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Dialogues between basic and clinical researchers: hypophosphatasia

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Abstract

Most serum alkaline phosphatase (ALP) (> 90 %) originates from the liver and bone. Normally, the contribution from other tissues, such as the intestine or kidney, is much smaller, although the placenta is an important source during pregnancy. Elevated ALP levels are usually indicative of liver or bone disease.

The analysis of other liver enzymes, particularly GGT—which is elevated in liver damage and normal in bone diseases—usually clarifies the origin. When in doubt, the bone isoform can be measured, or a profile of all isoenzymes can be conducted.

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ALKALINE PHOSPHATASE: BIOMARKER OF HYPOPHOSPHATASIA AND OTHER DISORDERS

Most of the serum alkaline phosphatase (ALP) (over 90 %) comes from the liver and bones. Normally, the contribution from other tissues, such as the intestine or kidney, is much smaller, although the placenta is an important source during pregnancy. Therefore, elevated ALP levels are usually indicative of liver or bone disease. The analysis of other liver enzymes, particularly γ-glutamyltransferase (GGT)—elevated in liver injury and normal in bone diseases—usually clarifies the origin. When in doubt, the bone isoform can be measured, or a profile of all isoenzymes can be conducted.

A decrease in ALP is a frequent transient finding in various acute diseases. More sustained decreases can be seen in various systemic disorders, such as celiac disease, myeloma, severe anemia (especially due to vitamin B_{12} deficiency), or various conditions associated with a slowdown in osteoblastic activity, such as hypoparathyroidism, hypothyroidism, hypercortisolism, certain skeletal abnormalities associated with advanced kidney disease, or treatment with antiresorptive drugs (bisphosphonates or denosumab). For being active, the ALP molecule requires the binding of certain cofactors, so the deficiency of cations such as zinc, magnesium, or calcium can be associated with a decrease in its serum activity (1).

If these acquired causes are excluded, a genetic cause for the ALP decrease should be considered. Among the hereditary diseases associated with low ALP are cleidocranial dysplasia and Wilson's disease. The associated abnormalities of the clavicles and liver, respectively, usually help in correctly diagnosing these rare disorders (Table I). If clinical and lab test results do not support these diagnoses and no secondary acquired cause is identified, low serum ALP levels may be indicative of the patient exhibiting hypophosphatasia (HPP) related to a mutation in the *ALPL* gene, which encodes the tissue-nonspecific ALP (TNSALP), including the liver and bone isoforms. Although these isoforms—encoded by the same gene—share the amino acid sequence, they differ in some post-translational modifications, such as glycosylation patterns.

However, when the coding regions of the *ALPL* gene are sequenced, variants are observed in only about 60 % of patients with low ALP (2). In other cases, the cause of low ALP levels remains unclear. Perhaps this decrease is related to other genomic or epigenomic changes or to post-translational changes of the protein (3).

A useful parameter for confirming ALP deficiency is the determination of pyridoxal 5-phosphate (PLP) in plasma. This is the main circulating form of vitamin B_6 and is hydrolyzed by ALP, so when ALP activity is deficient, the levels of PLP go up. Of note that PLP levels depend on vitamin B_6 intake. Therefore, they may increase if the patient is on vitamin supplements. Conversely, levels may be normal or even low—despite the patient having an ALP deficiency—if there is an associated vitamin B_6 deficiency (4). Hence, PLP determination is not a perfect diagnostic test; nevertheless, it can be very helpful, especially if it is not possible to sequence the *ALPL* gene.

Pathogenic variants of the *ALPL* gene lead to HPP, a disorder with a varied clinical spectrum. Infantile forms are usually severe with a a pronounced rickets-like condition, as ALP is necessary for bone mineralization. In adults, signs are usually much milder. They often present as stress fractures, chondrocalcinosis, tendinopathies, or dental abnormalities (5,6). Some patients may be completely asymptomatic, making it difficult to distinguish cases with mild signs from those who are simply "asymptomatic carriers" of the genetic variant. Therefore, the diagnosis of HPP requires the integration of clinical, biochemical, and genetic data, as we have recently reviewed (3). In any case, caution should be exercised in the use of antiresorptive drugs—particularly bisphosphonates—in these patients, as it has been suggested—although not clearly demonstrated yet—that they may have a higher risk of atypical fractures (7).

Table I. Causes of persistent decrease in serum alkaline phosphatase

Acquired causes
Hormonal
Hypoparathyroidism
Hypothyroidism
Hypercortisolism
Drugs
Bisphosphonates
Denosumab
Corticosteroids
Clofibrate
Vitamin D (toxicity)
Nutritional
General malnutrition
Vitamin deficiencies (C and B_{12})
Mineral deficiencies (calcium, zinc, and magnesium)
Other diseases
Celiac disease
Multiple myeloma
Advanced kidney failure
Genetic
Hypophosphatasia
Cleidocranial dysplasia
Wilson's disease
Acrodermatitis enteropathica
Hemochromatosis

Treatment of patients with HPP with moderate clinical signs, as is generally the case in adult-onset cases, is generally symptomatic. However, in cases with severe signs, particularly in children and adolescents, enzyme replacement therapy with asfotase alfa may be indicated. This is a soluble glycoprotein of 726 amino acids obtained by cell engineering, combining the active part of TNSALP, the Fc domain of human IgG1, and a deca-aspartate peptide domain (8,9). Although some criteria for its use in adults have been suggested (10), and some studies with small groups of patients have provided promising results (11), its role after growth is completed is less established.

ALKALINE PHOSPHATASE AND HYPOPHOSPHATASIA: MOLECULAR MECHANISMS

The *ALPL* gene encoding TNSALP is located on chromosome 1p34-36 (12) and is expressed in various tissues, including bones, liver, and kidney (13). TNSALP is an ectoenzyme that binds to the plasma membrane through a glycosylphosphatidylinositol (GPI) anchor molecule (12). Its main function is to catalyze the hydrolysis of phosphomonoesters (14), such as PLP, inorganic pyrophosphate (PPi), adenosine triphosphate (ATP), diphosphorylated lipopolysaccharide (LPS), and phosphorylated osteopontin (p-OPN), releasing inorganic phosphate (12,15-17). TNSALP requires 2 Zn^{2+} ions and 1 Mg^{2+} ion to form as a homodimer (18) and work properly (19).

Regarding the genetics of HPP, it has been demonstrated that the *ALPL* gene has great allelic heterogeneity (12). According to ALPL (https://alplmutationdatabase.jku.at) and LOVD variant databases (https://databases.lovd), about 500 loss-of-function variants of the *ALPL* gene have been reported (20). This great allelic heterogeneity is related to a very variable clinical expression of HPP (20,21), leading to the categorization of HPP into different clinical forms, from the most severe to the mildest: lethal perinatal HPP, infantile HPP, childhood-onset HPP, adult HPP, odontohypophosphatasia, and benign perinatal HPP (22,23).

HPP can be inherited in an autosomal dominant or recessive manner. The more severe clinical phenotypes are transmitted as a recessive autosomal trait, while milder forms may result from recessive or dominant transmission. However, cases of adult HPP with a mutation in only one allele and a more severe phenotype may be found. These cases could be explained by the presence of other intronic mutations or mutations in the regulatory sequence or by the existence of a heterozygous mutation with a dominant negative effect (24,25). This can lead to a decrease in the activity of the wild-type monomer in the heterodimeric enzyme complex, altering the structural and functional properties of TNSALP. Approximately 13.4 % of the alleles identified in HPP patients have a dominant negative effect in the European population (26). Examination of the TNSALP 3D model reveals that the protein consists of 3 distinct areas: the active site (including the 3 metal binding points), the homodimer binding zone, the crown domain (involved in functions such as non-competitive inhibition, thermal stability, allosteric behavior, dimer stability, and collagen binding), the calcium binding domain (whose function is not yet fully understood), and the N-terminal alpha-helix (which contributes to the stability of the dimeric structure). Most mutations that have been experimentally shown to have a measurable dominant negative effect are found in the homodimer binding region, the active site, and the crown domain (20).

As mentioned earlier, it can be difficult to interpret cases with persistently low ALP levels without identifying pathogenic variants in the *ALPL* gene. Of note that, in addition to those already mentioned, there are other less common situations that can also decrease bone formation and, therefore, be associated with low ALP levels. For example, iron and ferritin have been shown to be potent inhibitors of osteogenesis, significantly inhibiting ALP activity. The ferroxidase activity of ferritin is thought to be central to this inhibition (27). Furthermore, other factors are involved in the regulation of ALP, such as the transcription factor RUNX2 (28), as well as other regulators of Pi levels, such as PHOSPHO1 or ENPP1, which acts as a phosphatase in the absence of TNSALP (1). In this context, the existence of other modifier genes related to the development of heterogeneous clinical signs in patients with decreased ALP levels cannot be ruled out. Additionally, epigenetic changes could contribute to the severity of clinical signs in patients with HPP. In this regard, DNA methylation has been shown to play an important role in regulating ALPL expression (29). Similarly, different lifestyles or behaviors appear to have a direct effect on ALP levels. Thus, physical activity has been directly related to increased ALP levels (30,31). Therefore, for studying the phenotype associated with HPP, it would seem reasonable to explore the role of these regulatory factors as well as the contribution of external factors, such as lifestyle. In any case, in patients with clinical and biochemical findings consistent with HPP, it would be interesting to perform whole-genome sequencing to identify possible mutations in the regulatory or non-coding regions of the *ALPL* gene, as well as possible mutations in other genes regulating the ALP activity. Additionally, functional studies should be conducted to characterize each genetic variant to explore the relationship with specific phenotypes to better understand the possible effects of these variants in the carrier patient. Although it is difficult to establish a genotype-phenotype correlation in HPP patients, the correct identification of new variants and the study of their phenotype would improve our understanding of this metabolic disorder, making it accessible to the scientific community, which would allow for better management of the disease.

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