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Hepatology

Diagnostic Value of Serum Alpha-L-Fucosidase in Detecting Hepatocellular Carcinoma in Cirrhotic Patient

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Abstract

Original Research Article

Background: Tumour markers are pivotal tools for the early diagnosis of tumours. Alpha Fetoprotein (AFP) is a major marker widely used for the detection of hepatocellular carcinoma (HCC). However, AFP shows poor sensitivity and specificity in the early stages of HCC. Moreover AFP levels are not elevated in some HCC patients, while they are elevated in other patients with benign liver disease. New biomarkers are urgently needed for specific early diagnosis of HCC patients. Alpha- L- Fucosidase (AFU), a lysosomal enzyme present in all mammalian cells, has been proposed as a tumour marker since many studies reported increased AFU serum levels in patients with cirrhosis with HCC patients. ***Objectives:*** This study aimed to evaluate the diagnostic value of AFU in detecting of HCC. ***Methods:*** This cross-sectional observational study was conducted in the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka during the period from December 2019 to August 2020. Thirty three HCC patients with cirrhosis and 33 patients with liver cirrhosis were included. All HCC patients underwent FNA for cytopathological examination. Serum AFU levels were measured by enzyme-linked immunosorbent assay (ELISA) method and compared. Data were processed and analyzed with the help of computer program SPSS (Statistical Package for the Social Sciences) 25 version. Quantitative data were analyzed by mean and standard deviation; and comparison was done between two groups by unpaired t test or Mann-Whitney U test. Qualitative data were analyzed by frequency and percentage; and comparison was done between two groups by Chi- Square ($\chi 2$) test or Fisher' s Exact Test. A statistically significant result has been considered when p value less than 0.05. ***Results:*** The study revealed significant differences in serum Alpha-L-Fucosidase (AFU) and Alpha-Fetoprotein (AFP) levels between hepatocellular carcinoma (HCC) and cirrhosis groups. The median AFU levels were significantly higher in the HCC group (6.38 ng/ml) compared to the cirrhosis group (2.13 ng/ml, $p<0.001$). Similarly, AFP levels were markedly elevated in HCC patients (755.0 ng/ml) versus cirrhosis patients (10.20 ng/ml, $p<0.001$). The diagnostic accuracy of AFU for detecting HCC was notable, with an area under the receiver operating characteristic (AUROC) curve of 0.956, indicating high diagnostic efficacy. At an AFU cutoff value of 3.995 ng/ml, the sensitivity was 90.9% and specificity 93.6%. Tumor size and number of lesions in HCC patients did not significantly correlate with AFU levels ($p=1.000$). Additionally, no significant correlation was found between AFP and AFU levels in HCC patients ($p=0.578$). These findings highlight the potential of AFU as a sensitive and specific biomarker for HCC detection in cirrhotic patients. ***Conclusion:*** Serum Alpha-L-Fucosidase (AFU) demonstrates high diagnostic accuracy in detecting hepatocellular carcinoma (HCC) among cirrhotic patients, with significant sensitivity and specificity at a cutoff value of 3.995 ng/ml. Its strong performance as a biomarker highlights its potential utility in complementing existing diagnostic methods for early HCC detection.

Keywords: Hepatocellular carcinoma (HCC), Serum Alpha-L-Fucosidase (AFU), Cirrhosis.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide, accounting for a significant burden of morbidity and mortality, particularly in regions with high prevalence of hepatitis B and C infections. Cirrhosis, the advanced stage of chronic liver disease, is a well-established risk factor for HCC, with the majority of cases developing in patients with cirrhotic livers [1-3]. Early detection of HCC in cirrhotic patients is critical for effective treatment and improved survival, yet remains challenging due to overlapping clinical features of HCC and cirrhosis. This necessitates the development of reliable diagnostic tools, particularly in resource-limited settings where advanced imaging techniques may not be readily accessible [4].

Alpha-L-Fucosidase (AFU), a lysosomal enzyme involved in the degradation of fucosylated glycoproteins, has recently emerged as a promising biomarker for HCC. Elevated AFU levels have been observed in patients with HCC compared to those with cirrhosis alone, suggesting its potential as a non-invasive diagnostic tool [5-6]. Unlike traditional markers such as alpha-fetoprotein (AFP), which has limited sensitivity and specificity, AFU may offer enhanced diagnostic accuracy, especially in distinguishing HCC from benign liver conditions.

The pathophysiological basis for elevated AFU in HCC stems from the altered glycosylation patterns and increased turnover of glycoproteins associated with tumorigenesis. In cirrhotic livers, the hepatic microenvironment fosters chronic inflammation and fibrosis, which are critical drivers of oncogenesis. The resultant changes in glycosylation pathways may contribute to the diagnostic utility of AFU in differentiating HCC from cirrhosis [7-11].

Existing studies have explored the role of AFU as a diagnostic marker, often in combination with AFP, to improve sensitivity and specificity. While some research has demonstrated the superior diagnostic performance of AFU compared to AFP, the findings remain inconsistent across different populations and healthcare settings. The variation in diagnostic accuracy underscores the need for region-specific validation studies to determine the clinical utility of AFU in HCC surveillance programs [12-15].

Moreover, the cost-effectiveness and accessibility of AFU testing make it a viable option in low- and middle-income countries, where advanced imaging techniques like dynamic contrast-enhanced CT or MRI may not be universally available. Integrating AFU into routine diagnostic algorithms could enhance early detection rates, facilitating timely intervention and improving outcomes for patients with HCC.

Objective

This study aims to evaluate the diagnostic value of serum Alpha-L-Fucosidase in detecting HCC among cirrhotic patients, comparing its sensitivity and specificity with conventional diagnostic markers.

METHODOLOGY

Study Design

This cross-sectional observational study aimed to evaluate specific outcomes in a defined population, focusing on the diagnostic value of serum Alpha-L-Fucosidase (AFU) in hepatocellular carcinoma (HCC).

Study Setting

The research was conducted at the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

Study Duration

The study was carried out over nine months, from December 2019 to August 2020.

Study Population

The study included patients with suspected HCC attending the outpatient or inpatient departments of Hepatology. Cirrhotic patients without evidence of HCC were recruited as controls, creating a comparative framework for analysis.

Sample Size

The sample size was determined based on power analysis for a single proportion, assuming a hypothesized AFU sensitivity of 90%. Using an expected sensitivity of 70% (Montaser *et al.*, 2012), with 85% power and a 5% alpha error, the required sample size was 36 per group. Accounting for a 10% attrition rate, the target was adjusted to 40 per group. However, due to COVID-19 constraints, 33 participants were ultimately enrolled in each group.

Sampling Technique

Participants were selected using consecutive convenient sampling.

Inclusion Criteria

Eligible participants met the following criteria:

- **Group I:** Cirrhotic patients with HCC, diagnosed through clinical stigmata, laboratory findings (e.g., prolonged prothrombin time, low serum albumin), ultrasound, or endoscopic signs of varices. HCC was confirmed by fine-needle aspiration (FNA) of hepatic lesions.
- **Group II (Controls):** Cirrhotic patients without HCC.
- Aged 18 years or older.

Exclusion Criteria

Participants were excluded if they:

- Had prior or ongoing treatment for HCC.

- Presented with other malignancies.
- Had inflammatory/septic conditions, diabetes mellitus, or thyroid disorders.
- Had co-morbidities precluding FNA.
- Had acute viral hepatitis, alcoholic hepatitis, or were pregnant.

Grouping

Participants were divided into two groups:

- **Group I:** Cirrhotic patients with HCC (n=33).
- **Group II:** Cirrhotic patients without HCC (n=33).

Data Collection

Data were collected through a pre-designed questionnaire, developed from literature reviews and expert input, encompassing both quantitative and qualitative measures.

Data Collection Procedure

Eligible participants were identified in outpatient and inpatient settings using clinical, biochemical, and radiological evaluations. Patients with inflammatory, infectious, or malignant conditions other than HCC were excluded through specific tests (e.g., CRP, serum CEA, CA 19.9, CA 125). Imaging modalities such as abdominal ultrasound and triphasic CT documented liver cirrhosis and focal lesions. Laboratory evaluations, including liver function tests and viral markers (HBsAg, Anti-HBc, Anti-HCV), were conducted at BSMMU. FNA was performed for HCC confirmation, with informed consent obtained from all participants.

Study Variables

- **Independent Variables:** Cirrhosis with or without HCC.
- **Outcome Variables:** Liver function (ALT, bilirubin, INR), viral markers, Child-Pugh class, tumor characteristics (size, number), and biomarkers (AFU, AFP).
- **Demographic Variables:** Age and sex.

Statistical Analysis

Data were analyzed using SPSS (version 25). Continuous variables were summarized as means ± standard deviations or medians with interquartile ranges and compared using t-tests or Mann-Whitney U tests. Categorical data were analyzed using Chi-Square or Fisher's Exact tests. ROC analysis assessed the sensitivity and specificity of AFU, while correlations between AFU and AFP were evaluated using Spearman's correlation coefficient. Statistical significance was set at $p < 0.05$.

RESULTS

The demographic characteristics of the study revealed no significant differences between the hepatocellular carcinoma (HCC) and cirrhosis groups in terms of age and sex. In both groups, the majority of patients were male, with 87.9% in the HCC group and 72.7% in the cirrhosis group (p=0.12). Regarding age distribution, 51.5% of HCC patients were aged ≤50 years, compared to 67.7% in the cirrhosis group, while 48.5% of HCC patients were older than 50 years, compared to 32.3% in the cirrhosis group (p=0.131). These differences were not statistically significant.

Table-1: Demographic characteristics of the study

Variables	Hepatocellular car-, n=33	Cirrhosis, n=33	p-value
Age			
≤ 50 years	17 (51.5%)	23 (67.7%)	p=0.131ns
> 50 years	16 (48.5%)	10 (32.3%)	
Sex			
Male	29 (87.9%)	24 (72.7%)	p=0.12 ns
Female	4 (12.1%)	9 (27.3%)	

The median serum Alpha-L-fucosidase (AFU) value were 6.38 (interquartile range, 2.76-16.85) ng/ml and 2.13 (interquartile range, 1.65-2.90) ng/ml in HCC and cirrho- sis groups respectively. The median serum AFU value was significantly higher in HCC group compared to cirrhosis group (p<0.001).

The median serum Alpha fetoprotein (AFP) value were 755.0 (interquartile range, 46.0-25876.0) ng/ml and 10.20 (interquartile range, 5.45-17.85) ng/ml in HCC and cirrhosis groups respectively. The median serum AFP value was significantly higher in HCC group than that of cirrhosis group (p<0.001).

Table-2: Comparison of Alpha-L-fucosidase (AFU) and Alpha fetoprotein (AFP) valued between HCC and cirrhosis groups

Variables	Hepatocellular car-, n=33	Cirrhosis, n=33	p-value
AFU (ng/ml)	6.38 (2.76-16.85)	2.13 (1.65-2.90)	p<0.001s
AFP (ng/ml)	755.0 (46.0-25876.0)	10.20 (5.45-17.85)	p<0.001s

The area under the receiver-operating characteristic (AUROC) curve for prediction HCC was 0.956. It was found that the best cut-off value for AFU was 3.995 ng/ml (Figure-4.1).

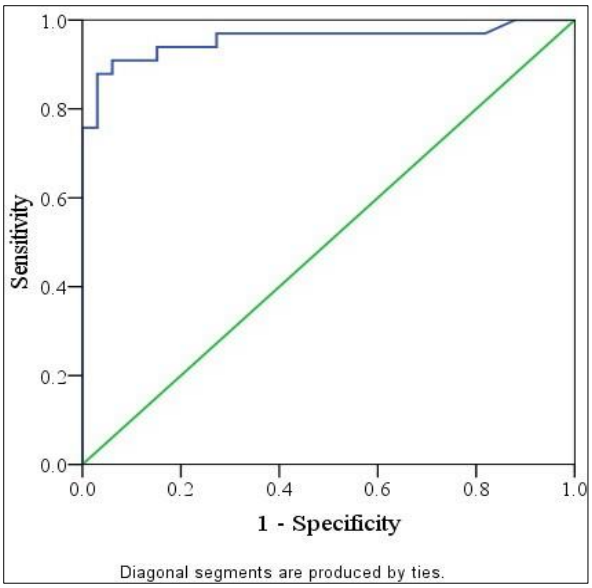

Figure 1. The receiver operating characteristic (ROC) curve of AFU plotted for the diagnosis of HCC. The area under the curve (diagnostic efficacy index) is 0.956

Regarding diagnosis of HCC by AFU level, at a cut-off value of 3.995 ng/ml through area under ROC curve the sensitivity was 90.9% and specificity was 93.6%.

Table 3: Receiver-operating characteristic (ROC) curve of AFU for prediction of HCC

	Cut off value	**Sensitivity**	**Specificity**	**Area under ROC curve**	**95% Confidence interval (CI)**	
					Lower bound	Upper bound
AFU	3.995	90.9%	93.6%	0.956	0.901	1.010

In HCC group 23 (76.7%) cases tumour size had above 5 cm and 7 (23.3%) cases tumour size had up to 5 cm when AFU value of ≥ 3.995 ng/ml; whereas 3 (100.0%) cases tumour size had above 5 cm and none cases tumour size had up to 5 cm when AFU cut off value of <3.995 ng/ml; difference was not significant (p=1.000) (Table- 4.8). In HCC group 22 (73.3%) patients had single lesion and 8 (26.7%) cases had multiple lesion when AFU value of ≥ 3.995 ng/ml; whereas 2 (66.7%) patients had single lesion and 1 (33.3%) case had multiple lesion when AFU value of <3.995 ng/ml; difference was not significant (p=1.000).

Table-4: Association of serum AFU levels with tumour size and number of le- sion in patients with hepatocellular carcinoma (n=33)

Variables	AFU Levels		**p-value**
	≥ 3.995 (n=30)	**< 3.995 (n=3)**	
Tumour size			
> 5 cm	23 (76.7%)	3 (100.0%)	p=1.000ns
≤ 5 cm	7 (23.3%)	0 (0.0%)	
Number of lesion			
Single	22 (73.3%)	2 (66.7%)	p=1.000ns
Multiple	8 (26.7%)	1 (33.3%)	

Figure-2 showed that there was a non-significant correlation between AFP and AFU level in patients with HCC **(**rho=-0.100; p=0.578).

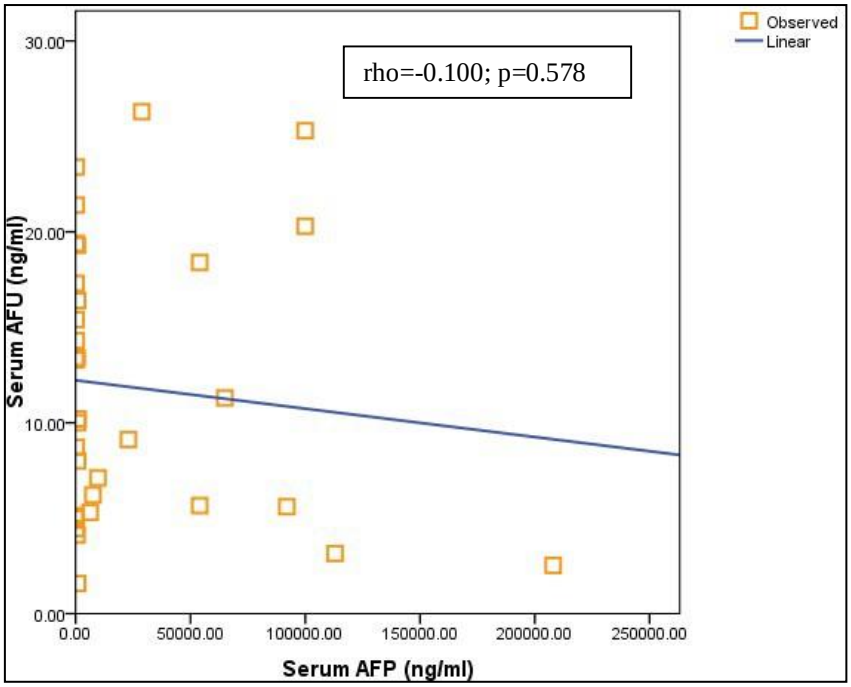

Figure 2 Correlation between serum AFP and AFU level in in patients with HCC (n=33)

*Spearman correlation was done to see the correlation between serum AFP and AFU level in patients with HCC.

DISCUSSION

This study found the median serum Alpha-L-fucosidase (AFU) value were 6.38 (interquartile range, 2.76-16.85) ng/mlin HCC and 2.13 (interquartile range, 1.65-2.90) ng/ml in cirrhosis groups respectively. The median serum AFU value was significantly higher in HCC group compared to cirrhosis group (p<0.001). This result was concordant with the study of Montaser *et al.*, (2012) showed that median values of serum AFU in the HCC group 9.28 (0.99–26.59µmol/ L/ min when compared with both the CLD 0.9 (0.11– 4.60 µmol/L/min and control 0.42 (0.1–1.2 µmol/L/min groups. This result was a statistically significant (p < 0.001).

Tangkijvanich *et al.,* (1999) 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), p < 0.05) [1].

This study stated that the median serum Alpha fetoprotein (AFP) value were 755.0 (interquartile range, 46.0-25876.0) ng/ml and 10.20 (interquartile range, 5.45-17.85) ng/ml in HCC and cirrhosis groups respectively. The median serum AFP value was significantly higher in HCC group than that of cirrhosis group (p<0.001). Montaser *et al.,* (2012) reported there was a highly significant difference between the HCC group (31.67ng/ml) and both the Cirrhosis (11ng/ml) and control group (2.03ng/ml) as re- gards the level of AFP activity (p < 0.001) [2].

In this study the prediction of HCC in cirrhotic patients by AFU level at a cut-off value of 3.995 ng/ml, the sensitivity was 90.9% and specificity was 93.6%. Montaser *et al.,* (2012) at a cut off value of 2.3005µmol/ L/min yielded a sensitivity and specificity of 90% and 97.5%, respectively in the detecting HCC. Tangkjvanich *et al.,* (1999) at a cutoff point 870 nmol/ml/hr (mean value of controls plus 3 standard deviations) found sensitivity and specificity of 81.7% and 70.7%, respectively in the detecting HCC [1].

In this study, the area under the receiver-operating characteristic (ROC) curve for pre- diction of HCC was 0.956. Montaser *et al.,* (2012) found area under the curve 0.966. Zhang *et al.,* (2015) found area under the curve for prediction of HCC was 0.718 [2-3].

When the patients with hepatocellular carcinoma were divided into two groups based on a cut-off AFU levels of ≥ 3.995 ng/ml tumour size was above 5 cm in most of the cases (76.7%) in AFU levels of ≥ 3.995 ng/ml and all patients in AFU levels of < 3.995 ng/ml; difference was not significant (p=1.000). Montaser *et al.,* (2012) did not find association between size of the lesion and AFU in hepatocellular carcinoma (p>0.05) [2].

This study revealed that the number of lesion was single in 22 (73.3%) patients of AFU levels ≥ 3.995 ng/ml and 2 (66.7%) patients in AFU levels < 3.995 ng/ml; difference was not significant (p=1.000). Montaser *et al.,* (2012) did not find association between number of the lesion and AFU in hepatocellular carcinoma (p>0.05) [2].

In our study, there was a non-significant correlation between AFP and AFU level in patients with HCC (rho=-0.100; p=0.578). This indicates that AFP and AFU are independent variables. This result was similar with several other studies that there was no significant correlation between AFU and AFP [1, 4]. Montaser *et al.,* (2012) found that AFU activity was negatively correlated with AFP level (r =-0.314, p = 0.049) [2].

CONCLUSION

The study highlights the diagnostic potential of serum Alpha-L-Fucosidase (AFU) in distinguishing hepatocellular carcinoma (HCC) from cirrhosis with high sensitivity (90.9%) and specificity (93.6%) at a cutoff value of 3.995 ng/ml, supported by an AUROC of 0.956. AFU levels were significantly elevated in HCC patients compared to cirrhotic controls, demonstrating its robustness as a biomarker. While Alpha-Fetoprotein (AFP) also showed significant differences between the groups, there was no significant correlation between AFU and AFP levels. Tumor size and lesion number did not show a significant association with AFU levels. These findings establish AFU as a reliable biomarker for early and accurate detection of HCC in cirrhotic patients, complementing existing diagnostic tools.

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