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Correlation of Hepatocellular Carcinoma with Differents Levels of Alpha-Fetoprotein-A Comparative Analytical Study

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Author`s Contribution

[1,3]Substantial contributions to the conception or design of the work, [2,4]Drafting the work or revising it critically for important intellectual content
[5] Active Participation in active methodology, Analysis
[6]Final approval of the version to be published

Funding Source: None
Conflict of Interest: None

Received: April 10, 2021
Accepted: Oct 27, 2021

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ABSTRACT

Objective: To assess pathological, biochemical and clinical parameters in HCC patients with low, normal and high AFP levels.

Methodology: This comparative analytical study was conducted in Liver and Gastric Clinic, Holy Family Hospital, Rawalpindi from June 2019-June 2020. Sample size was calculated as 225 by WHO sample size calculator. Non randomized convenient sampling was used. Patients were divided into three groups according to AFP values; <200 ng/ml, 200-400ng/ml and >400 ng/ml. Patients demographic details, medical history, clinical symptoms and signs, biochemical parameters and pathological findings on ultrasonography and CECT were assessed. Data was analyzed by SPSS version 21. Descriptive statistics were calculated for categorical variable. p Value less than 0.05 was considered significant.

Results: Out of 256 patients, 161 (62.9%) were males and 95 (37.1%) were females. The mean age of patients was 60.5 years ± 10.5. 94.4% of patients with HCC had Hepatitis C. Anorexia, jaundice and abdominal distension, hepatomegaly and splenomegaly were more frequent in patients having high AFP level. Serum bilirubin, tumor size, number of lesions, BCLC staging and other pathological parameters associated with HCC worsens as AFP levels increase.

Conclusion: Alpha-fetoprotein levels are significantly associated with clinical and pathological parameters of hepatocellular carcinoma thus can be used as a better diagnostic and prognostic tool.

Keywords: Hepatocellular carcinoma, Alpha fetoprotein, Hepatitis C, Liver cirrhosis, BCLC staging.

Cite this article as: *Munir M, Maqbool M, Ayyaz J, Anis S, Maqbool S, Krishan J. Quality of Life in Patients with Stoma. Ann Pak Inst Med Sci. 2021; 17(3):260-265. doi. 10.48036/apims.v17i3.565*

Introduction

Hepatocellular carcinoma is the sixth most prevalent tumor worldwide and the third leading cause of cancer-related death. [1]Detection of HCC at an early stage predicts the greater response of patient to treatment therapy and thus, better survival rate.[2] Alpha fetoprotein is a glycoprotein that is a component of serum proteins in early embryonic life but tends to fall before birth. However, it begins to rise in patients with HCC or germ cell tumors.[3] Alpha-fetoprotein (AFP) was described as a fetal form of albumin for the first time in 1953.The best-described and the most used marker for hepatocellular carcinoma (HCC) is AFP.[4] It has been recognized due to its usefulness in prognosis for HCC, its relationship to various indices of HCC human biology and for non-biopsy HCC diagnosis.[5] Combining AFP with new diagnostic modalities increases the sensitivity and specificity to diagnose HCC.[6] It also helps in assessing the response towards treatment therapy.[7] AFP levels along with abdominal ultrasound is used as screening tool in patients with hepatitis or cirrhosis who are at risk of development of HCC.[6] Serum AFP levels of more than 400 ng/ml are considered diagnostic.[8]

Alpha fetoprotein acts by interacting with Retinoic acid receptors-β within the cytoplasm and prevents the shifting of Retinoic acid receptorsβ into the nucleus through bidding to Retinoic acid receptors-β with all-trans-retinoic acid. Hence, cytoplasmic Alpha fetoprotein acts as an inhibitor within the retinoic acid receptor signaling pathway and is responsible for causing resistance of retinoid in cancer chemotherapy.[9]However, not all Hepatocellular tumors are associated with elevated amount of AFPs in blood. According to American Association for Study of Liver Diseases, only ultrasonography can be used to screen hepatocellular carcinoma. It also suggested sub-optimal sensitivity of Alpha fetoprotein to screen for hepatocellular carcinoma. AFP levels remain normal among approximately 40% of early cases of HCC and even 15-20% of advanced cases.[10] The accuracy of the AFP levels used as diagnostic tool and in surveillance programs for HCC was challenged, AFP was removed from updated international guidelines for HCC surveillance. However, many reports suggest a rationale for the continued use of AFP.

This study aims at assessing a comprehensive relationship between different AFP levels and pathological, biochemical and clinical behavior of patients with hepatocellular carcinoma. It will help in examining all the possible determinants of serum AFP values. It will assess the difference in clinical and biochemical behavior of patients with low, normal and high AFP values. The goal of this study is construction of models that predict serum AFP values. It will help in establishing high diagnostic and prognostic value of AFP.

Methodology

A comparative analytical study was conducted in Liver and Gastric Clinic, Holy Family Hospital, Rawalpindi from June 2019 to June 2020. Patients with hepatocellular carcinoma presenting to Liver and Gastric clinic were conservatively included in the study. Non-probability convenient sampling was used for selection of patients. Confirmed cases of hepatocellular carcinoma diagnosed by triphasic_CT scan or MRI were included in the study. Patients having metastatic hepatocellular carcinoma were excluded from the study. After approval from Institutional Research Forum (IRF) and permission from authorities of Rawalpindi Medical University, a structured checklist was used for data collection which included age, sex, hepatitis B and hepatitis C status, bilirubin level, albumin level, MELD score, BCLC staging, pathological parameters of lesion and AFP levels. Prior verbal consent was taken and patient anonymity was maintained. Patients were divided into three groups on basis of their AFP levels i.e., below 200ng/ml, 200-400ng/ml and greater than 400ng/ml defined as low AFP, moderately high AFP and high AFP levels. Patient's clinical symptoms and signs, findings on laboratory investigations, pathological findings of disease on ultrasonography, CECT and liver biopsy were assessed in relation to AFP values. Data was entered and analyzed using SPSS version 21. Sample size was calculated using WHO sample size calculator with 95% confidence level and margin of error was kept at 0.03%.[11] The sample size was calculated to be 256. For categorical variables, descriptive statistics was calculated using chi-square test. P values less than 0.05 was considered significant.

Results

Out of 256 patients, 161 (62.9%) were males and 95 (37.1%) were females. (Table I) The mean age of patients was 60.5 years ± 10.5. There were 65 (24.4%) patients with medical history of diabetes and 83 (32.4%) had hypertension. Only 14 (5.5%) each were found having IHD and HBV. There were 240 (94.9%) patients with Hepatitis C. Almost one-fourth of the patients were smokers. (Table I)

Table I: Demographic respondents of patients(n=256)

	No of cases	% age
Gender		
Male	161	62.9%
Female	95	37.1%
Medical History		
Diabetes mellitus		
No	191	74.6%
Yes	65	24.4%
Hypertension		
No	173	67.6%
Yes	83	32.4%
Ischemic Heart Disease		
No	242	94.5%
Yes	14	5.5%
Smoking		
No	190	74.2%
Yes	63	24.6%
Hepatitis B virus		
Negative	239	94.5%
Positive	14	5.5%
Anti-hepatitis C virus antibodies		
Negative	13	5.1%
Positive	240	94.9%

There was no significant variation was noted in age and gender distribution between AFP categories. Frequency

Table II: Association of demographic characteristics and medical history with AFP.

	Alpha Feto-protein			
	<200 (n=132)	200-400 (n=54)	>400 (n=80)	P-value
Gender				
Male	80 (65.6%)	34 (63.0%)	47 (58.8%)	
Female	42 (34.4%)	20 (37.0%)	33 (41.3%)	0.61
Diabetes Mellitus				
No	88 (72.1%)	37 (68.5%)	66 (82.5%0)	0.13
Yes	34 (27.9%)	17 (31.5%)	14 (17.5%)	
Hypertension				
No	85 (69.7%)	40 (74.1%)	48 (60%)	0.18
Yes	37 (30.3%)	14 (25.9%)	32 (40%)	
Ischemic heart disease				
No	133 (92.6%)	49 (90.7%)	80 (100%)	0.03
Yes	9 (7.4%)	5 (9.3%)	0 (0%)	
Smoking				
No	95 (77.9%)	38 (70.4%)	57 (71.3%)	0.19
Yes	27 (22.1%)	16 (29.6%)	23 (28.8%)	
Hepatitis B virus				
Negative	110 (92.4%)	54 (100%)	75 (93.8%)	0.12
Positive	9 (7.6%)	0 (0%)	0 (0%)	
Anti hepatitis C virus antibodies				
Negative	3 (2.5%)	2 (3.7%)	8 (10.0%)	0.06
Positive	116 (97.5%)	52 (96.3%)	72 (90.0%)	

Table III: Association of Clinical Presentation with AFP

	Alpha feto protein			
	<200 (n=132)	200-400 (n=54)	>400 (n=80)	**p-value**
Pain right hypochondrium				
No	25 (20.5%)	26 (48.1%)	26 (32.5%)	0.001
Yes	97 (79.5%)	28 (51.9%)	54 (67.5%)	
Weight loss				
No	45 (36.9%)	12 (22.2%)	14 (17.5%)	0.006
Yes	77 (63.1%)	42 (77.8%)	66 (82.5%)	
Fever				
No	58 (48.7%)	39 (81.3%)	39 (54.9%)	0.001
Yes	61 (51.3%)	9 (18.8%)	32 (45.1%)	
Lethargy				
No	23 (19.3%)	13 (25.5%)	11 (15.5%)	0.38
Yes	96 (80.7%)	38 (74.5%)	60 (84.5%)	
Anorexia				
No	37 (30.3%)	3 (5.9%)	9 (11.3%)	<0.001
Yes	85 (69.7%)	48 (94.1%)	71 (88.8%)	
Jaundice				
No	63 (51.6%)	37 (68.5%)	33 (41.3%)	0.008
Yes	59 (48.4%)	17 (31.5%)	47 (58.8%)	
Abdominal distension or mass				
No	53 (43.4%)	20 (37%)	17 (22.1%)	0.006
Yes	69 (56.6%)	34 (63%)	60 (77.9%)	
Liver & spleen on examination				
Unremarkable	70 (57.4%)	18 (33.3%)	34 (42.5%)	
Hepatomegaly only	3 (2.5%)	10 (18.5%)	3 (3.8%)	<0.001
Splenomegaly only	46 (37.7%)	17 (31.5%)	27 (33.8%)	
Both hepato-splenomegaly	3 (2.5%)	9 (16.7%)	16 (20%)	
Ascites				
None	56 (45.9%)	21 (38.9%)	15 (18.8%)	
Mild	40 (32.8%)	25 (46.3%)	35 (43.8%)	<0.001
Marked	26 (21.3%)	8 (14.8%)	30 (37.5%)	

of IHD was found significantly greater in AFP (< 200) and AFP (200-400) categories (p-value, 0.03). Similarly, HCV frequency was higher in lower AFP categories (<200) and (200 to 400), however, it was statistically significant (p-value, 0.06). (Table II)

Clinical symptoms were worsen in patients having high AFP levels. Pain and fever was more frequent in patients with AFP < 200 whereas weight loss was more common in patients having AFP levels of 200 to 400 and > 400 (p-value, 0.001). Anorexia, jaundice and abdominal distension were significantly more frequent in patients with AFP > 400 (p-value, <0.001). Hepatomegaly and splenomegaly were significantly associated with high AFP level (200 to 400) and (> 400) (p-value, <0.001). Moreover, marked as cities were also found significantly associated with higher AFP (>400). (Table III)

The pathological findings of patients with HCC were also found significantly variable according to AFP categories in this study. Serum bilirubin was found significantly raised (> 3 mg/dl) in patients with high AFP (>400). Whereas serum albumin was found significantly reduced in patients with high AFP levels (>400) (p-value <0.001). Prothrombin time > 6 second prolonged was significantly greater in AFP >400 group compared to low AFP levels (p-value, 0.002). Similarly, Child turcotte class C was significantly higher in AFP > 400 group (p-value, <0.001). Furthermore, the locations of lesions on CT and stages of liver cancer according to Barcelona score and Hong Kong Liver cancer score were also found variable between different categories of AFP (p-value, <0.001). (Table IV)

Discussion

This comparative analytical study conducted on 256 patients having hepatocellular carcinoma showed that clinical symptoms, biochemical parameters and pathological features of disease worsen with raised values of alpha-fetoprotein. According to our study, 240 patients with hepatocellular carcinoma have hepatitis C with cirrhotic liver disease. Similarly, in several studies from Pakistan; 4383.3% Hepatocellular carcinoma cases have been noticed to have anti-hepatitis C antibody positive.[12,13] According to Butt; 67.9% of hepatocellular carcinoma cases were hepatitis C positive.[14] HCV frequency is higher in patients having low AFP levels which is statistically significant (p-value, 0.06). This similar to a study conducted in United States of America which described strong association of presence of cirrhosis, high MELD scores and increased levels of ALT with high AFP levels.[15] Similarly, a multi variate analysis conducted in Japan showed that AFP level >20ng was an independent and significant risk factor for the development of HCC in patients with liver cirrhosis.[16]

This is in accordance with results of our study which showed significant rise in AFP levels in patients with hepatitis C and cirrhosis. Our study showed that HCC was more common in male patients as compared to females however the result is not statistically significant. This is in accordance with results of Abbasi A et al,[17]

documented that 70.4% males and 29.6% females have HCC. According to this study, hepatitis B and smoking do not have significant association with HCC.

According to this study, clinical symptoms worsen as AFP levels increase in blood of patients with HCC. It shows that weight loss, anorexia, jaundice and abdominal distension were significantly more frequent in patients with high AFP levels i.e, >400 (p-value, <0.001). Similarly, hepatomegaly and splenomegaly were significantly associated with high AFP level (200 to 400) and (> 400) (p-value, <0.001). Our study shows that serum bilirubin was found significantly raised in patients with high AFP (>400). Whereas serum albumin was found significantly reduced in patients with high AFP levels (>400) (p-value <0.001). Prothrombin time > 6 second prolonged was significantly greater in AFP >400 group compared to low AFP levels (p-value, 0.002). Similarly, Child turcotte class C was significantly higher in AFP > 400 group (p-value, <0.001). These findings are similar to a study conducted in Italian Liver center which indicated that cirrhosis, liver disease with viral etiology, raised alanine aminotransferase level, a low albumin level and a tumor size> 2cm were independently associated with elevated AFP levels.[18] According to our study, AFP levels increase with increase in size and number of lesion. This is in accordance with result of a study of Thailand which revealed that HCC patients with high AFP tend to have greater tumor size , bi lobar involvement and portal vein thrombosis.[11]Similarly, a logistic regression analysis conducted in China revealed that the AFP level at diagnosis was an independent risk factor of pathological grade, TNM size and tumor size.[19] Similarly, a study conducted in Hyderabad, Pakistan concluded that elevated AFP levels are associated with old age, Hepatitis C virus, child Pugh group C and tumor size greater than 7cm.[20]

Table IV: Association of Pathological Findings with AFP

	Alpha Feto-Protein			p-value
	<200 (n=132)	200-400 (n=54)	>400 (n=80)	
Bilirubin				
<2mg/dl	88 (77.1%)	20 (37%)	24 (30%)	<0.001
2-3mg/dl	9 (7.9%)	29 (53.7%)	23 (28.8%)	
>3mg/dl	17 (14.9%)	5 (9.3%)	30 (37.5%)	
Albumin				
>3.5 g/dl	40 (36%)	23 (42.6%)	11 (13.8%)	<0.001
2.8-3.5 g/dl	57 (51.4%)	25 (46.3%)	41 (51.3%)	
<2.8 g/dl	14 (12.6%)	6 (11.1%)	28 (35%)	
Prothrombin time				
<4 seconds prolonged	53 (49.3%)	29 (53.7%)	26 (32.5%)	0.002
4-6 second prolonged	40 (37%)	25 (46.3%)	36 (45%)	
>6 second prolonged	15 (13.9%)	0 (0%)	18 (22.5%)	
Child turcotte class				
Class A	21 (19.4%)	10 (18.5%)	3 (3.8%)	<0.001
Class B	69 (63.9%)	36 (66.7%)	42 (52.5%)	
Class C	18 (16.7%)	8 (14.8%)	35 (43.8%)	
No of lesions on USG				
0	9 (7.7%)	0 (0%)	0 (0%)	0.02
1	66 (56.4%)	30 (55.6%)	49 (63.6%)	
2	22 (18.8%)	11 (20.4%)	17 (22.1%)	
3	15 (12.8%)	8 (14.8%)	11 (14.3%)	
4 or more	5 (4.3%)	5 (9.3%)	0 (0%)	
Size of lesion on USG				
<3cm	44 (41.9%)	5 (9.3%)	19 (25.7%)	0.02
3-5cm	30 (28.6%)	26 (48.1%)	27 (36.5%)	
>5cm	31 (29.5%)	23 (42.6%)	28 (37.8%)	
No of lesion on CECT				
1	52 (61.2%)	28 (63.6%)	37 (62.7%)	0.95
2	22 (25.9%)	9 (20.5%)	14 (23.7%)	
3	4 (4.7%)	4 (9.1`%)	4 (6.8%)	
4 or more	7 (8.2%)	3 (6.8%)	4 (6.8%)	
Size of lesion on CECT				
<3cm	18 (43.5%)	2 (9.1%)	6 (23.1%)	0.14
3-5cm	8 (19.5%)	8 (36.4%)	8 (30.8%)	
>5cm	15 (36.5%)	12 (54.5%)	12 (46.2%)	
PST performance status (ECOG)				
0	28 (25.7%)	6 (11.5%)	10 (12.8%)	<0.001
1	66 (60.6%)	37 (71.2%)	42 (53.8%)	
2	9 (8.3%)	6 (11.5%)	23 (29.5%)	
3	6 (5.5%)	3 (5.8%)	3 (3.8%)	
Barcelona clinic for liver cancer				
A	32 (29.6%)	3 (5.6%)	3 (3.8%)	<0.001
B	31 (28.4%)	19 (35.2%)	14 (17.5%)	
C	31 (28.4%)	27 (50%)	34 (42.5%)	
D	15 (13.8%)	5 (9.3%)	29 (36.3%)	
Hong Kong liver cancer staging				
Stage I	17 (16%)	0 (0%)	3 (3.8%)	<0.001
Stage IIa	16 (15.1%)	6 (11.1%)	3 (3.8%)	
Stage IIb	13 (12.3%)	3 (5.6%)	5 (6.3%)	
Stage IIIa	11 (10.4%)	6 (11.1%)	9 (11.3%)	
Stage IIIb	12 (11.3%)	21 (38.9%)	6 (7.5%)	
Stage Iva	0 (0%)	5 (9.3%)	9 (11.3%)	
Stage IVb	19 (17.9%)	8 (14.8%)	22 (27.5%)	
Stage Va	9 (8.5%)	0 (0%)	13 (16.3%)	
Stage Vb	9 (8.5%)	5 (9.3%)	10 (12.5%)	
Liver biopsy				
Not performed	112 (97.4%)	50 (98%)	79 (98.8%)	0.88
Consistent with CT	2 (1.7%)	1 (2%)	1 (1.3%)	
Not consistent with CT	1 (9%)	0 (0%)	0 (0%)	

This study showed that AFP levels can be used as a diagnostic and prognostic tool for patients with HCC. This study can form basis to generate future research in this novel area to determine the effects raised levels of alpha-fetoprotein in blood with clinical and pathological presentation of hepatocellular carcinoma and use it for early diagnosis of patients.

Conclusion

Alpha-fetoprotein levels are significantly associated with clinical and pathological parameters of hepatocellular carcinoma thus can be used as a better diagnostic and prognostic tool.

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