Intestinal origin alkaline phosphatase activity in plasma for differential diagnosis of jaundice

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Abstract

Intestinal origin alkaline phosphatase activity (ALP) in plasma was measured by a sensitive immunocapture assay in 104 jaundiced patients—84 with intra-hepatic and 20 with post-hepatic jaundice. Increased enzyme activities were observed in those with intrahepatic disease and subnormal values in those with post-hepatic disease. At a discriminant level intestinal origin ALP showed a diagnostic sensitivity of 77% for intrahepatic cholestasis, with a diagnostic accuracy of 75% for its differentiation from post-hepatic jaundice. This diagnostic accuracy is not as good as that derived with other techniques—for example, imaging—and the technique is therefore not recommended as a supplement or replacement.

Intestinal origin alkaline phosphatase (ALP; EC 3.1.3.1) may be increased in plasma in intrahepatic disease, but is unusual in post-hepatic cholestasis.¹⁻³ Using polyacrylamide gel electrophoresis, Warnes, Hine, and Kay found intestinal (β -globulin mobility) ALP in the plasma of 45% of jaundiced patients with intrahepatic disease, but not in those with post-hepatic lesions.⁴ They suggested that in jaundiced patients its presence was indicative of an intra-hepatic aetiology and was a useful differential diagnostic aid. They did not, however, study the effect of blood group, a factor that is known to affect the activity of this isoenzyme.⁵⁻⁷

Studies from our laboratory, using both polyacrylamide gel and cellulose acetate membrane electrophoresis, indicated poor diagnostic sensitivity of intestinal ALP for intrahepatic jaundice, especially in patients not of blood group O, and incomplete specificity for post-hepatic disease, so that overall efficiency for the differentiation of jaundice was poor.⁸ This poor differentiation by these qualitative procedures was supported by quantitative studies of activity remaining after inhibition of p-bromotetramisole.⁸ These showed substantial overlap in intestinal origin ALP activity between the two diagnostic groups.

The development of more sensitive measurements of intestinal origin ALP activity by immunoassay has increased interest in possible differences between the two forms of jaundice. Using such an assay, Domar *et al* noted a pronounced increase in intestinal origin ALP in patients with non-

biliary or primary biliary cirrhosis but no increase in patients with other causes of cholestasis.⁹ The numbers studied were small, however, and again blood group was not considered.

In addition to high analytical sensitivity, immunological measurement of intestinal origin ALP has the advantage of measuring both intestinal (β -electrophoretic mobility) ALP and the so-called intestinal variant fraction.^{10,11} This latter fraction is normally obscured by liver and bone isoforms on electrophoresis. It is thought to represent intestinal ALP that is released with attached membrane-binding domain.¹¹ Though p-bromotetramisole inhibition also quantifies both fractions,¹² immunoassay provides greater sensitivity and precision at low activity.

We therefore re-evaluated the measurement of intestinal origin ALP in the plasma for the differentiation of intra- and post-hepatic jaundice using a highly specific and sensitive immunoassay¹¹ that reacts with both intestinal and intestinal variant fractions, which together comprise intestinal origin ALP in plasma.

Methods

Non-fasting plasma samples were studied from 104 consecutive patients admitted to a specialist liver unit, with total bilirubin greater than 50 $\mu\text{mol/l}$: 84 had intrahepatic and 20 post-hepatic jaundice. Diagnosis (table 1) was based on clinical findings, together with liver function and serological tests either alone ($n = 17$) or supplemented by ultrasonography ($n = 46$), radiological examination ($n = 65$), or liver

Table 1 Clinical diagnosis in jaundiced patients studied

Intrahepatic jaundice	
Non-biliary cirrhosis: alcoholic	27
other	13
Hepatitis: alcoholic	8
drug	4
other	5
Primary biliary cirrhosis	9
Carcinoma	4
Miscellaneous	15
Total	84
Post-hepatic jaundice	
Gallstones	3
Benign stricture	3
Carcinoma: pancreas	9
bile duct	1
Miscellaneous	4
Total	20

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Intestinal origin ALP in patients with intrahepatic jaundice (A, ●), post-hepatic jaundice (B, □), and healthy students (C, ▲). Values are shown for all subjects and for those of blood groups A and O.

histology (n = 51). Samples were also obtained from 61 healthy volunteer medical students. Plasma samples were stored at -18°C and examined within two months of collection. All samples were ABO blood grouped by reverse grouping.

Intestinal origin ALP in plasma was quantified by a microtitre plate immunocapture assay, as described previously.¹¹ The assay was linear up to 20 IU/l (IFCC method at 37°C)¹³ and samples with activities above this range were appropriately diluted. Interbatch (n = 13) imprecision (CV) was 8.6% at a mean activity of 1.9 IU/l. The detection limit for intestinal origin ALP was 0.2 IU/l.

Results

The distribution of intestinal origin ALP values in both patients and students was non-Gaussian (figure). Median activities in the patients for all blood groups considered

together, and for blood groups O and A considered separately, are shown in table 2. Median values on the student samples are also shown, together with their upper reference limits (98th percentile).

Compared with the reference population, intestinal origin ALP was increased in the patients with intrahepatic jaundice ($p < 0.005$, overall, by Mann-Whitney U testing) and decreased ($p < 0.05$) in post-hepatic jaundice, resulting in a significant ($p < 0.005$) difference in activity between those with intrahepatic and those with post-hepatic jaundice.

The clinical sensitivity and specificity of intestinal origin ALP measurement for the diagnosis of intrahepatic jaundice were calculated (table 3) using the blood group related upper reference limits (12 IU/l for blood group O, 2 IU/l for blood group A). A discriminant value was also calculated to give the fewest number of misclassifications between intra- and extra-hepatic disease. This level was IU/l for all patients considered together and for the individual blood groups O and A. Using this discriminant, clinical sensitivities and specificities were calculated again (table 3).

Discussion

Our findings confirm the finding of higher intestinal origin ALP in plasma in healthy subjects and in patients with liver disease of blood group O compared with blood group A.⁵⁻⁷ With such differences taken into account, increased intestinal origin ALP was seen in plasma in intrahepatic jaundice and reduced activity in post-hepatic disease. This agrees with a previous suggestion that post-hepatic biliary obstruction may interfere with the absorption of intestinal ALP; hepatocyte damage may decrease its plasma clearance.¹⁴

To assess the diagnostic value of the measurement we first considered activity in relation to the relevant blood group upper reference limit, 12 IU/l for blood group O and 2 IU/l for group A, respectively. Other blood groups were insufficiently represented in the reference population to derive reference values. The diagnostic sensitivity for intrahepatic jaundice of increased intestinal origin ALP activity was poor (45%), as was diagnostic accuracy (54%), though specificity (89%) and the predictive value of a positive test (94%) in the population (prevalence of intrahepatic jaundice 81%) were good.

The need to identify increased activity by referring to the patients' blood group was a disadvantage. Fortunately, the activity value giving the fewest misclassifications between intra- and post-hepatic jaundice (1 IU/l) was the same for the blood groups A and O as for the total patient population, permitting the use of a single diagnostic discriminant value. Using this discriminant, diagnostic sensitivity improved (to 77%), as did accuracy (75%), though at the expense of reduced specificity (65%).

This diagnostic accuracy of 75% is a considerable improvement on the less than 60% value previously obtained by electrophoretic demonstration of intestinal ALP.⁸ As it was

Table 2 Intestinal origin ALP in healthy subjects and in patients with intra- and post-hepatic jaundice

	Intestinal origin ALP (IU/l)			
	Healthy subjects		Jaundiced patients	
	Median	Upper reference limits	Intra-hepatic median	Post-hepatic median
Blood group				
O	2.9 (n = 36)	12.0	5.5 (n = 41)	0.5*† (n = 10)
A	0.6 (n = 19)	2.0	3.0* (n = 30)	0.6 (n = 8)
All	1.5 (n = 61)	12.0	3.5** (n = 84)	0.6*† (n = 20)

* $p < 0.05$, ** $p < 0.005$ compared with healthy subjects by Mann-Whitney U test.

† $p < 0.005$ compared with intra-hepatic jaundiced patients by Mann-Whitney U test.

Table 3 Diagnostic value of intestinal origin ALP in plasma for distinguishing intrahepatic from post-hepatic jaundice

	Upper reference limit*	Discriminant value†
	(Number) %	(Number) %
Sensitivity	(32/71) 45	(65/84) 77
Specificity	(16/18) 89	(13/20) 65
Predictive value of positive	(32/34) 94	(65/72) 90
Predictive value of negative	(16/55) 29	(13/32) 41
Accuracy	(48/89) 54	(78/104) 75

*12 IU/l for blood group O; 2 IU/l for blood group A.

†1 IU/l.

obtained using a quantitative immunoassay of excellent analytical performance further improvement of diagnostic accuracy is unlikely.

In conclusion, an overall diagnostic accuracy of (at best) 75% was obtained by a specific and sensitive measurement of intestinal origin ALP. As this method remains inferior to accuracies close to 90% that can be obtained with other techniques used for the differential diagnosis of jaundice such as ultrasonography, computed tomography, and liver biopsy,¹⁵ we do not recommend that these procedures be replaced or supplemented by the measurement.

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