Benign Inherited Hyperphosphatasemia of Intestinal Origin: Report of Two Cases and a Brief Review of the Literature

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Two families with benign hyperphosphatasemia of intestinal origin were studied and compared with six other cases reported in the literature. No evidence of clinical abnormalities or explanations for the unusual enzyme concentrations were found. Agarose gel electrophoresis of alkaline phosphatase (ALP, EC 3.1.3.1) isoenzymes in serum demonstrated markedly increased intestinal isoenzymes (the "soluble" and the "hydrophobic" forms), which accounted for ~60% of total ALP activity. The description of these families demonstrated patterns suggesting autosomal-dominant inheritance, even if the precise genetic background of the abnormality affecting the enzyme production or the control mechanisms for its entry into the circulation could not be determined. Exact recognition of this benign biochemical abnormality should help to avoid unnecessary investigation.

Additional Keyphrases: alkaline phosphatase isoenzymes - genetics - "Ulysses" syndrome

The activity of human alkaline phosphatase (ALP, EC 3.1.3.1) in serum is commonly measured in clinical chemistry laboratories. Increased values are often associated with disorders of the skeletal or hepatobiliary system (1). Apart from clinically important conditions, asymptomatic persistent increases in ALP have been reported (2). In 1981, McEvoy et al. (3) described the case of a family with increased concentrations of serum ALP in the absence of disease, with a preponderance of intestinal isoenzyme. Subsequent reports supported the possible familial nature of this syndrome (4-7). Here I describe two new cases of abnormal ALP activity with isoenzyme data and review the published literature. I call attention to this condition to avoid unnecessary extensive investigation of such cases and to avoid the subsequent "Ulysses" syndrome (8), in which complex, prolonged investigations result from the pursuit of a clinically insignificant abnormality.

Patients and Methods

Patients

Case 1: PS, a 23-year-old man (blood group A, Rh+), with a history of atopic dermatitis, was referred to our hospital for investigation. He denied any drug intake.

On physical examination, no clinical abnormality was present and abdominal ultrasonography was normal. Results of laboratory investigations including serum biochemical and hematological tests were within reference limits, but serum ALP, measured by the method recommended by the International Federation of Clinical Chemistry at 37 °C with a Cobas-Bio centrifugal analyzer (Roche, Nutley, NJ) (9), showed an activity of 560 U/L (adult reference interval, 30–85 U/L). Two months later, his serum ALP was 645 U/L, and two years after the original observation was 483 U/L. Serum ALP five years after the initial presentation was persistently increased (320 U/L) in the absence of any associated illness. ALP isoenzyme analysis (see below) showed a tremendous increase in the intestinal fractions in all samples (46% to 60% of total ALP activity), and a systematic survey of the whole family showed an identical isoenzyme pattern in some family members (Figure 1).

Case 2: OB, a 16-year-old asymptomatic boy (blood group A, Rh-), had a routine automated blood chemistry panel (SMAC II; Technicon, Tarrytown, NY) performed at a local laboratory. All results were within the reference limits, except for serum ALP, which was 542 U/L (age-related reference interval, 60–300 U/L). Measurement five months later confirmed that the concentration of serum ALP was persistently increased (383 U/L) and laboratory consultation was requested. Consequently, sera were obtained from the propositus, his parents, sister, and members of the immediate family, for enzyme and isoenzyme studies. Intestinal ALP activity was increased in many samples (Figure 2). In particular, the mother and two maternal aunts had...
normal total ALP activity but an abnormal isoenzyme pattern.

Methods

Alkaline phosphatase studies. ALP isoenzymes were determined by agarose gel electrophoresis, as described by Van Hoof et al. (10), and quantified by densitometric scanning with a Cliniscan densitometer (Helena Labs., Beaumont, TX). The detection limit of the agarose system for intestinal fractions was 2 U/L. Polyclonal antiserum, raised against placental ALP, which cross-reacted with intestinal ALP but not with other ALP isoenzymes, was kindly provided by Beckman Analytical S.p.A., Milan, Italy. Neuraminidase (from Vibrio cholerae; EC 3.2.1.18) was from Behringwerke AG, Marburg, F.R.G. Phospholipase C (from Bacillus cereus; EC 3.1.4.3), bromelain (EC 3.4.22.4), papain (EC 3.4.22.2), and L-phenylalanine were purchased from Sigma Chemical Co., St. Louis, MO. ALP from a specimen of human small intestine obtained at surgery was extracted into n-butanol within 1 h of resection; the aqueous phase obtained after centrifugation was used in subsequent assays (11). For treating samples with neuraminidase, I added 1 kU/L neuraminidase solution to a serum sample and incubated for either 15 min (25 µL of serum plus 5 µL of neuraminidase) or 24 h (25 µL of serum plus 25 µL of neuraminidase) at 37 °C (12). I added phospholipase C to samples (final activity, 80 kU/L) and incubated the specimens at 37 °C for 3 h (13). For papain hydrolysis of the intestinal ALP, I added a suspension containing 100 g of papain per liter of 0.01 mol/L Tris-borate buffer (pH 8.6) to an equal volume of the sample and incubated the mixture at 37 °C for 15 min (14). Samples were also incubated overnight at 37 °C with bromelain, 10 g/L in Tris-HCl buffer, 0.02 mol/L, pH 7.6 (15). When using anti-placental ALP antibodies, I added 5 µL of the antibody solution to 25 µL of serum, as described earlier (10). To investigate the effect of L-phenylalanine on the pattern of ALP isoenzymes, I included the inhibitor in the staining reagent (final concentration, 10 mmol/L) and assessed its effect on the intensity of staining.

Figure 3 shows the isoenzyme patterns of the two cases studied. In both samples, I encountered a marked increase in the common intestinal fraction (in the β-region of serum proteins) and the presence of a second fraction that co-migrated with bone ALP in the slow-moving α2-region, but it was less diffuse and neuraminidase-resistant (Figure 3, lanes 2 and 7). The change in electrophoretic mobility after incubation with the anti-placental/intestinal antisera in association with the resistance to neuraminidase identified both fractions as intestinal. The additional fraction of intestinal-type isoenzyme corresponded to the intestinal "variant" ALP described by Van Hoof et al. (10, 16) in most conditions where the main intestinal ALP was also present. Like intestinal ALP, this fraction showed a marked decrease in staining intensity when L-phenylalanine was incorporated in the staining medium, whereas liver and bone ALP were minimally affected. Pretreatment with papain and bromelain (not shown) and with phospholipase C (Figure 4) reduced the electrophoretic mobility of this fraction to exactly that of the common intestinal isoenzyme.

Discussion

Persistent idiopathic increased ALP activity was first described in an American family by Wilson (17), who reported increased activity of both liver and bone isoenzymes and an autosomal-dominant form of inheritance. Conversely, in the cases I present, the preponderant ALP isoenzyme was the intestinal form (~60% of total activity). A slight increase in intestinal ALP isoenzyme is usually found in apparently healthy fasted individuals of blood groups O or B who are secretor positive (18). In these subjects, intestinal ALP may account for ~10% of total ALP activity. An increased intestinal isoenzyme can also be seen in cirrhosis and end-stage chronic liver disease, owing to failure to eliminate this protein be-
increase in intestinal ALP could be explained by a mutation in the structural gene of the intestinal isoenzyme located on chromosome 2; in the gene regulating its expression; in the gene involved in the synthesis of glycosylphosphatidylinositol, the phospholipid that attaches ALP to the cellular membrane; or in the gene coding for the phospholipase that cleaves glycosylphosphatidylinositol, releasing ALP into the circulation (22). Regarding this hypothesis, it is interesting that increased concentrations of the more anodal intestinal isoform (the so-called variant) are present in these patients, probably representing the hydrophobic form of the intestinal isoenzyme with remaining phospholipid residues attached (16, 23). Pretreatment with proteases or phospholipase C eliminated this anodal band without affecting the mobility of the major intestinal band, suggesting that the latter is a modified form of the fast-moving enzyme. In particular, phospholipase C led to the release of the ALP covalently attached to membranes in a soluble form (24), allowing the previously membrane-associated ALP (the variant) to migrate with normal intestinal isoenzyme (the soluble form), as do serum ALPs of liver origin (22). Intestinal ALP is also liberated from the cellular membrane in an enzymatically active form by bromelain or papain treatment (14, 15), with the small segment removed by protease digestion probably being the hydrophobic domain involved in membrane binding in vivo (15, 22).

One can only speculate about the possible biological importance of the two isoforms of intestinal ALP. Within the cell, intestinal ALP could be released by intracellular phosphatidylinositol–phospholipase action, and converted further by partial proteolysis to a smaller form (25). The enzyme released from enterocytes is reabsorbed in the thoracic lymph and, after entering the circulation, is rapidly cleared by the liver. Thus, although the rate of removal from circulation is the major factor in determining the physiological activity of the intestinal isoenzyme in serum, the increased concentrations of intestinal ALP in patients with inherited hyperphosphatasemia are probably due to an abnormality affecting its production or to the control mechanisms for its entry into the circulation, with a relative increase in membrane-associated intestinal ALP.

In conclusion, although an inherited increase in ALP activity is considered rare, the real prevalence and importance of this benign familial condition are unknown. However, its recognition may prevent unnecessary and extensive investigation. If an increased concentration of ALP is not easily explained by physiological or clinical conditions, determination of ALP isoenzymes by improved methodologies should be more prominent in differential diagnosis.

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