# Fast Liver Alkaline Phosphatase Isoenzyme in Diagnosis of Malignant Biliary Obstruction

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**Abstract**

Fast liver alkaline phosphatase isoenzyme was measured by cellulose acetate electrophoresis in the sera obtained from 84 patients with specific hepatobiliary diseases and 10 control subjects. The mean value of this isoenzyme in patients with malignant extrahepatic obstruction was 130.58±107.08 U/L, significantly higher than that of patients with benign extrahepatic obstruction (65.63±34.14 U/L), as well as patients with intrahepatic cholestasis and infiltrative liver cancers (65.31±38.11 U/L and 48.47±36.85 U/L, respectively). Furthermore, we could not detect this isoenzyme in normal individuals. When 100 U/L was used as a cut-off value to discriminate between patients with malignant extrahepatic obstruction and the remaining hepatobiliary disorders, the sensitivity, specificity and accuracy of the test were 63 per cent, 84 per cent and 80 per cent, respectively. It is concluded that the fast liver isoenzyme could be a useful marker in diagnosis of malignant extrahepatic obstruction.

**Key word :** Alkaline Phosphatase, Isoenzymes, Jaundice, Liver Disease, Biliary Tract Disorders

Alkaline phosphatase (ALP; EC 3.1.3.1) comprises a group of isoenzymes present in a variety of tissues, including liver, bone, intestine, placenta, kidney and leukocytes. All enzymes in the family are glycoproteins that require zinc for activity, but they are otherwise immunologically distinct with difference physiochemical properties but overlapping substrate specificities(1). In a normal situation, a small amount of alkaline phosphatase is present in serum due to normal destruction of tissue. Whereas, in the setting of abnormal cellular damage, detection of increased isoenzymes activities by electrophoresis permits identification of the specific organs involved(2).

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The serum alkaline phosphatase activity rises in many types of liver disease. Nevertheless, ALP measurement poses the disadvantage that elevation of enzymatic activity is not specific enough in differentiating various hepatobiliary disorders. There appears to be two major hepatic isoenzymes of ALP which differ in their physicochemical and electrophoretic properties, one from hepatocytes that migrates to a position correlating to the alpha 2 serum protein fraction on electrophoresis. This regular 'liver' isoenzyme is found in the serum of all normal individuals and elevation occurs in a wide variety of conditions including hepatocellular disease, intrahepatic cholestasis and biliary obstruction. Therefore, the elevated levels of this isoenzyme are thought to be a nonspecific finding.

The other isoenzyme, 'fast liver' also known as the biliary or high-molecular-weight ALP isoenzyme, originates from plasma membrane and appears in the alpha1 region. Interestingly, this isoenzyme is rarely found in normal individuals. It is also a very sensitive and early marker of intra and extrahepatic cholestasis, even when total alkaline phosphatase activity remains normal[1,3]. Furthermore, it has been reported to be of merit as a tumor marker for hepatic metastasis even in the absence of jaundice[4,5]. The fast liver isoenzyme may coexist with the regular liver ALP and ultrafast liver ALP isoenzyme, which is now known as ALP lipoprotein X complex[6]. ALP lipoprotein X complex' is an unusual ALP isoenzyme migrating in an ultrafast position close to the albumin band on cellulose acetate and agarose elctrophoresis. Although this isoenzyme is more sensitive for cholestasis than fast liver isoenzyme, it seems to be a less specific indicator of biliary obstruction[7].

The purpose of our study was to determine the clinical usefulness of the fast liver isoenzyme by measurement on cellulose acetate electrophoresis. The study included different groups of patients who presented with jaundice, such as infiltrative hepatic disorders (primary or metastatic tumor) and cholestatic disorders (e.g. intrahepatic and extrahepatic cholestasis).

## PATIENTS AND METHOD

The study population comprised 84 patients who attended Chulalongkorn University Hospital between April and November 1998.

Group 1 consisted of 16 patients with malignant extrahepatic obstruction by cholangiocarcinoma or other periampullary tumors [aged 43-85 years, mean 62.3±11.4 years, M:F ratio 10:6]. All patients in this group had dilated intrahepatic and/or common bile duct on imaging study.

Group 2 consisted of 20 patients with nonmalignant extrahepatic obstruction [aged 18-78 years, mean 49.9±14.7 years, ratio of males to females (M:F) 11:9]. Among these, 18 patients had stones obstructing the common bile duct and the remaining 2 patients each had a choledochal cyst. As in group 1, all patients in this group had dilated intrahepatic and/or common bile duct on imaging study.

Group 3 consisted of 31 patients with intrahepatic cholestasis. [aged 23-80 years, mean 47.5± 18.4 years, M:F ratio 17:14]. All patients in this group had serum bilirubin > 2 mg/dl and total alkaline phosphatase exceeded the upper limit level (279 U/L). There was also no evidence of biliary tract obstruction or hepatic infiltration on imaging study. The causes of cholestatic jaundice in this group included systemic infection (n=15), drug-induced jaundice (n=5) and alcoholic hepatitis or cirrhosis (n=11).

Group 4 consisted of 17 patients with infiltrative hepatic disorders [aged 38-73 years, mean 57.8±11.4 years, M:F ratio 15:2]. Fourteen patients had hepatocellular carcinoma, diagnosed by histology or imaging study combined with serum alpha-fetoprotein (AFP) level exceeding 400 ng/dl. The remaining three patients had liver metastasis, diagnosing based on imaging study combined with positive histology.

All blood samples were analyzed for total alkaline phosphatase and its isoenzymes by cellulose acetate electrophoresis (Helena Laboratories, Beaumont, USA) using Electronic Data Center (EDC) Densitometer (Helena Laboratories, Beaumont, USA). In addition, sera from 10 healthy volunteers were included in this study to establish the normal value in the Thai population.

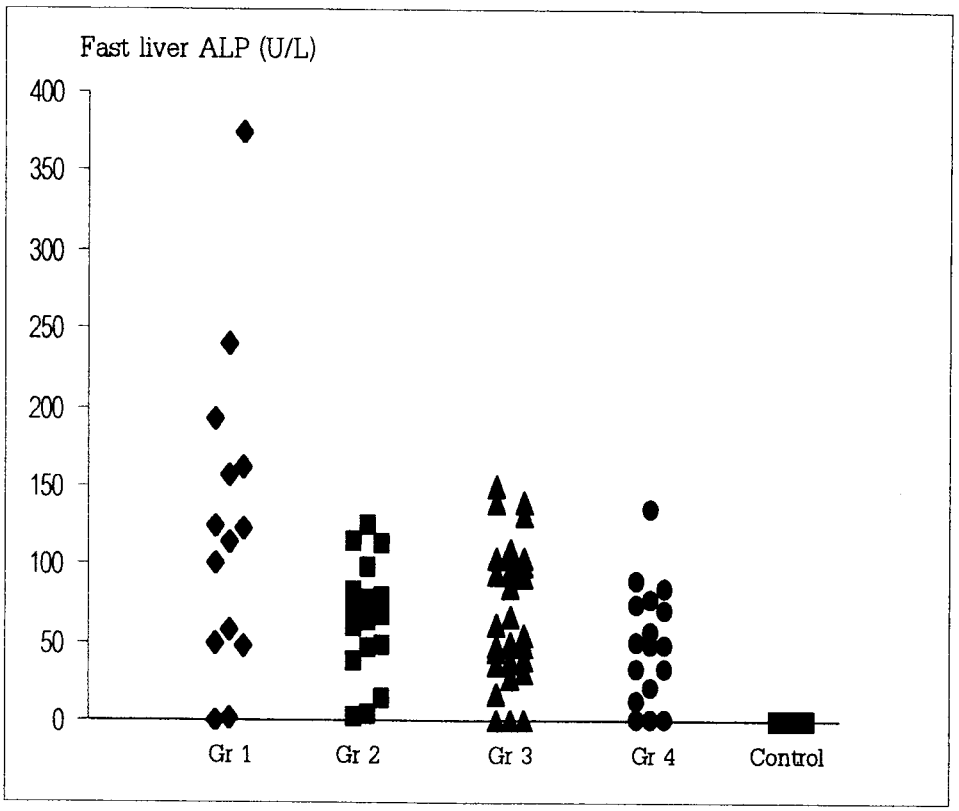
### Statistical Analysis

The data were expressed as mean value ± standard deviation and percentage as their appropriateness. The analysis of variance and Scheffe test was used to test the difference among groups of patients.

**Table 1. Serum total ALP and its isoenzymes in groups studied (values are expressed as mean ± standard deviation).**

| Group | Total ALP (U/L) | Fast liver ALP (U/L) | Liver ALP (U/L) |
|---|---|---|---|
| 1 | 874.56±633.62 | 130.58±107.08 # | 451.5±381.02 |
| 2 | 721.89±516.55 | 65.63±34.14 | 242.1±139.21 |
| 3 | 488.77±230.90 | 65.31±38.11 | 266.81±139.35 |
| 4 | 682.47±625.05 | 48.47±36.85 | 301.18±305.32 |
| Control | 205.41±101.32 | 0 | 75.60±82.40 |

# $p<0.05$ *versus* group 2, 3, 4 and control




NB: Gr 1: Malignant extrahepatic obstruction, Gr 2: Benign extrahepatic obstruction, Gr 3: Intrahepatic cholestasis, Gr 4: Infiltrative hepatic disorders

**Fig. 1. Fast liver isoenzyme level in groups studied.**

## RESULTS

In our study we could not detect the fast liver ALP isoenzyme in serum of normal individuals. The mean value of the fast liver ALP isoenzyme in group 1 was 130.58±107.08 U/L, significantly higher than that found in the other three groups (group 2; 65.63±34.14 U/L, group 3; 65.31±38.11 U/L and group 4; 48.47±36.85 U/L, ($p<0.05$). On the other hand, there was no significant difference of total ALP and the liver ALP isoenzyme among the four groups studied. (Table 1 and Fig. 1)

Comparing the fast liver ALP isoenzyme level in group I and the other groups, the ideal cut-off point for a diagnostic value of the fast liver ALP was determined by selection of multiple points from the receiver-operating characteristic (ROC) curve. The point considered best was 100 U/L. At this level, it had a sensitivity and specificity of 63 per cent and 84 per cent, respectively and the accuracy was 80 per cent.

## DISCUSSION

The mechanism by which different physiological states and disease processes cause an elevation of the serum ALP has been generally accepted that it originates in tissues that undergo metabolic stimulation[8]. Concerning the mechanism of the serum elevation in liver diseases, it is believed that the damaged liver releases the hepatic ALP isoenzyme into serum rather than the damaged liver failing to excrete it and the other isozymes from serum[9]. New protein synthesis appears to be a major factor accounting for the raised hepatic ALP isoenzymes activity in liver disease.

The fast liver ALP isoenzyme was first recognized in 1972 by Fritsche et al, who called it the 'high molecular-weight fraction' of alkaline phosphatase[10]. This fraction 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[11,12]. An increase in this isoenzyme activity in serum is frequently associated with hepatobiliary diseases and malignant tumors. Furthermore, it appears to be more sensitive and specific for diagnosing hepatobiliary disorders when compared to total alkaline phosphatase, gamma-glutamyl transferase and 5' nucleotidase[13-15].

The biochemical determination of total ALP and its isoenzymes is technically easy and reliable. For electrophoretic separation of ALP isoenzymes, various supporting media have been proposed: agar, agarose, starch, polyacrylamide and cellulose acetate[16-20]. The cellulose acetate method offers several district advantages over other identification methods. The technique is inexpensive and suitable for the electrophoretic fractionation of ALP in large numbers of sera, provides ease of handling, stability of supporting membranes and media and reproducibility of results. Both colorimetric and fluorometric methods are available for isoenzyme visualization.

In our study, as shown in Table 1, the mean level of the fast liver ALP in extrahepatic malignancies (group 1) was significantly higher than that found in the other groups. However, the extent of the enzymes elevation of total ALP and the liver ALP do not distinguish among groups studied. The difference of the mean level of the fast liver ALP could be explained that tumor obstruction is a slower process than obstruction by benign conditions such as stones in the common bile duct (CBD). In addition, the etiologic causes of intrahepatic cholestasis in our study are mainly from infections and medications, most of which occur in relatively shorter duration compared with that of malignant obstructions.

Previous data from Viot et al and subsequent studies, suggested that that fast liver ALP was highly correlated with liver metastasis and the presence of this isoenzyme could be a predictive marker of such a condition[4,5,14]. However, in our study, the mean value of the fast liver ALP in this group was comparable to that of benign liver conditions and even significantly lower than that found in malignant biliary obstructions. These results could, therefore, undermine the diagnostic usefulness of the test in detecting liver metastasis in patients with known underlying liver disease. Furthermore, the increasing availability of hepatic imaging, such as ultrasonography (US), computerized tomography (CT) or magnetic resonance imaging (MRI), has made it possible to diagnose hepatic metastasis more accurately.

In general, although US is the modality of choice in the noninvasive study of hepatobiliary tract, its sensitivity in detecting the distal CBD obstruction (stone or tumor) is disappointing. In this case, CT or MRI is far superior to US in differentiate the distal CBD and periampullary cancers from benign lesions. However, an accurate diagnosis is not always satisfactorily achieved and the next appropriate step with a more invasive technique, for example, cholangiography is required[21]. Hence, a simple screening test, such as the fast liver ALP as shown in our study, is considered to be a useful adjunct to non-invasive imaging in order to discriminate between benign and malignant biliary obstructions.

As a diagnostic test, the cut-off value should be reduced to the extent possible to increase the sensitivity of the test, but not too low to decrease its specificity and diagnostic accuracy. Using the fast liver ALP level between 10-17 U/L as an arbi-

trary cutoff point as mentioned in previous studies (4,5,15), we could not differentiate malignant extrahepatic obstructions from those of the remaining groups. In our study, the ideal cut-off point selected from the ROC curve showed that the best point was 100 U/L. At this level, although it had a relatively low sensitivity, the diagnostic specificity and accuracy could be considered acceptable.

## SUMMARY

The fast liver ALP isoenzyme seems to be useful in differentiating between malignant and benign extrahepatic obstructions, as well as intrahepatic cholestasis and liver cancers. It should be considered as an adjunct to diagnosis when noninvasive hepatobilary imaging assessment is inconclusive.

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## REFERENCES

1. Friedman LS, Martin P, Munoz SJ. Liver function tests and the objective evaluation of the patient with liver disease. In Hepatology: a textbook of liver disease Edited by Zakim D, Boyer TD. Philadelphia, 3rd edition W.B. Saunders company 1996: 791-833.
2. Crofton PM. Biochemistry of alkaline phosphatase isoenzymes. CRC Crit Rev Clin Lab Sci 1982; 16: 161-94.
3. McIntyre N, Rosalki S. Biochemical investigations in the management of liver disease. In Oxford textbook of clinical hepatology. Edited by McIntyre N, et al. Oxford, Oxford University press 1991: 293-309.
4. Nishio H, Sakuma T, Nakamura SI, et al. Diagnostic value of high molecular weight alkaline phosphatase in detection of hepatic metastasis in patients with lung cancer. Cancer 1986; 57: 1815-9.
5. Wei J, Chung NC, Wei LLL, et al. High-molecular mass alkaline phosphatase as a tumor marker of colorectal cancer: comparison of two test methods. Clin Chem 1993; 39: 540-3.
6. Wolf P. High-molecular-weight alkaline phosphatase and alkaline phosphatase lipoprotein X complex in cholestasis and hepatic malignancy. Arch Pathol Lab Med 1990; 114: 577-9.
7. Siede WH, Seiffert UB. Relative merits of the biliary alkaline phosphatase isoenzyme and lipoprotein-X in diagnosis of cholestasis. Clin Chem 1983; 29: 698-700.
8. Reichling JJ, Kaplan MM. Clinical use of serum enzymes in liver disease. Dig Dis Sci 1988; 33: 1601-14.
9. Kaplan MM. Serum alkaline phosphatase –another piece is added to the puzzle. Hepatology 1986; 6:526-8.
10. Fritsche Jr HA, Adams-Park HR. Cellulose acetate electrophoresis of alkaline phosphatase isoenzyme in human serum and tissue. Clin Chem 1972;18:417-21.
11. De Broe ME, Roels F, Nouwen EJ, et al. Liver plasma membrane: the source of high molecular weight alkaline phosphatase in human serum. Hepatology 1985;5:118-28.
12. Crofton PM, Smith AF. High-molecular-mass alkaline phosphatase in serum and bile: nature and relationship with lipoprotein-X. Clin Chem 1981;27:867-74.
13. Crofton PM, Elton RA, Smith AF. High molecular weight alkaline phosphatase: a clinical study. Clin Chim Acta 1979;98:263-75.
14. Viot M, Thyss A, Viot G, et al. Comparative study of gamma-glutamyl transferase, alkaline phosphatase and its alpha-1 isoenzyme as biological indicators of liver metastases. Clin Chim Acta 1981; 115:349-58.
15. Yeh CT, Wel JS, Liaw YF. Biliary alkaline phosphatase measured by mini-column chromatography on DEAE-cellulose: application to detection of hepatobiliary diseases. Clin Chem 1989;35: 1684-7.
16. Haije WG, De Jong M. Isoenzyme patterns of serum alkaline phosphatase in agar-gel eletrophoresis and their clinical significance. Clin Chim Acta 1963; 8: 614-20.
17. Hagerstrand I, Skude G. Improved electrophoretic resolution of human serum alkaline phosphatase isoenzymes in agarose gel by Triton X-100. Scand J Chin Lab Invest 1976; 36: 127-9.
18. Jennings RC, Brocklehurst D, Hirst M. A comparative study of alkaline phosphatase enzymes using starch-gel electrophoresis and Sephadex gel-filtration with special reference to high molecular weight enzymes. Clin Chim Acta 1970; 30: 509-17.
19. Kaplan MM, Rogers L. Separation of human serum alkaline phosphatase isoenzymes by polyacrylamide gel elctrophoresis. Lancet 1969; 2: 1029-31.
20. Fritsche HA, Adams-Park HR. High molecular

weight isoenzymes of alkaline phosphatase in human serum. Demonstration by cellulose acetate electrophoresis and physico-chemical characterization. Clin Chim Acta 1974; 52: 81-9.

21. Call GA, Ros PR. Imaging of the liver and biliary tract. In Kaplowitz N, eds. Liver and biliary diseases. Baltimore, 1st edition, Williams & Wilkins 1992; 207-25.

# ไอโซเอ็นซัยม์ชนิดฟาสท์ ลิฟเวอร์ ของอัลคาไลน์ ฟอสฟาเทส ในการวินิจฉัยภาวะอุดตันของท่อน้ำดีจากโรคมะเร็ง

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คณะผู้ทำการวิจัยได้ตรวจวัดไอโซเอ็นซัยม์ของ alkaline phosphatase ชนิด fast liver ด้วยวิธี cellulose acetate electrophoresis ในน้ำเหลืองของผู้ป่วยโรคของตับและท่อทางเดินน้ำดีจำนวน 84 ราย รวมทั้งคนปกติจำนวน 10 ราย ผลการศึกษาพบว่าค่าเฉลี่ยของระดับ fast liver ในผู้ป่วยกลุ่มที่มีการอุดตันของท่อทางเดินน้ำดีจากโรคมะเร็ง (malignant biliary obstruction) เท่ากับ 130.58 ± 107.08 U/L ซึ่งสูงกว่าค่าเฉลี่ยของกลุ่มผู้ป่วยที่มีการอุดตันของ ท่อทางเดินน้ำดีจากสาเหตุอื่น ๆ ที่ไม่ใช่มะเร็ง (non-malignant biliary obstruction, 65.63 ± 34.14 U/L) กลุ่มผู้ป่วย intrahepatic cholestasis (65.31 ± 38.11 U/L) และกลุ่มผู้ป่วยโรคมะเร็งตับชนิด hepatocellular carcinoma และมะเร็งที่ลุกลามมาที่ตับ (liver metastasis) 48.47 ± 36.85 U/L) เมื่อใช้ fast liver ที่ระดับสูงกว่า 100 U/L เพื่อเป็นเกณฑ์สำหรับวินิจฉัยแยกโรคระหว่างกลุ่มผู้ป่วยที่มีการอุดตันของท่อทางเดินน้ำดีจากโรคมะเร็งกับผู้ป่วยกลุ่มอื่น ๆ พบว่ามีความไว (sensitivity) ความจำเพาะ (specificity) และความถูกต้องของการทดสอบ (accuracy) เป็นร้อยละ 63, 84 และ 80 ตามลำดับ สรุปได้ว่าการตรวจ fast liver มีประโยชน์ในการวินิจฉัยภาวะการอุดตันของท่อทางเดินน้ำดีที่เกิดจากโรคมะเร็ง

**คำสำคัญ** : อัลคาไลน์ ฟอสฟาเทส, ไอโซเอ็นซัยม์, ดีซ่าน, โรคตับ, ภาวะท่อน้ำดีอุดตัน

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