

Serum Alpha-L-Fucosidase Enzyme Activity as a Marker for Hepatocellular Carcinoma: Comparison with AFP Using ROC Analysis

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms. It is associated closely with cirrhosis and its prognosis is very poor because the diagnosis is generally late, when the disease is so advanced that any effective treatment is precluded. Therefore, early detection is important in the management of this type of cancer. Alpha-L-fucosidase enzyme has been recommended as a marker for HCC at an early stage in many countries all over the world.

Purpose: To evaluate Alpha-L-fucosidase activity level as an early detection of HCC.

Materials and Methods: Alpha-L-fucosidase enzyme. Levels were measured in 140 cases of chronic liver diseases including 50 patients with liver cirrhosis, 40 patients with chronic hepatitis and 50 patients with hepatocellular carcinoma (HCC).

Results: Alpha-L-fucosidase enzyme levels were significantly higher in patients with HCC than those with chronic hepatitis ($p < 0.01$) and cirrhosis ($p < 0.001$), no significant difference was found between patients with cirrhosis or with chronic hepatitis and controls. In patients with hepatocellular carcinoma, α -L-fucosidase enzyme activity was correlated to the size of the tumor and the histopathologic grades but not to the Child Pugh grades and most of the liver function tests. Using the receiver operative characteristic (ROC) and the differential positive rate (DPR) curves, 10 μ mol/L/min was the optimal cut-off value that differentiate patients with HCC from those with cirrhosis. At this level, the sensitivity, the specificity, and the diagnostic accuracy were 70%, 86%, and 73%, respectively. AFP was found to be significantly higher in patients with HCC than those with chronic hepatitis and cirrhosis. The best cut-off value of AFP was 100 ng/ml, at which the sensitivity, specificity, and diagnostic accuracy were 52%, 96%, and 74%, respectively. The simultaneous determination of AFP and α -L-fucosidase enzyme raised the sensitivity of the test to 80% with specificity of 100%

and diagnostic accuracy of 90%. The area under the ROC curve for AFP and α -L-fucosidase enzyme were 0.764 and 0.846, respectively, the difference is insignificant.

Conclusion: Both AFP and α -L-fucosidase enzyme are good markers for HCC and their simultaneous determination may improve the detection of HCC in cirrhotic patients negative for AFP.

Key Words: Alpha-L-fucosidase enzyme - AFP - Hepatocellular carcinoma.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms. It is associated closely with cirrhosis [2,7,9,21] and its prognosis is very poor because the diagnosis is generally late, when the disease is so advanced that any effective treatment is precluded. Therefore, early detection is important in the management of this type of cancer.

Surveillance programs have been conducted in many countries to detect HCC at an early stage. AFP and ultrasonography are usually used as diagnostic tools [4,17]. However, not all HCC secrete AFP and AFP levels may be normal in as many as 40% of patients with early HCC [11,12]. Ultrasonography is very effective in the early diagnosis of HCC and because of its improved performance, HCC was detected in 76% of HCC cases in a surveillance program [23]. However, ultrasonographic findings sometimes are not specific [10] and contrasting data have been reported in the past regarding the utility of ultrasonography in the diagnosis of

HCC at an early stage, even when performed together with serum AFP level determination [12,13]. Therefore, more sensitive diagnostic tools for detecting HCC are desirable, particularly in the screening of cirrhotic patients, because it has been suggested that the disease may respond more favorably to treatment at an early stage [12].

Alpha-L-fucosidase enzyme (EC 3. 2. 1. 51) is a lysosomal enzyme present in all mammalian cells. In 1984, Deugnier et al. [3] showed that serum alpha-L-fucosidase activity was significantly increased ($p < 0.001$) in patients suffering from hepatocellular carcinoma when compared to healthy and cirrhotic subjects. The authors recorded a sensitivity and specificity of 75% and 90%, respectively. This was the first time to use alpha-L-fucosidase enzyme as a marker for HCC [3]. The validity of alpha-L-fucosidase as a marker of HCC has been confirmed by many investigators [1,5,18,22]. Serial determinations of serum α -L-fucosidase activity was found to be useful in the early detection of HCC in cirrhotics [4,6].

To investigate the clinical usefulness of α -L-fucosidase in our population, we assayed this marker in patients with HCC, chronic hepatitis and cirrhosis.

PATIENTS AND METHODS

Between January 1998 and July 1999, one hundred and forty patients with chronic liver diseases were selected from the outpatient clinics and inpatient departments of the Tropical Medicine Department, Al-Hussein Hospital, Al-Azhar University and the Medical Oncology Department, National Cancer Institute, Cairo University. The study population was classified into the following patient groups.

Group 1: Includes 50 patients with histologically proven hepatocellular carcinoma (HCC), 39 males and 11 females.

Group 2: Includes 50 patients with cirrhosis, 27 males and 23 females.

Group 3: Includes 40 patients with chronic active hepatitis, 26 chronic active hepatitis C virus and 14 chronic active hepatitis B virus 33 males and 7 females.

Group 4: Includes 35 normal age and sex-matched healthy adults used as controls, 22 males and 13 females.

Serum samples were taken from fasting patient subjects and controls and stored at -80°C until needed. Table (1) summarizes all the characteristics of patients and controls.

Each patient was subjected to the following:

- Clinical assessment including history taking and physical examination.
- Routine laboratory investigations including urine and stool analysis, complete blood picture and serum creatinine.
- Liver function profile (serum bilirubin, AST, ALT, alkaline phosphatase, albumin and prothrombin time).
- The severity of the underlying disease was assessed by the Child-Pugh score based on serum albumin, bilirubin, prothrombin time, the presence of ascites and encephalopathy [13].
- Serum activity of α -L-fucosidase enzyme was assayed by a modification of the method of Zielke et al. [22]. 10 μl of serum were added to 50 μl of the substrate mixture, p-nitrophenyl- α -L-fucoyanoside dissolved in phosphate buffer (pH 5) and incubated for 1 hour at 37°C . The reaction was stopped by adding 3.5 ml of stop buffer (pH 10.5) (15 mM sodium carbonate, 15 mM glycine and 7.5 mM sodium chloride). Blanks were prepared in the same way, but the incubation step was omitted. Absorbance of p-nitrophenol was read at 400 nm. The change in absorbance of the liberated p-nitrophenol was directly proportional to the activity of α -L-fucosidase enzyme.

Statistical analysis:

All data were expressed as mean value \pm standard deviation. The relationship between continuous variables were analyzed by Pearson's correlation coefficient. Mean values of continuous variables were compared using ANOVA or *t*-test. The significance level was set at *p*-value less than 0.05. The Mann-Whitney U-test was used to compare the medians of continuous variables.

Sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios and diagnostic accuracy were calculated according to the following formula [14].

$$\text{Sensitivity} = a / (a+c)$$

$$\text{Specificity} = d / (b+d)$$

Accuracy = $(a+d) / (a+b+c+d)$

Positive predictive value = $a / (a+b)$

Negative predictive value = $d / (c+d)$

Positive likelihood ratio = Sensitivity / (1-specificity).

Negative likelihood ratio = (1-sensitivity) / specificity.

Where: a = true positive cases, b = false positive cases, c = false negative cases, d = true negative cases.

Receiver operating characteristic (ROC) curves were constructed by calculating the sensitivities and specificities of AFP and α -L-fucosidase enzyme at several cut-off points. Also, the differential positive rate plot (DPR) (true positive rate-false positive rate) was constructed by calculating DPR at different decision levels. The highest point of the plot is at the optimum cut-off or decision level [19]. The differences in diagnostic accuracy between the marker tests were measured by McNemar's test.

RESULTS

Table (1) summarizes all characteristics of patient groups and controls.

AFP levels in patients and controls:

The median level of AFP in patients with HCC (122; range 1.5-20143 ng/ml) was significantly higher than that in patients with cirrhosis (11.5; range 1-203 ng/ml, $p < 0.001$), chronic hepatitis (14.1; range 1.13-939.6 ng/ml, $p < 0.001$) and in controls (9.6; range 1-19 ng/ml, $p < 0.001$). No significant differences were found between patients with cirrhosis or with chronic hepatitis and controls ($p > 0.05$ in both cases).

Serum α -L-fucosidase enzyme:

The mean value of serum α -L-fucosidase enzyme in patients with HCC was significantly higher (14.6 ± 8.21 μ mol/L/min) than that found in patients with cirrhosis (5.67 ± 4.07 μ mol/L/min) or with chronic hepatitis (5.6 ± 2.88 μ mol/L) and in control subjects (5.01 ± 2.71 μ mol/L/min, $p < 0.0001$). No significant differences were found between controls and patients with cirrhosis or with chronic hepatitis (Table 2).

Fig. (2) illustrates the individual values for serum α -L-fucosidase enzyme in patients and controls. There are 35 patients with HCC, 7 patients with cirrhosis and 3 with chronic hepatitis

who had serum α -L-fucosidase enzyme above the cut-off value, while only two healthy individuals showed slight elevations above the cut-off value.

Serum α -L-fucosidase enzyme and AFP as diagnostic markers for HCC evaluated by ROC curve:

Based on the significant increase of both AFP and α -L-fucosidase enzyme in patients with HCC than the other patients and controls, an attempt was made to differentiate HCC from cirrhosis by two markers. ROC curves for both markers are shown in Fig. (1). The calculated area under the ROC curve was 0.764 for AFP and 0.846 for α -L-fucosidase enzyme. The sensitivity of each marker was determined at several specificity levels. The corresponding sensitivities and actual cut-off points producing Fig. (1) are given in Table (4). The optimal cut-off values selected by ROC curves were 100 ng/ml for AFP and 10 μ mol/L/min for α -L-fucosidase enzyme.

For AFP, 100 ng/ml was the best cut-off value at which the resulting specificity was 96% and sensitivity 52%, with a diagnostic accuracy of 74%, a positive likelihood ratio of 13 and a negative likelihood ratio of 0.5 (Table 4). The optimal cut-off value of serum α -L-fucosidase enzyme was 10 μ mol/L/min, which gave a specificity of 86% at a sensitivity level of 70%. The calculated diagnostic accuracy and positive and negative likelihood ratios were 70%, 21 and 0.59, respectively. The area under the ROC curve of AFP (0.764) was slightly less than that of α -L-fucosidase enzyme (0.846), the difference is insignificant. When both AFP and α -L-fucosidase enzyme were determined in parallel, 14 (58.3%) of 24 patients with HCC negative for AFP (less than 100 ng/ml) could be diagnosed. The resulting sensitivity and diagnostic accuracy were 80% and 90%, respectively. The specificity was 100% with a positive likelihood ratio of > 80 and a negative likelihood ratio of 0.2.

Correlation between AFP and serum α -L-fucosidase enzyme:

No correlation could be found between AFP and serum α -L-fucosidase enzyme. The regression equation correlating the two variables is $y = 93.006x + 699.31$ ($r = 0.0316$, $p > 0.05$). This

indicates that AFP and serum α -L-fucosidase enzyme are independent variables and so their simultaneous determination may increase the sensitivity of the test. The simultaneous determination of serum α -L-fucosidase enzyme activity and serum AFP increased the sensitivity to 80% and hence reduced a good fraction of the false negative results of serum AFP. The specificity, the diagnostic accuracy, positive and negative predictive values, positive and negative likelihood ratios and the differential positive rate at the simultaneous determination of serum α -L-fucosidase enzyme and serum AFP were 100%, 90%, 100%, 93.33%, > 80, 0.2 and 80%, respectively.

Serum α -L-fucosidase enzyme and the tumor size:

Serum α -L-fucosidase enzyme levels were significantly correlated to tumor size. In patients with tumor size > 5 cm, α -L-fucosidase enzyme concentration was (16.375 \pm 8.16 μ mol/L/min) and (11.72 \pm 7.63 μ mol/L/min) in patients with tumor size < 5 cm. The difference was statistically significant ($p < 0.05$) (Table 3).

Serum α -L-fucosidase enzyme and the Child-Pugh grades of HCC:

Serum α -L-fucosidase enzyme levels were not correlated to the Child-Pugh grades. In patients with Child A, α -L-fucosidase enzyme showed an insignificant decrease (14.1 \pm 8.59

μ mol/L/min) than Child grades B and C (14.9 \pm 8.12 μ mol/L/min, $p > 0.05$) (Table 3).

Serum α -L-fucosidase enzyme and the histopathologic grades of HCC:

With respect to tumor grades, no statistically significant difference was found between patients with grade I and II (19.62 \pm 8.81 μ mol/L/min and 14.75 \pm 7.52 μ mol/L/min, respectively, $p > 0.05$) and between grade II and III (10.8 \pm 7.85 μ mol/L/min, $p > 0.05$). Significant difference was found only between patients with grades I and III ($p < 0.05$) (Table 3).

Serum α -L-fucosidase enzyme and liver function tests:

In patients with HCC, α -L-fucosidase enzyme was positively correlated to serum ALT ($r = 0.2649$; $p < 0.01$), negatively correlated to alkaline phosphatase ($r = -0.3106$; $p < 0.01$) and prothrombin concentration ($r = -0.354$; $p < 0.001$) but not correlated to albumin ($r = -0.1698$; $p > 0.05$), AST ($r = 0.0386$; $p > 0.05$), nor total bilirubin ($r = -0.1467$; $p > 0.05$). In patients with cirrhosis, α -L-fucosidase enzyme was positively correlated only to serum albumin ($r = 0.2396$; $p < 0.05$) and prothrombin concentration ($r = 0.274$, $p < 0.01$) but not correlated to the other liver function tests; AST, ALT, alkaline phosphatase and total bilirubin ($r = 0.124$, 0.129, 0.178 and 0.036, respectively $p > 0.05$ in all cases).

Table (1): Main characteristics of the three patient groups; HCC, cirrhotics, chronic hepatitis and controls. HCC: Hepatocellular carcinoma, CH: Chronic hepatitis, SD: Standard deviation.

Group	Number of patients		Age (years)		
	Males	Females	Mean \pm SD	Median	Range
HCC	39	11	57.69 \pm 8.96	55	40-71
Cirrhosis	27	23	50.68 \pm 10.95	52.5	21-75
CH	33	7	45.80 \pm 12.3	48	17-80
Controls	22	13	38.37 \pm 11.46	36	21-61

Table (2): Serum levels of α -L-fucosidase enzyme in patients with hepatocellular carcinoma (HCC), patients with cirrhosis and patients with chronic hepatitis (CH) as well as in controls.

Group	Total number of patients	Alpha-L-fucosidase enzyme activity (μ mol/L/min) Mean \pm SD	Fvalue	p
HCC	50	14.6 \pm 8.21 B	36.35	< 0.0001
Cirrhosis	50	5.67 \pm 4.07 A		
CH	40	5.6 \pm 2.88 A		
Controls	35	5.01 \pm 2.71 A		

Table (3): Serum α -L-fucosidase enzyme activity in patients and controls and their relation to the tumor size, Child-Pugh grades and histopathologic grades as compared by the student *t*-test.

	Alpha-L-fucosidase enzyme activity ($\mu\text{mol/L/min}$) Mean \pm SD	<i>p</i> -value
<i>Tumor size:</i>		
> 5 cm	16.375 \pm 8.16	< 0.05
< 5 cm	11.72 \pm 7.63	
<i>Child Pugh grade:</i>		
A	14.1 \pm 8.59	> 0.05
B or C	14.9 \pm 8.12	
<i>Histopathologic grade:</i>		
I	19.62 \pm 8.81	> 0.05 (I & II)
II	14.75 \pm 7.52	< 0.05 (I & III)
III	10.8 \pm 7.85	> 0.05 (II & III)

Table (4): Sensitivity, specificity, diagnostic accuracy, positive and negative predictive values (PV), positive and negative likelihood ratios (LR) and differential positive rates (DPR) of AFP and α -L-fucosidase enzyme activity at different cut-off levels and the simultaneous use of both markers in the diagnosis of HCC in cirrhotic patients. A: AFP > 100 ng/ml, B: α -L-fucosidase enzyme > 10 $\mu\text{mol/L/min}$.

Cut-off value	Sensitivity %	Specificity %	Diagnostic accuracy %	Positive PV %	Negative PV %	Positive LR	Negative LR	DPR
<i>AFP (ng/ml):</i>								
40	60	78	69	73.2	66.1	2.72	0.51	38
60	56	86	71	80	66.15	4	0.51	42
100	52	96	74	92.9	66.6	13	0.5	48
160	46	98	72	95.8	64.5	23	0.55	44
200	42	98	70	95.4	62.8	21	0.59	40
<i>α-L-fucosidase ($\mu\text{mol/L/min}$):</i>								
7.5	80	68	74	71.42	77.3	2.5	0.29	48
8.33	78	72	76	73	75	2.78	0.3	50
10	70	86	78	83.33	74.13	5.0	0.35	56
11.6	60	92	76	88.2	69.7	7.5	0.43	52
13.3	46	96	71	92	64	11.5	0.56	42
A	52	96	74	92.9	66.6	13	0.5	48
B	70	86	78	83.33	74.13	5	0.35	56
A or B	80	100	90	100	80.7	> 80	0.2	80

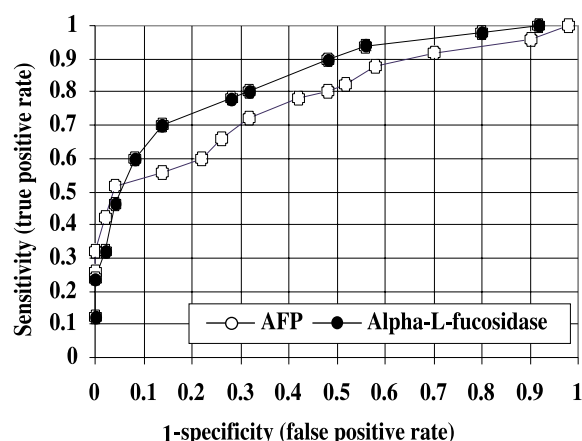


Fig. (1): Receiver operating characteristic (ROC) curves of AFP and α -L-fucosidase enzyme activity plotted for the diagnosis of HCC in cirrhotics. The area under the curve (diagnostic efficacy index) is 0.764 for AFP and 0.846 for α -L-fucosidase enzyme. The difference is not significant.

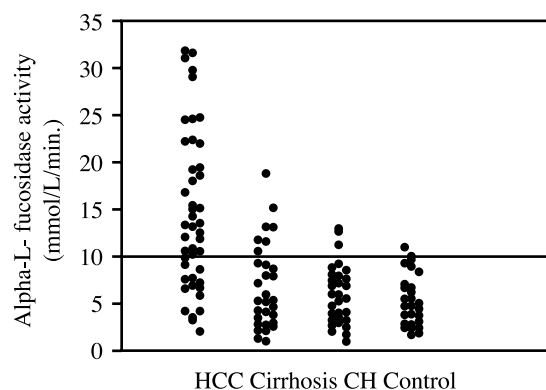


Fig. (2): Dot diagram of α -L-fucosidase enzyme activity in patients with HCC, cirrhosis and CH. The horizontal line represents the cut-off value (10 $\mu\text{mol/L/min}$) obtained from the ROC and DPR curves.

DISCUSSION

Alpha-L-fucosidase enzyme acts mainly on L-fucose (6-deoxy-L-galactose) containing glycoproteins [10]. It has been proposed as a marker of HCC [1,4,6,18]. The results of the present study confirm the work of the previously mentioned studies of an increased serum activity of α -L-fucosidase enzyme in patients with HCC. The mean value of α -L-fucosidase enzyme activity levels in patients with HCC was significantly higher than those found in patients with cirrhosis, chronic hepatitis and controls. The reason for the increase of α -L-fucosidase is still unknown. One possible explanation is an increased synthesis of proteins by tumor with a consequent increase in fucose turnover [3]. This possibility was not supported by another report [8] of a significant decrease in α -L-fucosidase levels in the tumoral liver tissue compared with non-hepatoma tissue.

For clinical decision making, the selected cut-off value of a laboratory test should provide the best diagnostic performance for either ruling out or ruling in the particular disease. The receiver operating characteristic curve (ROC) is a graphic method which can be used to determine this optimal cut-off level. In addition, it is a precise and valid measure of diagnostic accuracy [15]. The differential positive rate curve (DPR) is also useful in determining the cut-off level where the maximum point of the curve is that of choice [20].

Using ROC and DPR analysis, the optimal cut-off level of α -L-fucosidase activity and AFP were 10 μ mol/L/min and 100 ng/ml, respectively. At 10 μ mol/L/min, α -L-fucosidase showed a moderate sensitivity (70%), a good specificity (86%) and a moderate diagnostic accuracy (73%). The area under the ROC curve was 0.846 & 0.764 for AFP & α -L-fucosidase, respectively. The areas under ROC curve were between 0.7 and 0.9 indicating the validity of α -L-fucosidase and AFP as markers for HCC in cirrhotics. There are seven cirrhotic patients with α -L-fucosidase activity above the cut-off value; all have AFP concentrations < 100 ng/ml. It cannot be excluded that they might be affected by HCC at an early stage, not yet detectable by common imaging techniques such as ultrasonography and computed tomography.

Determination of AFP and α -L-fucosidase enzyme activity in parallel increased the diagnostic accuracy to 90% in spite of 74% of AFP alone. Although, each test may not have sufficient sensitivity, the simultaneous use of both tests may be highly discriminatory for the detection of HCC. The sensitivity of AFP alone was 52% and that of α -L-fucosidase enzyme alone was 70% while the sensitivity of both tests when carried out simultaneously increased to 80%. Parallel detection of AFP and α -L-fucosidase increases the number of tests performed and this may have cost implications. As the cost of determining α -L-fucosidase enzyme is low and the test is easy to perform, an assay for α -L-fucosidase enzyme activity should be performed to improve the detection of HCC in AFP-negative cirrhotics.

Alpha-L-fucosidase enzyme showed no correlation to AFP or to the Child-Pugh grades of patients with HCC. A positive correlation was found between α -L-fucosidase enzyme activity and the size of the tumor. This finding came in agreement with Tangkijvanish et al. [18] but in contrast to Takahashi et al. [16] who found no relationship between α -L-fucosidase enzyme activity and tumor size. With respect to tumor grades, α -L-fucosidase enzyme activity was significantly higher in patients with grade I than those with grade III which was unexpected but may possibly indicate changes in the metabolic cascade pathway of cells in the different grades of the tumor. The positive correlation between α -L-fucosidase and ALT indicate that α -L-fucosidase may be affected by the hepatic cell damage. Alpha-L-fucosidase enzyme activity was negatively correlated to alkaline phosphatase in patients with HCC, a condition may be explained by the fact that alpha-L-fucosidase is a lysosomal but not a cell membrane enzyme.

In conclusion, this study revealed that the addition of an assay for α -L-fucosidase enzyme to AFP gives a significant improvement in detection of HCC in patients with cirrhosis. The optimal cut-off value for AFP in the diagnosis of HCC is 100 ng/ml and that of α -L-fucosidase enzyme is 10 μ mol/L/min. In addition, a test for serum α -L-fucosidase enzyme should be used as an adjunctive tool in the detection of HCC in cirrhotics negative for AFP.

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