female with a heterozygous mutation. There was significant overlap in ALP activities between study-positive and 11 current patients with hypophosphatasia. We propose a diagnostic algorithm for children with low ALP activities based on clinical and biochemical variables.

Conclusions Patients with persistently low ALP activity require further clinical, biochemical, and radiological assessment for hypophosphatasia, even in the absence of clinical symptoms. The proposed diagnostic algorithm for children with low ALP will facilitate early detection of cases of hypophosphatasia, which, with the availability of enzyme replacement for hypophosphatasia, can be life-saving or avoid years of undiagnosed morbidity. *(J Pediatr 2016;172:181-6)*.

Serum alkaline phosphatases (ALPs) are a group of glycoprotein enzymes that catalyze the hydrolysis of phosphoesters to release inorganic phosphate.[1] There are 4 different ALP isoenzymes, 3 tissue-specific ALPs (placenta, intestine, germ cell), and 1 tissue nonspecific ALP (TNSALP). The latter, encoded by the *ALPL* gene on chromosome 1, is widely expressed in various tissues including bone, liver, and kidney.[2] TNSALP cleaves extracellular substrates such as inorganic pyrophosphate (PPi) and pyridoxal-5′-phosphate (PLP).[3]

Reduced serum ALP activities are associated with a variety of conditions.[4-7] Hypophosphatasia is a rare, inherited, potentially life-threatening bone disorder caused by inactivating mutations in the *ALPL* gene resulting in low ALP activity and accumulation of the enzyme substrates PPi, PLP, as well as an additional biochemical marker, urinary phosphoethanolamine (PEA).[1] PPi inhibits bone mineralization leading to skeletal abnormalities such as rickets, osteomalacia,[8] fractures,[9] and systemic complications.[3] Based on age at presentation and severity of symptoms, hypophosphatasia is classified into perinatal lethal, prenatal benign, infantile, childhood, adult,[10] and odontohypophosphatasia, which is limited to dental manifestations (no skeletal involvement).[11] Patients with the recessively inherited perinatal and infantile forms of hypophosphatasia come to medical attention before age 6 months because of pyridoxine-responsive seizures,[12,13] failure to thrive, muscular hypotonia, hypercalcemia, nephrocalcinosis, craniosynostosis, fractures, or respiratory failure, and have a high mortality rate.[14] However, the milder, usually dominantly inherited[15] childhood and adult forms may have only subtle symptoms such as early tooth loss[16] or chronic pain. The clinical phenotype varies, and all forms of hypophosphatasia can be associated with disability and/or poor quality of life.[17]

ALP	Alkaline phosphatase
PEA	Phosphoethanolamine
PLP	Pyridoxal-5′-phosphate
PPi	Inorganic pyrophosphate
TNSALP	Tissue nonspecific ALP

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The diagnosis of hypophosphatasia is made on the basis of clinical, biochemical, and radiologic features.[18] The hallmark biochemical feature of low serum ALP is typically associated with elevated serum phosphate, PLP, and urinary PEA levels, but diagnostic criteria for hypophosphatasia based on biochemical markers alone are not well established.[3] In fact, asymptomatic carriers of recessive *ALPL* gene mutations also can have these biochemical abnormalities, although to a lesser extent.[19] With the arrival of a novel enzyme replacement therapy for hypophosphatasia,[20] early detection of hypophosphatasia can be life-saving or avoid years of undiagnosed morbidity. Therefore, establishing better diagnostic criteria for children is vital for early detection and appropriate management of all forms of hypophosphatasia, including avoidance of inappropriate treatment.

We conducted a laboratory audit of children with low ALP activities in a large tertiary children's hospital. The aims were to explore potentially missed diagnoses in our institution, evaluate the role of laboratory screening for hypophosphatasia, and design a diagnostic algorithm for children presenting with either low ALP activity or clinical signs of hypophosphatasia.

Methods

We assessed all recorded serum ALP measurements between August 2004 and October 2012 at the Department of Clinical Chemistry, Birmingham Children's Hospital, United Kingdom. Appropriate pediatric ALP reference ranges are used in our hospital.[21] Results from children under the age of 16 years were collated using the hospital's Telepath system. Patients with a low ALP concentration, arbitrarily defined as <100 U/L (based on levels from cases of severe hypophosphatasia in the literature),[20] in whom repeat ALP results either remained <100 U/L or where no repeat sample was obtained, were included. Patients >16 years of age were not included because ALP values <100 U/L fall within the normal reference range.[22] As reference ranges for ALP activity are age dependent, patients were grouped into 5 age categories: neonates, 1 month-9 years, 10-11 years, 12-14 years, and 15-16 years.

Following identification of patients with persistently low ALP ("study positive"), approval from the hospital ethics advisory group was obtained to contact these individuals and arrange further investigations. All study-positive patients and their healthcare providers were contacted via letter and/or telephone requesting a repeat blood sample for ALP measurement. Urine PEA was performed in patients with persisting low ALP level on repeat sampling (below the reference range for age), and plasma PLP was measured in those patients with elevated urinary PEA concentrations. Those with low ALP and elevated PEA and PLP had radiographs and genetic testing of the *ALPL* gene performed.

Serum ALP activities were determined using a dye-based assay, which measures the enzyme activity by monitoring the rate of hydrolysis of p-nitrophenylphosphate to p-nitrophenol at 410/480 nm in the presence of magnesium on an Olympus AU640 (Beckman Coulter, High Wycombe, United Kingdom). Urinary PEA was measured by amino acid quantification by ion exchange high performance liquid chromatography on a Biochrom 30+ amino acid analyzer (Biochrom Ltd, Cambridge, United Kingdom). Plasma PLP was measured by an external laboratory using high performance liquid chromatography following derivatization with fluorometric detection using a kit (Chromsystems, Munich, Germany).

To illustrate the overlap between ALP activities of study-positive patients and cases with hypophosphatasia, longitudinal ALP data from 11 children with confirmed hypophosphatasia, all patients currently managed at Birmingham Children's Hospital, were selected for comparison. Together with the outcome of this laboratory study, these comparative data contributed to the design of the proposed diagnostic algorithm.

Results

Over an 8-year period, 323 064 serum samples (from 62 285 patients) were analyzed for ALP activity, which showed a median concentration of 450 (range 17-29 600) U/L. Of the total samples, 1526 (0.47%) had ALP concentrations <100 U/L. The majority (75%) of these samples were from intensive care, 15% from ward inpatients, 5% from Accident and Emergency department, and remainder (5%) were from outpatient referrals. Sixteen samples from 4 known patients with hypophosphatasia, and 1317 samples (392 patients) with subsequent repeat ALP activity >100 U/L were excluded (**Figure 1**; available at www.jpeds.com).

Of the remaining 50 patients (193 samples), 17 (34%) had previously recorded normal ALP activity for age and 15 (30%) had previously recorded ALP activities of >100 U/L, thus, their low ALP activity on this occasion was considered secondary to acute severe illness. The remaining 18 (36%) study-positive patients never had ALP activities of >100 U/L. Three patients were less than one month old, 4 patients were 1 month-9 years of age, 2 patients were 12-14 years of age, and 9 patients were 15-16 years of age. A considerable overlap in serum ALP activities was observed between these 18 study-positive patients (median 84 [range 55-96] U/L) compared with the 4 patients with known hypophosphatasia (69.5 [41-94] U/L) (**Figure 2**). None of these 18 patients had had further diagnostic clinical or biochemical evaluations performed to exclude hypophosphatasia at the time of measurement.

On reviewing the medical notes of these 18 patients, 3 had died of severe head injury, fulminant sepsis, and acute renal failure. Thirteen of the 15 remaining patients were traceable and on repeat testing, 4 patients were found to have persistently low ALP activities for age with a median of 25.5 (range 24-65) U/L. Urine PEA was elevated in 2 (50%) of them, and on subsequent testing, their plasma PLP levels were also elevated. In view of the laboratory findings, genetic testing was performed that identified mutations in the *ALPL* gene in both patients.