A Supplement to Alkaline Phosphatase Fractionations: Utilization of Gamma-Glutamyl Transpeptidase and Hydroxyproline Assays

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ABSTRACT

Fractionation of serum alkaline phosphatase by either biochemical or electrophoretic techniques is often insufficient to determine the source of the elevated serum enzyme. In 31 unselected patients with high serum alkaline phosphatase a simultaneous determination of serum gamma-glutamyl transpeptidase and urinary hydroxyproline excretion rates was performed, in addition to urea denaturation and L-phenylalanine inhibition tests. Of 25 patients with definite diagnosis, the urea denaturation test correctly identified the source of the elevated serum alkaline phosphatase (ALP) in 16 (64 percent). The serum gamma-glutamyl transpeptidase (GCTP) alone predicted the correct diagnosis in 64 percent of the cases and the urinary hydroxyproline (HOP) alone in 69 percent. When all three tests were performed simultaneously, the combination of results identified the source of the ALP in 88 percent of the cases. It is believed that this combination offers better sensitivity and specificity than any of the three tests used individually.

Introduction

Serum alkaline phosphatase (ALP) is a mixture of various tissue specific isoenzymes among which the principal ones are produced in the liver, bone, placenta and intestine. In order to ascertain the origin of an elevated ALP when either the liver or the skeleton may be involved by disease, laboratories have used a variety of procedures such as heat fractionation, urea denaturation and electrophoretic separation. All such techniques have been criticized because of poor correlation between the results of the assay and the presumed organ(s) of involvement and also because there is much overlap of results between groups of patients with different organ involvement.

Urea denaturation, the procedure utilized in this study, classifies the results into three categories: liver pattern, bone pattern and mixed pattern. When a mixed pattern is obtained, the physician is unable to ascertain whether bone or liver or both are responsible for the elev-
vation, and the test is therefore of no clinical utility. In order to substantiate the validity of the urea denaturation test in assigning a patient to the liver or bone categories, and to assist in resolving the origin(s) of the ALP in the mixed patterns, serum gamma-glutamyl transpeptidase (GGTP) and hydroxyproline (HOP) assays were utilized in all cases of elevated ALP where fractionation by the urea method seemed indicated.

GGTP was chosen because it is a sensitive indicator of hepatobiliary disease and, further, because it is not elevated in primary diseases of the skeleton, the placenta or the intestinal mucosa. Several investigators have therefore utilized a determination of GGTP in order to ascertain whether, in a particular case, the elevation in ALP was secondary to hepatic or hepatobiliary disease. Although this additional assay aids in the differentiation of the source of the elevated ALP, two unanswered questions remain: (1) if the GGTP is elevated, can the ALP elevation still be the result of elevations of both liver and bone isoenzymes? and (2) if the GGTP is normal, is the ALP elevation necessarily due to bone involvement?

Increased urinary HOP excretion rates, on the other hand, are observed only in conditions associated with increased collagen turnover rates and are, therefore, a good indicator of osteoblastic activity, since over 70 percent of the total body collagen is located in the bone matrix. In localized lesions such as metastatic disease and fractures, there is a clear relationship between the urinary HOP excretion rate and the extent of bone involvement. In metabolic bone disease, patients with elevated HOP values have universally increased bone turnover rates, whereas in cases of osteoporosis in which the turnover rate is usually normal, the excretion of HOP is normal.

Diseases in which elevated HOP values may be encountered include Paget’s disease of the bone, Marfan’s syndrome, acromegaly, osteomalacia, hyperthyroidism and skeletal malignancy, both primary and secondary. Several investigators have demonstrated excellent correlation between urinary hydroxyproline excretion rates and the extent of pathological involvement in metastatic bone disease, in many instances even before radiological evidence of bone metastasis is observed.

Methods and Materials

The population studied consisted of 31 unselected patients with elevated ALP of at least 200 IU per L. ALP and GGTP were measured kinetically at 37° C on a Gilford Model 2400-S spectrophotometer. The ALP and GGTP were assayed by the method of Bowers and McComb and Szasz, respectively. The method for the urea denaturation of ALP was based on the procedure of Posen. Serum (0.2 ml) and 6 M urea (0.2 ml) were mixed, incubated for 18 minutes at 37° C and then 0.1 ml of the mixture was utilized in the ALP assay. The L-phenylalanine (LPA) inhibition of intestinal ALP was based on the procedure of Fishman. Serum (0.2 ml) and LPA/AMP (2.7 ml) were incubated at r.t. for 20 minutes. Exactly 0.2 ml of p-nitrophenylphosphate substrate was then added and the reaction assayed kinetically at 37° C. The serum assays were performed on fresh serum or serum stored for less than two days at 4° C. Urinary hydroxyproline was determined according to the method of Prockop and Udenfriend. All patients were placed on a hydroxyproline-free diet for 48 hours with the urine collected in HCl during the second 24 hour period.

The normal GGTP ranges for males and females were 0 to 45 IU per L and 0 to 40 IU per L while ALP and HOP were

* Gilford Instrument Labs, Inc., Oberlin, OH 44074.
60 to 110 IU per L and 9 to 45 mg per 24 hours, respectively. ALP urea fractionations, which are expressed as percentage of activity remaining, are interpreted as follows: bone (0 to 15 percent), mixed (16 to 34 percent) and liver (35 to 60 percent). LPA remaining activities below 80 percent are considered suggestive of intestinal origin of the ALP.15

Results

The 31 patients were divided accordingly with the results of the urea denaturation test in 3 groups: liver, bone or mixed patterns as shown in tables I, II and III. The discharge diagnoses were arrived at by a combination of clinical, radiological and laboratory findings and were verified by biopsy, autopsy and/or complete metabolic workup when appropriate. The person interpreting the results of the tests which form part of this study had no knowledge of the results of the other studies or of the final diagnosis.

Of the 31 patients with elevated alkaline phosphatase, the urea-resistant fraction was greater than 35 percent (liver pattern) in nine. Eight had definite and one had probable liver disease. A bone pattern (urea resistant fraction of 15 percent) was observed in seven patients. All seven had demonstrable bone disease with osteoblastic activity. In 15 patients, the urea denaturation pattern was between 16 and 35 percent (mixed pattern). Of these 15 patients, definite liver disease was present in five and in all five the elevated GGTP correctly predicted the diagnosis. In three patients with osteoblastic bone disease and no documented liver or biliary tract abnormalities, the GGTP was elevated in all three and the HOP in two. One patient with metastatic disease of both skeleton and liver had elevated GGTP but normal HOP. No definite diagnosis could be established in the remaining six cases. All six had mixed urea denaturation patterns. There were four instances of elevated GGTP and two of elevated HOP in this last group.

Discussion

Addition of the GGTP test to patients with elevated alkaline phosphatase contributed to an additional diagnosis of liver disease in five patients and addition of hydroxyproline excretion test contributed to an additional diagnosis of bone disease in two. Altogether there were nine elevations of GGTP in patients

<table>
<thead>
<tr>
<th>Case</th>
<th>ALP† (Percentage)</th>
<th>LPA§ Activity (Percentage)</th>
<th>GGTP‡</th>
<th>HOP§</th>
<th>Discharge Diagnosis</th>
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*Figures are percent of original activity remaining after denaturation with urea or inhibition with L-phenylalanine. GGTP values are in IU per L (normal up to 45) and HOP in mg per 24 hours (normal up to 45). For interpretation of denaturation pattern, see text.
†Serum alkaline phosphatase
‡Gamma-glutamyl transpeptidase
§L-phenylalanine
xHydroxyproline
TABLE II

Patients with Osteoblastic Pattern in the Urea Denaturation Test*

<table>
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<tr>
<th>Case</th>
<th>ALPi (Percentage)</th>
<th>LPAS (Percentage)</th>
<th>GGTPf</th>
<th>HOPx</th>
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<td>4</td>
<td>96</td>
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<td>17 Compression fracture of lumbar spine</td>
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</table>

*Figures are percent of original activity remaining after denaturation with urea or inhibition with L-phenylalanine. GGTP values are in IU per L (normal up to 45) and HOP in mg per 24 hours (normal up to 45). For interpretation of denaturation patterns, see text.

Of the 25 patients with definite diagnosis, the urea denaturation test correctly identified the source of the elevated ALP in 16 (65 percent). A determination of GGTP confirmed the diagnosis in an additional five and a test for urinary HOP excretion rate contributed two more positive diagnoses. When all three tests are performed, the source of the ALP was identified in 22 of the 25 patients (88 percent). The combination of the urea denaturation test plus a determination of GGTP and HOP offers a better combination of sensitivity and specificity than any of the three tests performed alone.

References


