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Why should the neonatologist give a closer look at the alkaline phosphatase?

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ABSTRACT

Background. Alkaline phosphatase (ALP) has been intensively studied and proved to be useful in Neonatology. The first condition studied in correlation with increased ALP values was osteopenia of prematurity. In recent years there was an increase interested in correlations between ALP and bilirubin levels. In this article, we want to review the literature to find other benefits of ALP dosing in neonatal patients.
Methods. Clinical trials were searched and analyzed by a single individual (via dedicated search engines such as Google Scholar, PubMed, and Scopus). We have included clinical studies from the last 7 years that present statistically significant results regarding the role of ALP dosing in the screening or diagnosis of other neonatal pathologies (jaundice with hyperbilirubinemia, necrotizing enterocolitis, liver dysfunction, renal failure and oncological diseases).
Results. After excluding the clinical trials that did not meet the eligibility criteria, 6 clinical trials remained (n = 859). Following the analysis of these studies, correlations were highlighted between high ALP values in the umbilical cord and the need for phototherapy. It was also shown that all newborns with elevated ALP values required treatment for hyperbilirubinemia, as well as being a good predictor of severe jaundice and helping to predict its onset. Also, evidence about the correlation between high values of ALP and prematurity osteopenia was reaffirmed in recent studies.
Conclusions. ALP is an easily dosed biomarker that can be successfully used in Neonatology. The existing studies to date encourage the use of ALP as a biomarker especially for the detection of osteopenia of prematurity and for the early detection of newborns who will develop intense forms of jaundice. There are still differences regarding the serum ALP values considered as cut-offs for the diagnosis of various neonatal conditions, but performing multicenter studies on a large number of cases could establish a valid consensus in order to use the same ALP cut-off values in all Neonatology departments.

Keywords: alkaline phosphatase, neonates, jaundice, metabolic bone disease of prematurity, treatment, statistical correlations

INTRODUCTION

Alkaline phosphatase (ALP) defines a group of plasma enzymes that hydrolyze monophosphoric esters at an optimal pH releasing inorganic phosphates. The enzyme was first isolated from bone and cartilage by Robert Robinson in 1923. ALP present in mammals contains metalloenzymes encoded by a multigene family and functions as dimeric molecules. Three metal ions including two Zn^{2+} molecules and one Mg^{2+} molecule in the active site are essential for enzyme activity. However, these metal ions also contribute to the conformation of the ALP monomer [1].

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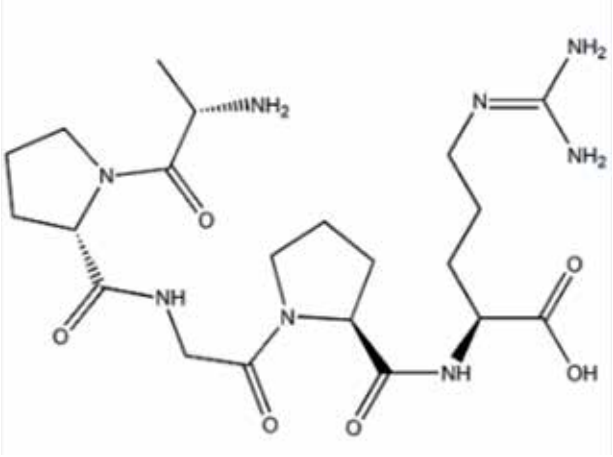

FIGURE 1. Molecular structure of ALP

In humans, ALP acts at an optimal alkaline pH of 8.2 to 10.7, it is bound to the cell membrane and is inhibited by amino acids and peptides [2]. The specific properties differ for each isozyme, causing the appearance of the characteristic functions of each. ALP can be non-competitively inhibited by a wide range of compounds, which include amino acids, tetramysol, theophylline and NADH.

ALP has been shown to play a role in regulating phosphate transport in the intestine, kidney, and calcium transport in the intestine and kidney. In recent years, a large number of studies focused on the complexity of ALP functions have been carried out.

ISOENZYMES OF ALP IN THE HUMAN BODY

Four types of ALP isozymes have been identified in the human body (Figure 2), namely: placental [1], intestinal [2], non-tissue specific (bone/liver/kidney) [3] and from germ cells [4].

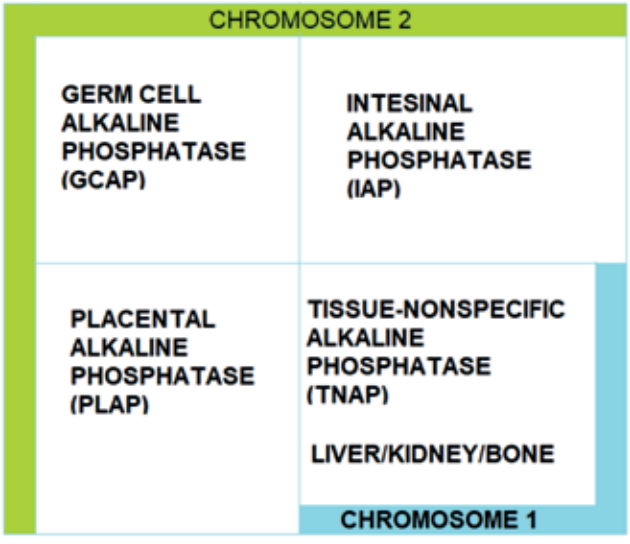

FIGURE 2. Isoenzymes and their genetic coding [3]

1) Placental ALP

It is a thermostable enzyme present in large amounts in the syncytiotrophoblast during the third trimester of pregnancy. Some of the serum activity comes from neutrophils. The genes that code it are located on chromosome 2 and contain 3 specific alleles (1,2 and 3) and can be reactivated by cancer cells. It is the most polymorphic enzyme, with up to 18 allozymes resulting from point mutations. Placental ALP has been shown to be involved in the transfer of maternal IgGs to the embryo during pregnancy, as well as in DNA synthesis and cellular profiling in association with insulin, zinc, and calcium [2]. Being synthesized in the placenta, the effects of placental ALP on fetal cell growth and survival suggest a key role in the regulation of embryo growth.

2) Intestinal ALP

The gene encoding intestinal ALP is located on the long arm of chromosome 2. It has a different structure than the other isoenzymes, its side chains do not end with salicylic acid, and it is less heat resistant. The isozyme has been shown to play a role in the absorption and transport of lipids and nucleotides. Different forms have been isolated in the adult and fetal intestines in both protein and carbohydrate content. The fetal form can be reactivated in some cases of cancer.

3) Tissue-nonspecific ALP (bone/liver/kidney)

The gene encoding tissue-nonspecific ALP (bone, liver, kidney) is located on the short arm of chromosome 1. It is a heat-labile isozyme present in liver, bone, and kidney tissues, encoded by related genes. It has been shown to play a role in preventing the rapid elimination of sugars from circulation by binding carbohydrates to a liver sialoglycoprotein. Bone isozyme has been shown to play a role in bone and tooth mineralization, and the binding of calcium and collagen fibers in bone. Non-specific tissue ALF is closely related to hypophosphatemia and vitamin B6 deficiency causing seizures and neurological impairment.

4) ALP from germ cells

It is a heat-stable isozyme present at low levels in embryonic germ cells and some neoplastic tissues. It can be identified in the testes and thymus. Like the other forms, it can be reactivated in some types of cancer.

ALP DOSING TECHNIQUES

Three traditional methods of ALP quantification have been reported, namely: fluorescence-based methods (flow cytometry, histochemistry, and immunohistochemistry), methods based on mRNA identification by RT-PCR, and immunoreaction methods by RIA or ELISA. The sample precipitate with substrates such as enzyme-labeled fluorescence-97 (ELF-97) is coupled with some of the salts and dyes to produce insoluble colored substances detec-

ted by fluorescence. These methods have high sensitivity and allow rapid detection. However, their expensive instrumentation hinders widespread use, along with the need for highly skilled personnel [4,5].

Second, methods based on mRNA, Northern blot, and RT-PCR [6] can detect the level of ALP in real-time. The former method is an old and classical method used to detect a specific phosphatase isoform based on its mRNA level [7]. The RT-PCR approach is also based on RNA expression, but combines with a single nucleotide range extension analysis to determine phosphatase isoenzymes.

Thirdly, the immunoreaction-based method including Western blot, radioimmunoassay (RIA) and ELISA assay were performed to achieve more specificity and sensitivity [8]. Western blotting involves an electrophoretic selection, which allows the phosphatase to be separated by size and then the results are transferred to membrane bands. The membrane is incubated with specific antibodies. This method, although sensitive, is time-consuming and dependent on the experience of the examiner. In addition, it requires multiple optimizations of the experimental conditions. In RIA, techniques are optimized using monoclonal antibodies to eliminate cross-reactivity. RIA requires radioactive isotopes of iodine as an indicator. Although RIA is a sensitive method, it requires careful preparation of radioactive antibodies as well as exposure to radiation. In addition, several steps are required to handle, store, and dispose of radioactive materials.

In 1971 Peter Perlman and Eva Engvall together with other researchers from the University of Stockholm published the first article on the ELISA immunoenzymatic technique. The article specified the quantitative chemical analysis of IgGs in rabbit serum using ALP as substrate enzyme [9]. The working principle of the method is based on the antigen-antibody immune reaction. The antigen or antibody is fixed on a solid substrate and subsequently contacted with a specific immunoglobulin. An enzyme is bound to the specific immunoglobulin that degrades the colorless substrate causing the appearance of a colored compound that can be determined by spectrophotometry [10]. This technique cannot identify the isozymes and can give false-positive or false-negative results [11,12].

ALP VALUES IN NEONATOLOGY

Growth, changes in bone metabolism and changes in the hepatobiliary system cause a great variability of ALP values according to age and sex, especially in childhood and puberty. Because of these increased dynamics, it is very important to identify when phosphatase values are increased as a result of the installation of pathology, or are physiologically adapted to age and sex (Figure 3). In the studies carried out over the years, an attempt was made to determine some reference intervals for the most concrete establishment of the appearance of the pathology. Continuous classification of test results with percentile plots from birth to adulthood may improve the use of ALP activity as a screening marker for hypophosphatasia and related conditions.

In 2017 Jakob Zierk et al. conducted a study on a sample of 124,440 patients and made percentile charts (between the 2.5th and 97.5th percentile) for the correct interpretation of ALP values according to age and sex [13].

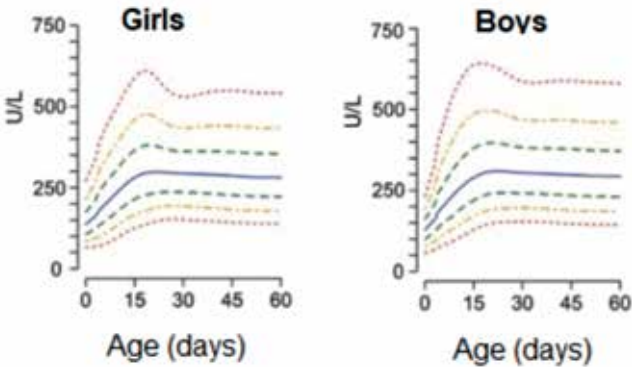

FIGURE 3. ALP values according to age and sex [13]

The use of reference intervals based on percentiles helps us to identify as precisely as possible the changed values in the context of the appearance of pathology and to follow the evolution of the patient against the strict numerical intervals.

ALP USES IN NEONATAL PATHOLOGY

Important modified ALP is an important biomarker in the diagnosis of many pathologies such as liver dysfunctions, bone diseases, renal failure and neoplasia.

ALP shows physiologically increased values in the newborns and in pregnant women when associated with macrosomia, but with normal blood glucose values [14]. Pathologically elevated values are found in biliary tract obstruction, diseases with increased osteoblast activity (such as Paget's disease of bone), increased calcium levels (such as hyperparathyroidism), vitamin D3 deficiency, hepatocytic damage, and celiac disease. The placental isozyme of ALP shows increased values in malignant tumors such as seminomas [15,16,17,18].

Lower levels of ALP are less common than high levels. Some conditions or diseases such as hypophosphatemia, malnutrition, magnesium deficiency, hypothyroidism, severe anemia, achondroplasia and cretinism, severe enteritis, pernicious anemia, aplastic anemia, chronic myeloid leukemia, Wilson's

disease can lead to a reduction in ALP levels. In addition, some drugs have been shown to reduce phosphatase levels [19].

Deficiency of the non-specific tissue isoenzyme of ALP (TNSALP), an isoenzyme that has the ability to hydrolyze phosphate groups from a wide spectrum of physiological substrates leads to hypophosphatemia (hPP), an inborn error of metabolism caused by abnormal vitamin B6 metabolism, characterized by epileptic seizures in the most severe cases, and by hypomineralisation of the skeleton and teeth with rickets and early tooth loss in children or osteomalacia and dental problems in adults caused by the accumulation of inorganic pyrophosphate (PPi) [20]. Subjects with hypophosphatemia have an isolated deficiency of TNSALP activity, and placental and intestinal isozyme activity is normal. The most severe cases are fatal in childhood, practically characterized by the complete absence of bone, kidney and liver phosphatase in all tissues [21].

Several studies have indicated the involvement of phosphatases in cellular events such as the regulation of protein phosphorylation, cell growth, apoptosis, and cell migration during embryonic development. ALP isozyme genes are regulated by distinct signals, as shown by clear differences in the expression of their actions. Ectopic expression of isozymes has been associated with a wide variety of human cancers (breast cancer, choriocarcinoma, ovarian, testicular, lung and gastrointestinal tract cancer, osteosarcomas, bone metastases, hepatocellular carcinoma) [22,23].

The use of ALP has been demonstrated as a biomarker for osteopenia of prematurity at values above 600-800 IU/l [24]. The disease has an increased incidence in premature infants under 1250 grams, especially those associated with broncho dysplasia, necrotic enterocolitis and requiring many days of hospitalization in neonatal intensive care. Elevated ALP values have been associated with severe osteomalacia in preterm infants [25].

Enas et al. concluded in a 2016 study that high ALP levels can be considered a reliable biomarker to predict bone mineralization status and the need for radiological evaluation in preterm infants, especially those <1000 g at birth and <32 weeks of gestation [26].

A study published in 2022 by Manoj Kumar et al. demonstrated a statistical correlation between the dose of caffeine citrate administered to premature infants and the small gestational age, with the onset of prematurity osteopenia, by using serum ALP as a biological marker [27].

In recent studies, the use of ALP as a treatment for some diseases with severe implications has been attempted. A soluble recombinant human non-specific alkaline phosphatase has been approved for the treatment of perinatal, infant, and juvenile hypophosphatemia and has been shown to be successful in improving symptoms and long-term survival [28]. ALP has also been used in the treatment of necrotic ulcerative colitis, which has proven its effectiveness in reducing intestinal inflammation in newborns at risk [29].

In recent years, several studies have been conducted in an attempt to determine a correlation between the presence of neonatal jaundice and ALP values. In 2015 Mousa Ahmadpour-Kacho et al. published the first documentation of a correlation between ALP values in the umbilical cord and the prediction of jaundice evolution. Their study included 102 full-term newborns. Their results showed a correlation of ALP values above 314 IU/L with the need for phototherapy [30]. In 2017 Assal et al. published a study of 200 normal-weight term neonates, and the results showed that all neonates with elevated ALP values required treatment for hyperbilirubinemia [31]. Also, in 2019 Elmonem et al. conducted a study on 100 newborns with a gestational age of more than 35 weeks in which they demonstrated that ALP values can be a good predictor of severe jaundice and can predict its onset [32]. In 2020, the study by El-Amin et al. showed a statistical correlation between increased levels of ALP (> 145IU/l) in the umbilical cord and intense forms of neonatal jaundice in a number of 60 term and premature newborns (groups equal in number) [33].

In 2020 Dima et al. showed a statistical correlation between APL values in the umbilical cord of over 319.55 U/l and the onset of intense forms of neonatal jaundice, with the need for phototherapy, studying a group of 250 normal-weight newborns, at term, without associated pathologies [34].

Also, in 2021 Gulishan D et al. found a positive correlation between ALP values in the umbilical cord and serum bilirubin values at 72 hours [35].

DISCUSSIONS

ALP has proven to be a useful tool in the evaluation of various conditions in the neonatal period. There are many studies that have shown a close connection between increased values for serum ALP with changes in other biological markers such as calcium and phosphorus, and the onset of osteopenia of prematurity.

Also, recent studies have emphasized the importance of using ALP as a biomarker for the early detection of newborns who will develop increased values of total bilirubin and implicitly the increased incidence of intense forms of jaundice.

Research continues in an attempt to establish other statistical correlations between ALP and various other conditions in the neonatal period.

The use of ALP as a treatment in certain neonatal or pediatric onset conditions should not be ignored either.

CONCLUSIONS

ALP is an easy-to-measure biomarker that can be successfully used in neonatology. The existing studies to date encourage the use of ALP as a biomarker especially for the detection of osteopenia of prematurity and for the early detection of newborns who will develop intense forms of jaundice.

There are still differences in the serum ALP values considered as cutoffs for the diagnosis of various neonatal conditions, but performing multicenter studies on a large number of cases could establish a valid consensus in order to use the same cutoff values of ALP in all Neonatology wards.

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