ALPHA-L-FUCOSIDASE AS A SERUM MARKER OF HEPATOCELLULAR CARCINOMA IN THAILAND

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Abstract. To evaluate the role of serum alpha-L-fucosidase (AFU) in the diagnosis of hepatocellular carcinoma (HCC), we simultaneously studied both AFU activity and alpha-fetoprotein (AFP) level in 60 patients with HCC, 60 patients with cirrhosis and chronic hepatitis each, 30 patients with other liver tumors and 60 healthy subjects. Serum AFU activity in patients with HCC (1,418.62 ± 575.76 nmol/ml/hr) was significantly higher than that found in cirrhosis (831.25 ± 261.13 nmol/ml/hr), chronic hepatitis (717.71 ± 205.86 nmol/ml/hr) or other tumors (706.68 ± 197.67 nmol/ml/hr) and in controls (504.18 ± 121.88 nmol/ml/hr, $p < 0.05$). With 870 nmol/ml/hr (mean value of controls plus 3 standard deviations) considered as the cut-off point, AFU was more sensitive (81.7 *vs* 39.1%) but less specific (70.7 *vs* 99.3%) than AFP at a level of > 400 ng/ml as a tumor marker of HCC. With both markers combined, the sensitivity was improved to as much as 82.6%. AFU activity in HCC patients was correlated to tumor size ($r = 0.3529$, $p = 0.006$) but not associated with tumor staging classified by Okuda's criteria ($p = 0.1$). The AFU activity in the viral hepatitis group (hepatitis B or C) was also significantly higher than in the non-viral group ($p = 0.0005$). We conclude AFU to be a useful marker, in conjunction with AFP and ultrasonography, for detecting HCC, particularly in patients with underlying viral hepatitis and cirrhosis.

INTRODUCTION

Hepatocellular carcinoma (HCC) constitutes one of the most serious tumors in Southeast Asia, including Thailand, where HBV, and to a lesser extent, HCV infection are prevalent. Most patients with HCC are diagnosed at an advanced stage and therefore, the prognosis is very poor. Nowadays, the diagnosis could be achieved at an earlier stage by regular screening programs among high risk populations, using ultrasonography and a serum tumor marker. Until now, alpha-fetoprotein (AFP) has been considered the best marker in detecting HCC. However, serum AFP is not always elevated to a diagnostic level in all patients, particularly in small HCC (Chen *et al,* 1984; Okuda, 1986) and approximately 30% of advanced HCC would be missed unless another diagnostic tool is used (Colombo, 1995; Tsai *et al,* 1995). Furthermore, it may be also elevated in benign chronic liver diseases, such as chronic hepatitis and cirrhosis (Di Bisceglie and Hoffnagle, 1989; Lok and Lai, 1989). A prospective study in Caucasian patients with cirrhosis found that AFP had a sensitivity of 21% using a cut-off value of more than 100 ng/ml, although the specificity was 93% (Pateron *et al,* 1994). As a result, the clinical usefulness of AFP in the diagnosis of HCC has to be re-evaluated.

Serum alpha-L-fucosidase (alpha-L-fucoside hydrolase; EC 3.2.1.5; AFU) is a lysosomal enzyme present in all mammalian cells which catabolizes fucose-containing glycoconjugates. AFU has been proposed as one of the useful serum marker in the diagnosis of HCC, although no definite hypothesis could be proposed to explain the increase of AFU in this tumor (Leray *et al,* 1989). Studies performed on patients in France and Italy reported a specificity of 90% and a significantly higher sensitivity (approximately 75%) compared with AFP sensitivity (approximately 40-60%) (Deugnier *et al,* 1984; Giardina *et al,* 1992). Contrasting that, in native South-African patients, even though AFU sensitivity and specificity were found as high as in these reports, it was less sensitive and specific than AFP (Bukofzer *et al,* 1989).

The purpose of our study has been to determine the clinical usefulness of this tumor marker in Thai patients diagnosed with HCC, compared to other liver cancers and chronic liver diseases, including chronic hepatitis and cirrhosis.

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PATIENTS AND METHODS

Subjects

Sera for AFP and alpha-L-fucosidase (AFU) analysis were obtained from 4 groups of subjects attending Chulalongkorn University Hospital between November 1997 and May 1998. The first group comprised 60 patients with HCC, 45 cases diagnosed based on histology, and the remaining ones based on clinical features such as liver mass detected by ultrasound or CT scan and serum AFP levels above 400 IU/ml. The tumor stages according to Okuda's criteria were stage I in 3 patients, stage II and III in 48 and 9 patients, respectively. The second and third groups consisted of 60 patients with cirrhosis and chronic hepatitis, respectively. The fourth group comprised 30 patients with liver cancers other than HCC, namely, cholangiocarcinoma (CC) in 14 cases, and the remaining cases were liver metastases established by clinical symptoms and confirmed by needle biopsy. Sera from 60 healthy voluntary blood donors, attending the National Blood Center of the Thai Red Cross, were assayed for AFP and AFU activity to established the normal value in the Thai population. The main characteristics of these subjects are summarized in Table 1.

Laboratory test

Sera from all subjects were separated by centrifugation, stored at -70°C and assayed at the latest within 8 weeks after collection because AFU activity levels would undergo alterations after this peroid (Bukofzer *et al,* 1989). Serum AFU activity was measured by using the modified Zielke method (Zielke *et al,* 1972). In brief, 20 µl serum were added to 100 µl 1 mmol/l *p*-nitrophenyl-α-L-fucopyranoside (Sigma Chemical Company, St Louis, MO), dissolved in 150 mmol/l citrate-phosphate buffer (pH 5). This mixture was incubated at 37°C for 60 minutes, and the reaction was stopped by the addition of 3.5 ml glycine-NaOH buffer (pH 10.5). Blanks were prepared and incubated identically, except for the sample being added just before addition of the glycine-NaOH buffer. Absorbance of *p*-nitrophenol was read at 400 nm and enzyme activity was expressed as nanomoles of *p*-nitrophenyl-α-L-fucopyranoside cleaved per milliliter per hour at 37°C.

The precision of the assays on serum AFU activity was determined by analysis of intra-assay and inter-assay variations. The per cent coefficient variation (%CV) of the intra-assay analysis in 2 normal subjects and 2 HCC patients were 1.8% and 2.9%, respectively, and the %CV of the inter-assay analysis during 10 consecutive days were 8.7% and 6.9%, respectively.

The effect storage had on serum AFU activity was determined in sera from 2 normal subjects and 2 HCC patients. When stored at -20°C, slightly higher serum AFU concentrations were found in samples stored for more than 5 weeks in comparison with samples stored for less than 5 weeks. However, no significant differences were discernible when sera were stored at -70°C.

Serum AFP concentrations were assayed in the same patients by ELISA Kit (Cobas®Core, Roche Diagnostics, Basel, Switerland).

Statistical analysis

The data were expressed as mean values ± standard deviation and percentage as appropriate. Variance analysis and the Scheffe test were used to test the difference in AFP and AFU activities among groups of patients. Spearman correlation coefficients were used to find the correlation between the level of AFP, AFU and tumor size. The difference of AFP and AFU activities between two groups of HCC patients, those positive for viral hepatitis B or C markers and those without viral markers , were assessed using the Mann-Whitney U test. Receiver operating characteristic (ROC) curves were also constructed to establish the diagnostic cut-off level of AFU in patients with HCC.

RESULTS

AFU levels measured

In our study, the mean value of AFU activity in patients with HCC was 1,418.62 ± 575.76 nmol/ml/hr, significantly higher than that found in control subjects (504.18 ± 122.88 nmol/ml/hr) and the other three groups, cirrhosis (831.25 ± 261.13 nmol/ml/hr), chronic hepatitis (717.71 ± 205.86 nmol/ml/hr) and other liver cancers (706.68 ± 197.67 nmol/ml/hr) ($p < 0.05$). There was also a significant difference between cirrhosis and controls, as well as chronic hepatitis and controls ($p < 0.05$), but among cirrhosis,chronic hepatitis and other liver cancers, no significant difference was found ($p > 0.05$). (Table 2).

When comparing between AFU activity levels in HCC and benign liver disease, including other liver cancers, the ideal cut-off point for a diagnostic value of AFU was determined by selecting multiple

Table 1
Clinical characteristics of subjects.

Group	No.	Sex (M/F)	Mean age (yr)	HBV (HBsAg+)	HCV (Anti HCV +)	No viral marker
Hepatoma	60	50/10	56.5 (18-81)	35	8	17
Cirrhosis	60	43/17	52.8 (20-82)	28	8	24
Chronic hepatitis	60	45/15	41.5 (17-75)	40	10	10
Other tumors	30	18/12	55.7 (35-81)	0	1	29

Table 2
Serum AFU and AFP levels in each group studied (Values are expressed as mean ± standard deviation).

Group	AFU (nmol/ml/hr)	AFP (ng/ml)
Hepatoma	1,418.62 ± 575.76 *	11,114.27 ± 51,322.41*
Cirrhosis	831.25 ± 261.13 #	39.73 ± 107.76 #
Chronic hepatitis	717.71 ± 205.86 #	4.97 ± 4.06
Other liver tumors	706.68 ± 197.67	9.46 ± 14.34
Controls	504.18 ± 121.88	4.68 ± 4.10

* $p<0.05$ versus cirrhosis, chronic hepatitis, other liver cancers and control
# $p<0.05$ versus controls

Table 3
Comparison of upper limits of serum AFU activity.

Cut-off point (nmol/ml/hr)	Sensitivity	Specifity	Positive p-value	Negative p-value
626 (1 SD)	96.7	29.3	35.4	95.7
687 (1.5 SD)	93.3	40.0	38.4	93.8
748 (2 SD)	86.7	50.0	40.9	90.4
809 (2.5 SD)	83.3	60.7	45.9	90.1
870 (3 SD)	81.7	70.7	52.7	90.6

Table 4
Sensitivity and specificity of AFU and AFP in HCC* patients, differentiated from patients with other liver disease.

Accuracy	Cut-off	Point	Sensitivity	Specificity
AFU (nmol/ml/hr)	870	81.7	70.7	72.4
AFP (ng/ml)	400	39.1	99.3	85.2
Combination of two tests		82.6	70.7	73.5

* 45 cases, diagnosed based on histology

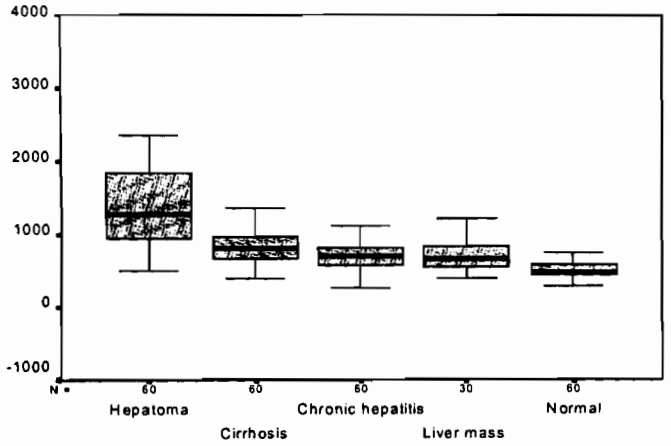

Fig 1–Serum AFU activity in the various groups studied AFU activity (nmol/ml/hr).

points from the ROC curve. The point considered best was three standard deviations above the mean value (Table 3). At this concentration, AFU had a sensitivity of 81.7% and a specificity of 70.7%. This was comparable with a sensitivity of 39% and a specificity of 99.3 % for AFP at a level of > 400 ng/ml(Table 4).

Relationship between AFU, AFP levels and size of HCC

As for tumor mass, no significant correlation between AFP activity and tumor size was found ($r = -0.1186$, $p = 0.35$). On the contrary, the increased frequency of an abnormal AFU concentration in larger tumors was significant in 45 cases of HCC diagnosed by histology ($r = 0.3529$, $p = 0.006$).

Relationship between AFU, AFP levels and staging of HCC

The mean value of AFU for Okuda stage I was 1,030.67 ± 228.66 nmol/ml/hr, for stages II and III the values were 1,387.93 ± 588.73 and 1,711.63 ± 486.61 nmol/ml/hr, respectively. However, no obvious association was found between tumor staging and AFU concentrations ($p = 0.1$). Likewise, there was no association between AFP level and staging of the tumor ($p = 0.42$).

Relationship between AFU, AFP levels and etiology of HCC

There was a significant correlation between AFU concentrations and viral markers of chronic liver disease (HBV or HCV). The AFU level in the viral hepatitis group was 1,310.45 ± 588.55 nmol/ml/hr, significantly higher than in the non-viral group (940.78 ± 388.35 nmol/ml/hr) ($p = 0.0005$). The same correlation was observed with regard to the AFP level ($p = 0.001$).

DISCUSSION

The precise mechanism underlying the increase in serum AFU in HCC is unknown. One possible explanation is that the tumor cells increase synthesis and secretion of proteins (Vischer and Reutter, 1978; Holmes and Hakamori, 1982). However, this possibility is not supported by a study (Laray *et al*, 1989) showing a significantly lower AFU level in HCC tissue compared to non-tumoral liver tissue. Moreover, a recent report by a Japanese group (Takahashi *et al*, 1994) found similar levels of AFU activity detected in culture supernatants of HCC cell lines and liver cells. Thus, it appears that an elevated serum level of AFU is not directly due to increased production by tumor cells.

Deugnier *et al* (1984) first reported the value of serum AFU as a useful tumor marker for HCC in the French population. In this study, a significantly higher AFU sensitivity was found (76.2%) compared with the AFP sensitivity (42.9%). Although no correlation could be established between the levels of AFU activity and disease severity according to Child scoring, it could be considered a simple complementary test for screening of HCC. A subsequent Italian study by Giardina *et al* (1992) has shown results consistent with the above-mentioned report, while a study in African black patients by Bukofzer *et al* (1989) rather argued in favor of AFU as a marker for HCC, even though AFU sensitivity and specificity were comparable to the previous report (Deugnier *et al*, 1984) as high as in the French report. However, a study performed in Asia by Takahashi *et al* (1994) suggested that AFU could be a promising marker for detecting HCC, especially at an early stage of the tumor, when compared to AFP and des-γ-carboxy-prothrombin.

The mean value of normal serum AFU activity obtained from healthy control groups in our study was 504.18 nmol/ml/hr (range between 206.10 and 865.70 nmol/ml/hr) which was comparable to those reported by Zielke *et al* (1972) and Marotta *et al* (1991). This level was higher than those observed by Italian and Japanese groups (Giardina *et al*, 1992; Takahashi *et al*, 1994) but lower than in native Africans (Bukofzer *et al*, 1989). Such a difference

may be explained by racial diversities in the distribution of AFU phenotypes (Bukofzer *et al,* 1989).

In our study, as shown in Table 2, serum AFU activity levels in the HCC group were significantly higher than those found in the other four groups tested. However, when compared to AFP (> 400 ng/ml) as a serum marker for HCC, AFU is much more sensitive (81.7 *vs* 39.1 %) but less specific (70.7 *vs* 99.3 %). Hence, our results are in accordance with those of Deugnie *et al* (1984), Giardina *et al* (1992) and Takahashi *et al* (1994) Moreover, due to the high AFU activity in the HCC group positive for viral markers, it appears that AFU could provide a useful screening test for viral hepatitis B or C associated cirrhosis.

No correlation was observed between AFU activity and AFP level. Frequently, however, even among patients with low or normal AFP levels, AFU activities were high. When used separately, diagnosis of HCC based on AFP above 400 ng/ml was feasible in only 39.1%, whereas upon combination with AFU (>870 nmol/ml/hr), the sensitivity was improved to as high as 82.6 %. In our study, the AFU activity in HCC patients was related to tumor size. Therefore, diagnostic application of AFU activity to small HCC seems to be limited. This result contradicted the study from Japan (Takahashi *et al,* 1994) which suggested that AFU could be a good marker for small HCC.

In conclusion, although AFP remains the "gold standard" serum marker for diagnosis of HCC, it is considered insufficient for the recognition of many cases of HCC. As shown in our study, serum AFU levels are higher in viral hepatitis associated HCC than in the non-viral group. Hence, the test may be useful for detecting HCC in cirrhotic patients who have viral hepatitis markers. Moreover, AFU activity does not correlate with serum AFP concentrations. Thus, the combination of both tests, complementary to ultrasonography, may improve the sensitivity for detection of HCC, especially in patients with a negative or low serum AFP concentration.

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