Biliary Alkaline Phosphatase Measured by Mini-Column Chromatography on DEAE-Cellulose: Application to Detection of Hepatobiliary Diseases

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Using chromatography on diethylaminoethyl (DEAE) cellulose, we measured biliary alkaline phosphatase (BALP; EC 3.1.3.1) activities in sera from 182 patients, most with hepatobiliary disorders but some with non-hepatobiliary diseases. Relative BALP activities were extremely low in otherwise healthy carriers of hepatitis B virus (mean: 5.4 U/L) and in patients with non-hepatobiliary diseases (mean: 5.3 U/L). Although BALP activities were detectable in some cases of liver cirrhosis and chronic hepatitis, these values were generally low (respective means: 12.6 and 12.0 U/L). High BALP activities were detected in patients with primary hepatocellular carcinoma, secondary metastatic liver tumors, and obstructive jaundice: mean values were 27.2, 37.2, and 73.6 U/L, respectively. There was no correlation between BALP activity and bilirubin concentration in patients with obstructive jaundice, nor between BALP activities in obstructive jaundice caused by stones and in those caused by extrahepatic tumor. Some patients with primary hepatocellular carcinoma had high BALP but low alpha-fetoprotein (AFP) values, some others the reverse. Based on AFP alone, the sensitivity for detecting hepatocellular carcinoma was 79%; adding BALP in parallel improved the sensitivity to 97%. We found mini-column chromatography on DEAE-cellulose useful for determining BALP activity in hepatobiliary diseases.

Additional Keyphrases: liver disease · bilirubin · isoenzymes · cancer · obstructive jaundice · cirrhosis · alpha-fetoprotein

The biliary isoenzyme of alkaline phosphatase (ALP; orthophosphoric-monoester phosphohydrolase [alkaline optimum]; EC 3.1.3.1) was first recognized by Fritsche and Adams-Park, who called it the "high-molecular-weight fraction" of alkaline phosphatase (1).3 Since then, various workers using different methods and electrophoretic media have given this enzyme fraction various names. Viot et al. (2, 3), using electrophoresis on cellulose acetate, studied this isoenzyme in patients with metastatic liver cancer and reported that the enzyme, which they called the "alpha-1 isoenzyme," showed 96% sensitivity and 93% specificity for the presence of cancer. Using the same analytical method, Burlina et al. (4) found 100% sensitivity for primary hepatocellular carcinoma, detecting the anodic "biliary" fraction of alkaline phosphatases (BALP) in all of 32 patients with primary hepatocellular carcinoma and in all of seven cases of secondary metastatic liver neoplasia. However, in the same year, and also using cellulose acetate electrophoresis, Siede and Seiffert (5) found a cathodic fraction, and they called it BALP. In their studies, only 12 of 24 primary metastatic liver cancers were positive for activity of the cathodic isoenzyme, and those cases were highly correlated with cholestasis (5, 6). In 1984, Karman et al. (7) used mini-column chromatography on DEAE-cellulose to detect the biliary fraction of alkaline phosphatase. They studied only patients having breast cancer with liver metastasis and noted a 70% sensitivity and an 80% specificity of this test (7).

Here we report measurement of BALP in seven groups of patients with hepatobiliary or non-hepatobiliary diseases, by use of mini-column chromatography on DEAE-cellulose.

Materials and Methods

Reagents. The DEAE-cellulose anion-exchanger (DE52, microgranular, preswollen) was from Whatman Inc., Clifton, NJ. The ALP diagnostic kit (ALP 10, single-reagent system, stock no. 245-10) and the ALP calibrator (Accutrol Abnormal Chemistry Control, catalog no. A 3034) were from Sigma Chemical Co., St. Louis, MO. The polystyrene chromatography mini-columns (0.8 × 8 cm) were from Pierce Chemical Co., Rockford, IL. The AFP RIA kit (alpha-FETO.RIABEAD) was from Abbott Laboratories Diagnostics Division, Abbott Park, North Chicago, IL.

Buffer A (pH 7.5) contained, per liter, 10 mmol of Tris and 100 mmol of NaCl. Buffer B was the same, except that the NaCl concentration was 300 mmol/L.

Serum samples. Patients' sera were collected at Chang Gung Memorial Hospital, Lin-Kou Medical Center, Taiwan. We divided the patients studied into seven groups.

Group 1 consisted of 33 patients (ages 40–79 years, mean 55; ratio of females (F) to males (M), 3:30) with hepatocellular carcinoma. The diagnosis was established by histology and (or) angiography in 20 of these patients, or by abdominal echography or other imaging techniques. Echocardiogram with hemoperitoneum was found in one patient. Four patients were diagnosed only by echographic pattern and clinical picture. AFP exceeded 400 µg/L in 17 of these patients.

Group 2 consisted of 15 metastatic liver cancer patients (ages 26–66 years, mean 53). The primary sites of occurrence were as follows: pancreas 2, gastric 3, lung 3, cervix 1, melanoma 1, breast 1, nasopharyngeal 1, cholangiocarcinoma 1, and unknown 2. Liver metastasis was evidenced by multiple masses in the liver detected by sonography.

Group 3 consisted of 23 obstructive jaundice patients (ages 25–70 years, mean 49, F:M 11:12). Nine of these patients had biliary tract obstruction by tumor other than primary hepatocellular carcinoma. Thirteen patients had stones obstructing the biliary tract and one patient had biliary tract structure.

Group 4 consisted of 29 patients (ages 31–67 years, mean 50, F:M 4:25) with cirrhosis. Hepatic cirrhosis was diagnosed from biochemical data (aspartate and alanine aminotransferases; albumin/globulin), histology, and (or) peritoneoscopy in seven patients, nodular surface in liver echograph in eight patients, and esophageal varices in

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3 Nonstandard abbreviations: ALP, alkaline phosphatase; BALP, biliary alkaline phosphatase; DEAE, diethylaminoethyl; AFP, alpha-fetoprotein; and HBV, hepatitis B virus.
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endoscopy in six patients. Eight of these patients were diagnosed only by their clinical picture, liver size, and biochemical data.

Group 5 consisted of 30 patients (ages 21–63 years, mean 38, F:M 5:25) with chronic hepatitis. In group 5, 27 patients were positive for hepatitis B surface antigen; for all 30 patients, alanine aminotransferase activity ranged from 11 to 673 U/L (mean 212 U/L). Liver biopsies were obtained from 26 of these patients within three years preceding the tests and gave the following results: one chronic persistent hepatitis, 10 chronic lobular hepatitis, 12 chronic active hepatitis, two nonspecific reactive hepatitis, one scarring stage.

Group 6 consisted of 30 patients (ages 18–67 years, mean 33, F:M 6:24) with hepatitis B virus (HBV) carrier. These sera were obtained from the Carrier Clinic in the Chang Gung Memorial Hospital.

Group 7 consisted of 22 patients with non-hepatobiliary disease, ages 19–68 years (mean 42), who presented in the Emergency Service.

**Mini-column chromatography for BALP.** We separated the BALP from the other ALP isoenzymes according to the method of Karman et al. (7), except that we used serum instead of plasma. We used 200 μL of the biliary-origin fraction for determination of enzyme activity.

**Determination of the BALP activity, AFP, and bilirubin.** The Sigma ALP test kit contains p-nitrophenyl phosphate (16 mmol/L), magnesium ion (4 mmol/L), mannitol (274 mmol/L), and pH 10.2 buffer. After mixing 0.2 mL of the sample with 1.0 mL of the reagent, the absorbance was measured at 405 nm vs a substrate/buffer solution, in a computerized spectrophotometer (Japan Spectroscopic Co., Ltd., Tokyo, Japan). We used various dilutions of Accutrol Abnormal Chemistry Control, containing ALP 147 U/L (30 °C) as the calibrator, to convert results for the samples into U/L (30 °C). In the primary hepatocellular carcinoma group we determined AFP by RIA with use of the Abbott alpha-FETO.RIABEAD kit. Total bilirubin was determined in an SMA-12 continuous-flow analyzer (Technicon Instruments Corp., Tarrytown, NY).

**Statistical methods.** We calculated the sensitivity of the assay by dividing the number of the patients with a disease and also with BALP above the cutoff value by the total number of the patients with that disease. The specificity of the assay was calculated by dividing the number of the patients who did not have the disease and had BALP below the cutoff value by the total number of the patients who did not have the disease. To assess significance we used the two-tailed Student’s t-test.

**Results**

The within-run precision of the mini-column procedure was determined by analyzing a serum sample from a cancer patient. Based on calculation from five runs, the CVs were 12.8% for the BALP peak and 10.9% for the other ALP isoenzyme peak.

Average analytical recovery of the total ALP activity after chromatography was 96.8%.

In the obstructive jaundice group, all patients showed BALP activities >17 U/L. On the other hand, 51 of 52 of the HBV carrier patients and non-hepatobiliary groups had relative BALP activities <17 U/L (Figure 1). We thus set 17 U/L as our cutoff value. The mean activities for the obstructive jaundice group, HBV carrier group, and non-hepatobiliary groups were 73.6 (SD 63.6) U/L, 5.4 (SD 3.5) U/L, and 5.3 (SD 3.1) U/L, respectively. The sensitivity in the obstructive jaundice group was 100%. When bilirubin concentrations of the obstructive jaundice group were compared with the BALP activities, there was no significant correlation. In nine patients, their obstructive jaundice was caused by extrahepatic tumor obstruction, whereas in 13 patients it was caused by stone impaction. The mean BALP concentrations in these two subgroups were 90.0 (SD 76.6) and 65.0 (SD 51.9) U/L (P >0.1). The hepatitis and cirrhotic groups had similar BALP activities. Six of 30 patients in the chronic hepatitis group and 10 of 29 patients in the cirrhosis group had BALP activities just exceeding 17 U/L, the respective highest values being 24 U/L and 29 U/L. The histological examination of one case in the chronic hepatitis group showed chronic active hepatitis with cholestasis; the value for BALP in this case was 24 U/L. The respective means for these two groups were 12.6 (SD 8.1) and 12.0 (SD 5.9). In the group with secondary metastatic liver tumors, the BALP activities in eight of the 15 patients exceeded 17 U/L, and the highest value was 103 U/L (sensitivity 53%, specificity 65%). The mean and standard deviation were 37.2 and 33.5 U/L. In the primary hepatocellular carcinoma group, 21 of the 33 patients showed BALP >17 U/L (sensitivity 64%, specificity 68%), the highest being 161 U/L. The mean and standard deviation were 27.2 and 30.4 U/L.

In the seven hepatoma patients, AFP concentrations were <20 μg/L. Six of these seven patients had BALP values >17 U/L. Twenty-six of the 33 patients had AFP >20 μg/L. If AFP alone is used to detect hepatoma, the sensitivity is 79%. Thirty-two of the 33 patients had either AFP values >20 μg/L or BALP values >17 U/L (Table 1). Thus, by

![Fig. 1. Activity concentrations of biliary alkaline phosphatase in serum of patients with various diseases](image)

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**Table 1. The BALP and AFP Activities in the Primary Hepatocellular Carcinoma Group**

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>BALP, U/L (rel.)</th>
<th>AFP, μg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>&gt;17</td>
<td>&lt;20</td>
</tr>
<tr>
<td>5</td>
<td>&gt;17</td>
<td>20–400</td>
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<tr>
<td>10</td>
<td>&gt;17</td>
<td>&gt;400</td>
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<tr>
<td>1</td>
<td>&lt;17</td>
<td>&lt;20</td>
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<td>4</td>
<td>&lt;17</td>
<td>20–400</td>
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<tr>
<td>7</td>
<td>&lt;17</td>
<td>&gt;400</td>
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using these two tests together, the sensitivity was improved to 97%.

The significance of BALP in these seven groups of patients was analyzed. The BALP concentration for either the primary hepatoma, secondary liver tumor, or obstructive jaundice group was significantly higher than for either the hepatitis or cirrhosis group \( P < 0.01 \). BALP values for either the hepatitis or the cirrhosis group significantly exceeded those for either the HBV carrier or non-hepatobiliary group \( P < 0.01 \). There was no significant difference between the hepatitis and cirrhosis groups, or between the HBV carrier and non-hepatobiliary groups \( P > 0.1 \). The BALP concentration in the obstructive jaundice group significantly exceeded that in the primary and secondary liver tumor groups \( P < 0.025 \), whereas there was no significant difference between the primary and secondary liver tumor groups \( P > 0.1 \).

Discussion

Because the biliary alkaline phosphatase isoenzyme was detected first, several different methods have been developed for measuring it. Regardless of the method used for the assay, it was found to be closely related to cholestasis. The sensitivity for detecting cholestasis reportedly exceeded 90% \( (5) \). The clinical significance in malignant diseases and metastatic liver cancers has also been discussed by several authors \( (3, 7, 8-11) \). This fraction of alkaline phosphatase is believed to originate from fragments of cell membranes containing alkaline phosphatase activity, or complexes of alkaline phosphatase and lipoprotein-X, which also frequently appear in cholestasis \( (12-16) \). Using mini-column chromatography on DEAE-cellulose, we were able to quantify this fraction of alkaline phosphatase activity.

In this study, we set 17 U of BALP activity per liter as the cutoff value, because only one of our healthy HBV carriers had BALP levels at 17.2 U/L and all of the patients with non-hepatobiliary diseases had BALP values <17 U/L, whereas all of the obstructive jaundice patients showed BALP values >17 U/L (Figure 1). The sensitivity for the obstructive jaundice group was 100%. In the chronic hepatitis and cirrhosis groups the enzyme activities were in low positive range, all of them <29 U/L. Thus, this method may be useful in differentiating hepatitis from biliary atresia, especially in neonates, which currently is a frequent challenge in pediatrics. The bilirubin concentrations are not correlated with the BALP values in our study. This means that severe intrahepatic cholestasis may not necessarily cause severe hyperbilirubinemia, because some of the biliary tree may still be patent and can clear the bilirubin efficiently, while others are completely obstructed. It does not make a significant difference in BALP activities whether the cause of obstruction is a tumor or stones, although the mean BALP value is higher in patients with obstruction by a tumor than in patients with obstruction caused by stones. This may be because tumor obstruction is a slower process than obstruction by stones, so the biliary tree has more time to induce higher BALP.

Another possible application of the present test would be in differentiating between jaundice caused by cholestatic hepatitis and that caused by hepatitis with liver failure. The chronic hepatitis patient who had the highest enzyme activity (24 U/L) had cholestasis observed in histological analysis. Another patient with secondary biliary cirrhosis had enzyme activity as high as 113 U/L (data not shown in the figure). This idea can be tested by collecting and measuring BALP for a group of cholestatic hepatitis patients.

The values for BALP did not reach significance between patients with primary and secondary liver tumors; however, without considering whether the cancer is primary or secondary, we can still use this isoenzyme to detect hepatic malignancy. Because of the high BALP activities in patients with obstructive jaundice, this enzyme is less specific in malignant liver diseases. However, if the obstructive jaundice group is excluded, the specificity for malignant liver disease, if primary and secondary are combined, can reach 85%. Obstructive jaundice can usually be diagnosed sonographically without difficulty.

In primary hepatocellular carcinoma, the 100% sensitivity that Burlina et al. reported \( (4) \) was not observed by us. Our finding of 59% sensitivity was close to that found by Siede and Seiffert \( (5) \). In their study, 50% sensitivity was noted for primary and metastatic liver cancer. The discrepancy could be due to different assay methods and patient groups. In the study of Karman et al. \( (7) \), the sensitivity for breast-cancer metastasis to liver was 70%. In our patients the primary sites for secondary metastatic liver cancers are heterogeneous. The sensitivity is only 53%. In the hepatoma group, six of seven low-AFP patients had a high BALP value; the reason for this is not known. However, because of it the diagnostic sensitivity of primary hepatocarcinoma can be improved by adding this test to the clinical-laboratory studies for such patients. We found that doing so improved the sensitivity from 79% and 97%. In one of our primary hepatocellular carcinoma cases, the hepatoma was small (<3 cm) and the BALP value was 8.9 U/L. More data are required before we can say whether BALP is useful in early detection for hepatocellular carcinoma.

Our chromatographic technique seems to be useful for assaying BALP activities in hepatobiliary diseases. The fractions collected can be used for further characterization, and the procedure is simpler than electrophoresis. However, more data are needed before it can be routinely applied clinically.

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References