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Original Article

Association of Serum Alpha-fetoprotein with the Tumor Characters and HBV Status of Hepatocellular Carcinoma to Assess its Resectability

Dr. Md. Najmul Haque,[1] Dr. Mst. Rupali Yasmin,[2] Dr. Shantona Rani Paul[3]

Abstract

Background: Serum alpha-fetoprotein (AFP) has long been used to complement imaging tests in the screening and diagnosis of hepatocellular carcinoma (HCC), its utility as a predictive marker of long-term risk for HCC in HBV patients is contentious.

Objective: The current study is aimed to assess the serum alpha-fetoprotein with the tumor character and HBV status of HCC to assess its resectability.

Methods: This was a single-center, cross-sectional study done from September 2020 to August 2021 at the department of Hepatobiliary, pancreatic, and liver transplantation surgery at BSMMU. A total of 27 HCC patients who met the inclusion criteria were enrolled in the study. Clinical, laboratory, and imaging findings were obtained using a standardized data collecting sheet with the patients' informed agreement. AFP was measured by automated chemiluminescence analyzer (LIAISON XL, DiaSorin, Italy) in the Department of Biochemistry & Molecular Biology, BSMMU. Statistical analyses were performed using SPSS version 22. Quantitative variables were expressed as mean standard deviation and examined using an unpaired t-test. The qualitative data were represented by frequencies and percentages, and the Chi-Square test and Fisher exact test (where applicable) were used to determine any association. The statistical tests were conducted with a 95% confidence interval, and P<0.05 was deemed statistically significant.

Results: Majority of the tumors were single and located in the right lobe. Local extension and distant metastasis were preset in unresectable patients of group 2.Hepatitis B virus was positive in 16 (59.3%) patients and DNA was detectable in 8 (29.6%) patients. HBV positivity and detectable HBV DNA was significantly associated with poor resectable status in group 2 but insignificant in group 1.

Conclusions: In this study, the overall resectability was 37%. HBV positive HCC patients are significantly associated with poor resectability when serum AFP is raised and it would be more indicative when HBV DNA is detected.

Key words: HCC, Alpha-fetoprotein, Tumor characters, HBV.

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Introduction

Patients with hepatocellular carcinoma (HCC) have a 7% chance of surviving for five years after diagnosis, making it the third leading cause of cancer death worldwide [1]. When patients present with overt clinical symptoms, typically the HCC has progressed from early stages to late stages and is rather large by the time they reach the hospital for treatment. Patients are left with a grim future

[1] Assistant Professor, Hepatobiliary Surgery, Rajshahi Medical College, Rajshahi.
[2] Registrar (Rheumatology), Rajshahi Medical College hospital, Rajshahi.
[3] Assistant Professor, Paediatric Surgery, Rajshahi Medical College, Rajshahi.

and poor prognosis as a result of the ensuing rapid growth and vascular invasion. Clinicians' ability to identify HCC at earlier stages, when it's more treatable, depends on their understanding of the disease's pathobiological aspects [2-3].

Ultrasound imaging and measures of serum alpha-fetoprotein (AFP) levels are currently the most widely utilized tools for screening for and diagnosing HCC. When comparing AFP to other serum markers, AFP has been used as the gold standard internationally, especially in low-resource, far-flung regions. Nonetheless, AFP's diagnostic worth is still debated because its sensitivity and specificity are inconsistent [4,5]. Additionally, AFP-related factors such AFP mRNA and AFP glycoforms have been investigated [6-8]. These AFP-related factors have recently been shown to have diagnostic value and are recommended as complementary tests. But their application is constrained by both cost and technology, and it is unlikely that they will displace serum AFP as the gold standard of diagnostic serum indicators for hepatocellular carcinoma [9-11].

Selection of candidates with HCC for Liver resection required adequate preoperative staging. Currently the resectability and prognostic predictors for HCC include tumor burden, liver functional reserve and functional status of the patients. Tumor burden that is the tumor size, number, location, vascular invasion, lymplnode involvement, distant metastasis are evaluated by a lot of imaging systems but diagnostic value of preoperative imaging (CT, MRI) approximates 80% and is limited for satellite nodule or sub-centric (<1 cm) lesions[12].

Prognostic markers for HCC have been established, in part, by their representation of the pathobiological characteristics of AFP in the serum [13-14]. According to some medical professionals, larger tumors are correlated with higher serum AFP levels [15-17], while smaller tumors appear to secrete less AFP into the blood. The researchers also note that there is no correlation between tumor size and AFP levels [18]. Lowly differentiated tumor cells typically had AFP levels of > 400 g/L [19], while those with well differentiated tiny tumors expressed undetectable amounts of serum AFP [14]. Serum AFP levels have been reported to correspond with a number of other variables, including tumor number, grade [14,16] and vascular invasion [14,20-22]. Several studies have indicated that an elevated serum AFP level is associated with HBV-related HCC [15]. However, these studies did not see the relation of AFP with the resectability of HCC. So, this study was designed to see the association of serum alpha-fetoprotein with the tumor characters and HBV status of hepatocellular carcinoma to assess the resectability of HCC.

Materials and Methods

This cross-sectional study was conducted from September 2020 to August 2021 at the Department of Hepatobiliary, Pancreatic and Liver Transplant Surgery, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, after approval of the Institutional Review Board (IRB) of BSMMU, Dhaka. During the study period, all clinically diagnosed HCC patients of any age and gender who were treated at the Department of Hepatobiliary, Pancreatic, and Liver Transplant Surgery at BSMMU were enrolled and HCC patients with undetectable serum alpha-fetoprotein, recurrent case of HCC, HCC patients received neo-adjuvant chemo-radiotherapy or any ablative therapy were excluded. Consecutive sampling method was used and a total of twenty-seven HCC patients were analyzed. Diagnosis of HCC was confirmed by histopathological examination of resected specimen or core-cut biopsy of unresectable HCC. Serum sample for the detection of AFP was taken up on entry into the study before initial treatment and AFP determined by automated chemiluminescence analyzer (LIAISON XL, DiaSorin, Italy) in the department of Biochemistry and Molecular Biology, BSMMU. The cut off value for normal AFP levels (≤ 20 ng/ml) were chosen on the basis of the EASL guidelines. Optimum AFP threshold was calculated 38.45 ng/ml by a receiver operator characteristic (ROC) curve constructed using AFP to predict resectability of HCC. Based on ROC curve of this study the finally enrolled patients

were divided into group 1 having AFP levels ≤ 38.45 ng/ml and group 2 AFP levels > 38.45ng/ml. Each AFP group was further divided into resectable and unresectable groups according to the resectable criteria (TNM stage I II , BCLC stage 0 A B, CTP grade A B and ECOG PS 01) set for the study.

Data Collection and Analysis

Data were collected in a structured data collection sheet only the patients who fulfilled the enrollment criteria. Finally, all the collected data were prepared for statistical analysis. Statistical analyses were carried out by using the Statistical Package for Social Sciences version 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Quantitative variables (like age, monthly income, BMI, tumor size) were presented as mean ± standard deviation and analyzed by the Un-paired t-test. The qualitative variables (gender, education, smoking, tumor location, single/multiple tumor, HBV positivity) were indicated by frequencies and percentages and Chi-Square test/Fisher exact test (as appropriate) was used to see any association. Receiver-operator characteristic (ROC) curve were constructed using AFP for the prediction of resectability of HCC. The statistical tests were conducted with the 95% confidence interval and $P<0.05$ was considered as statistically significant.

Results

Table 1 shows the mean age was 45.52±14.25 years ranged from 25 to 74 years. The mean monthly income was 24,520±14,160 (BDT) ranged from 8,000 to 60,000 taka and the mean BMI was 22.04±2.75 kg/m2 ranged from 18 to 27

Table 1: Socio demographic profile of the patients (n =27)

Variables	Number (n =27) Mean±SD	Percent (%) Range
Age in year	45.52±14.25	25-74
Monthly income (BDT)	24,520±14,160	8000-60000
BMI	22.04±2.75	18-27

Among the 27 patients the majority of the patients were male 20 (74.1%) and female were 7 (25.9%). M:F was 2.85:1

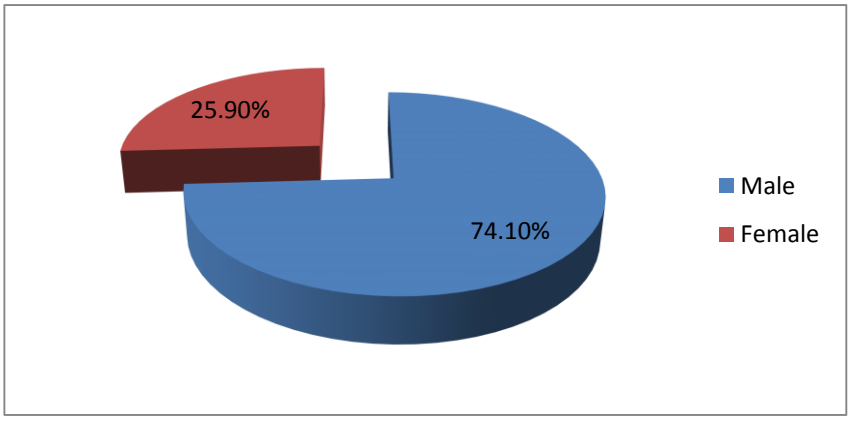

Figure 1: Gender Distribution of the patient

Table 2 showsmean tumor size was 8.17±4.02 cm. More than half (51.9%) of the patients had single tumor and located predominantly (51.9%) in the right lobe. Local extension and metastasis were absent in 21 (77.8%) and 25 (92.6%) patients respectively.

Table 2: Characteristics of tumors of the study patients (n =27)

Variables		**Number**	**Percent**
		(n =27)	**(%)**
		Mean±SD	**Range**
Tumour size (cm)		8.17±4.02	2.2-17.7
T/Number	Single	14	51.9
	multiple	13	48.1
T/Location	Rt lobe	14	51.9
	Lt lobe	6	22.2
	Both lobe	7	25.9
Local extension	Yes	6	22.2
	No	21	77.8
Metastasis	Yes	2	7.4
	No	25	92.6

T= tumor, cm = centimeter,n= Number, %= Percent Table 3 shows hepatitis B virus was positive in 16 (59.3%) patients and it was active in 8 (29.6%) patients.

Table 3: HepatitisBvirusinfectionstatusofthestudypatients(n =27)

HBV		**Number**	**Percent**
		n=27	**%**
HBV			
	Positive	16	59.3
	Negative	11	40.7
HBVDNA			
	Detected	8	29.6
	Undetected	8	29.6
	Notapplicable	11	40.7

HBV= Hepatitis B virus, DNA= Deoxy ribonucleic acid, n= Number, %= Percent Table 4 shows 37% of HCC patients were resectable and the rest of the patients were unresectable.

Table 4: Distribution of the study patients by overall resectability(n =27)

Resectability	Numberofthepatients	
	n=27	%
Resectable	10	37.0
Unresectable	17	63.0

n= Number, %= Percent

Table 5 shows the association of AFP level and the characteristics of tumors with the resectability. It was observed that, the mean tumor size (7.4±3.8) of resectable patients of group 1 was smaller than that (9.5±4.1) of unresectable patients of group 2. More than 60% of the patients had single tumor and were resectable in group 1 whereas, in group 2 almost same percentage of the patients had multiple tumors and were unresectable. Among the resectable patients in group 1 about 70% of the tumors were located in single lobe and no patient had both lobe involvement but in group 2 among the unresectable patients about 56% of the tumors were located in single lobe and more than 30% patients had both lobe involvement. Among the unresectable patients of group 2, local extension and distant metastasis were present in 31.3% and 12.5% respectively but it was absent among the resectable patients of group 1. These observations indicate that large tumor size, number, location within the liver, local extension and distant metastasis had an association with the AFP level and resectability, but the difference were statistically not significant (p>0.05) between two groups.

Table 5: Association of AFP level and the characteristics of tumors with the resectability (n=27)

	Group 1 (n=11)				*p* value	Group 2 (n=16)				*p* value
	Resectable		Unresectable			Resectable		Unresectable		
	n	%	n	%		n	%	n	%	
Tumor Size (cm)										
Mean±SD	7.4±3.8		6.6±4.2		[a]0.768[ns]	4.2±1.3		9.5±4.1		[a]0.099[ns]
T/Number										
Single	7	63.6	1	9.1	[c]0.145[ns]	2	12.5	4	25.0	[c]0.125[ns]
Multiple	1	9.1	2	18.2		0	0.0	10	62.5	
T/Location										
Right lobe	7	63.6	1	9.1		1	6.3	5	31.3	
Left lobe	1	9.1	0	0.0	[b]0.037[s]	1	6.3	4	25.0	[b]0.587[ns]
Both	0	0.0	2	18.2		0	0.0	5	31.3	
Local extension										
Yes	0	0.0	1	9.1	[c]0.272[ns]	0	0.0	5	31.3	[c]0.458[ns]
No	8	72.7	2	18.2		2	12.5	9	56.3	
Metastasis										
Yes	0	0.0	0	0.0	1.000[ns]	0	0.0	2	12.5	[b]0.758[ns]
No	8	72.7	3	27.3		2	12.5	12	75.0	

T= Tumor, cm= Centimeter, SD= Standard deviation, n = Number, % = Percents = significant, ns = notsignificant [a]p value reached from Unpaired t-test[b]p value reached from Chi-square test [c]p value reached from Fisher exact test Table 6shows the association of AFP level and the hepatitis B virus

infection status with the resectability. It was observed that in group I, more than 50% patients were HBV negative and were resec table. In contrast in group 2, seventy five percent patients were HBV positive and among them more than 30% patients had detectable HBV DNA and were unresec table. The difference was statistically significant (p= 0.032) in group 2. It indicates that AFP level is significantly associated with HBV positivity and detectable HBV DNA, and poor resectability.

Table 6: Association of AFP level and the hepatitis B virus infection status with the resectability (n=27)

	Group 1 (n=11)				*p* value	Group 2 (n=16)				*p* value
	Resectable		Unresectable			Resectable		Unresectable		
	n	%	n	%		n	%	n	%	
HBV										
Positive	2	18.2	2	18.2	0.201[ns]	0	0	12	75.0	0.009[s]
Negative	6	54.5	1	9.1		2	12.5	2	12.5	
HBV DNA										
Detected	2	18.2	1	9.1		0	0	5	31.2	
Undetected	0	0.0	1	9.1	0.190[ns]	0	0	7	43.8	0.032[s]
NA	6	54.5	1	9.1		2	12.5	2	12.5	

HBV= Hepatitis B virus, DNA= Deoxy ribonucleic acid, n= Number, %= Percent s=significant, ns=not significant p value reached from Chi-squaretest

Discussion

Alpha-fetoprotein (AFP) is a glycoprotein that consists of 591 amino acids and has a half-life that ranges from 5-7 days. High serum AFP levels are present in 60%-70% of early HCC cases and 80%-85% of late HCC patients [23, 24]. AFP has been used as the gold standard for serum markers on a global scale, and researchers have related it to the etiological and clinicopathological elements of HCC [26-28]. AFB is still indicated as a biomarker for HCC screening, prognosis, and recurrence monitoring in clinical practice [25, 29]. We used AFP, HCC characteristics, HBV status of HCC patients to assess whether or not the HCC could be resected. We found that there was an association of low resectability with the HBV positivity and active HBV infectionin patients with HCC who had high serum AFP level.

The mean age in this study was 45 years, which is comparable to studies [30] and [31], which had mean ages of 48 and 49 years, respectively. The majority of research found that individuals with HCC had a mean age of more than 50. There were 2.85 times as many males as females. The majority of people who participated in the study lived in metropolitan areas and their mean BMI was 22.04 2.75.

The mean tumor size (cm) in our study was 8.17±4.02, however the tumors in group 1 measured 7.43.8 and those in group 2 were 9.54.1, making them resectable and unresectable, respectively. According to the findings of our research, larger tumors are connected with higher AFP levels and poor resectability. In group 2 unresectable individuals had much larger tumors. The findings were in line with those of earlier analyses [24], [34], and [35], however they were

not significant statistically. According to the findings of [26], negative serum AFP values (less than 20 ug/L) decreased as the diameter of the tumor increased. This particular focus on tumor sizes and different cut off valuesmight be useful in suggesting criteria values for the screening and diagnosing of HCC [26]. As we set our AFP cut off value in relation with the respectability of HCC, so we could mention that our cut off value of AFP (38.45 ng/ml) might also be useful for the assessment of resectability of HCC especiallyin context of tumor size.

In this research, the location of the tumor in the liver, its local extension or its distant metastasis did not affect the amount of AFP in the serum. Despite this, a single tumor exhibited a lower level of AFP in the serum compared to multiple tumors.

As is common knowledge, HBV is the root cause of up to 80 percent of all cases of HCC worldwide. There are around 400 million chronic HBV patients in the world [32]. HCC may develop in 20% of these patients. The prevalence of HBV-positive HCC may vary depending on the study and the locale. Infection with HBV was the cause of 60% of cases in Africa and East Asia [33], but only 20% of cases in the West. According to the study done by Tangkijvanich et al (2000) about 58.1% HCC patients were HBsAg-positive but as high as 81.4% was mentioned by Liu et al (2013) [19, 29]. A total of 59.3% of our patients tested positive for HBV.

Just as the relationship between HBV and HCC has been clarified, AFP is foundhigherin HBV-related HCC thaninnon-HBV-related HCC. Mutual influence of the HCC and HBV might have effect on the level of AFP [26]. Hann et al (2012) concluded that elevated serum AFP was significantly associated with increased risk of HCC in HBV patients and that high levels of serum AFP were associated with the higher risk of developing HCC in non-cancer HBV patients. Our results indicated that HBV positive/negative in lower AFP group (group 1) was not associated with resectability but in higher AFP group (group 2) HBV positive patients were significantly associated with poor resectable status. Similarly, when HBV DNA was detected then it was also found in significant in lower AFP group but significant in higher AFP group. These indicate that HBV positive HCC patients are significantly associated with poor resectability when serum AFP is raised and it would be more indicative when HBV DNA is detected.

Conclusion

In this study, the overall resectability was 37%. Frequency of resectable patients were more in lower AFP level. Preoperative serum AFP level has significant association with HBV positive HCC and detectable HBV DNA. So, itcould be a good predictor for the resectability of HCC.

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Conflict of interest: None declared

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All correspondence to
Dr. Md. Najmul Haque
Assistant Professor
Hepatobiliary Surgery
Rajshahi Medical College, Rajshahi
E-mail: mnhaque2010@gmail.com