Evaluation of HCV-associated Hepatocellular Carcinoma Based on Alpha-fetoprotein Levels

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Background and study aim: Alpha-fetoprotein (AFP) cannot be relied on alone for diagnosis of hepatocellular carcinoma (HCC). However, it may have a prognostic value and can be used for monitoring response to different modalities of treatment for HCC. This study aimed to differentiate the clinical and pathological features of HCCs according to AFP levels.

Subjects and Methods: This retrospective study included 60 patients with HCC secondary to chronic hepatitis C (HCV). They were divided based on serum AFP into two groups; group I; included 30 patients with AFP lower than 302.5ng/ml. and group II; included 30 patients with AFP higher than 302.5ng/ml. clinical, laboratory and pathological differences between both groups were compared.

Results: Regarding the pathological features, patients with higher AFP secreting tumors have larger tumor size compared to lower AFP secreting tumors; (5.8 cm Vs. 4.5 cm, P value; 0.001). Number of lesions and tumor location were similar between the two groups.

Conclusion: HCC-secreting high levels of AFP are larger and aggressive tumors when compared to low secreting AFP-HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is among the most common malignant tumors in the world **[1]**. In Egypt, the incidence of HCC has been doubling over a decade and ranks the 2nd and 6th most common cancers among males and females, respectively **[2]**. This is attributed to high burden of chronic hepatitis C virus (HCV) infection. It has been reported as the highest prevalence worldwide **[3]**. The overall prognosis in HCC is poor as majority of patients with HCC are diagnosed late when there is no curative treatment **[4]**. So, HCC surveillance among high risk groups is recommended by all societies of hepatology to reduce mortality. Populations at highest risk are those having cirrhosis, which is present in about 85-95% of the patients with HCC **[5]**.

It is well established that abdominal ultrasonography with or without Alpha fetoprotein (AFP) every 6 months is the standard surveillance strategy **[6]**. However, the capability of HCC in secreting AFP is not predictable; some tumours do not produce AFP while others produce high levels. This has been attributed to different origins of HCC; proliferative type which produces high levels of AFP may be developed from progenitor cells while non-proliferative HCC may be developed from mature hepatocytes **[7]**. Due to this different behavior, the diagnostic levels of AFP is variable and not the same in different studies, levels more than 200ng/ml was used as a diagnostic value, however, currently it is accepted that levels of more than 20 ng/ml are diagnostic **[8]**. Clinical and pathological features of HCC may differ according to etiologies and origins.

Also, such issues are needed to be compared among patients with different AFP levels. This study aimed to evaluate the clinical, laboratory and pathological differences of HCC, secondary to chronic hepatitis C, based on their capability in secreting AFP.

PATIENTS AND METHODS

Patients' selection:

This is a retrospective study that analyzed 60 patients diagnosed to have HCC secondary to chronic HCV. After treatment of chronic HCV using direct-acting antiviral therapy (DAA), patients were underwent different treatment modalities for HCC; radiofrequency ablation (RFA), microwave, local alcohol injection and trans-arterial chemoembolization (TACE) at the tropical department, zagazig university hospitals, during the period between January 2017 and December 2018. Diagnosis of HCC was based on the results of dynamic imaging (triple phase computed tomography or magnetic resonance imaging). Since, this study was a retrospective one, the diagnosis of HCC was confirmed in all patients. Because of high AFP levels observed among this cohort of Patients before treatment of HCC, they were divided into two groups based on the median level of AFP among all patients.

Group I; included 30 patients with AFP levels (<302.5ng/ml(

Group II; included 30 patients with AFP levels (>302.5ng/ml(

The clinical, laboratory and pathological characteristics were reviewed between these 2 groups .

For each patient the following data were collected؛

1- Demographic and clinical data including; Age, gender, occupation, special habits, symptoms and signs of chronic liver disease (Child Pugh classification .(

2- Laboratory data; CBC, liver and kidney function tests, AFP, HCV PCR

3- Pathology data; number, size and location of lesions

Statistical Analysis

Statistical analysis was performed using SPSS statistics version 18.0. P<0.05 was considered significant value when comparing data. Continues variables are reported as median and range or mean and standard deviation as appropriate. Categorical variables are reported as numbers and percentages. Both groups were compared using Man-Whitney tests for categorical data and 2-sample t test for continuous data. Odds ratio and corresponding 95% confidence interval were calculated to evaluate the association between the AFP levels and HCC recurrence. Finally, receiver operating characteristic (ROC) curve was done to estimate the accuracy of the high AFP in predicting HCC recurrence.

RESULTS

The baseline characteristics of the included 60 patients are shown in table 1. The median age was 55 years (range; 48-65 years) and the male patients were 40 (66.7%). Splenomegaly was found in 32 (53.3%) patients and 45% of patients have chronic liver disease Child grade A.

The levels of AFP before HCC treatment were high and the patients were divided based on the median level of AFP 302.5 ng/mL, 30 (50%) patients had AFP levels (<302.5ng/ml) and 30 (50%) patients had higher AFP-secreting tumors (≥302.5ng/ml). Demographic and clinical differences between both groups are shown in table 2. There was no statistically significant difference between both groups regarding age, sex, occupation, neither special habits nor evidence of splenomegaly. However, patients with higher AFP levels had higher HCV viraemia compared to those with lower AFP levels (345700 vs. 123500, P value; 0.04). There was no statistically significant difference between both groups in terms of CBC, neither liver nor kidney function tests. Although occult hepatitis B virus infection was evident in 3 patients (10%) of the group I and 5 patients (16.7%) of the group II, this difference was non-significant.

Regarding the pathological features, patients with higher AFP secreting tumors have larger tumor size compared to those with lower AFP secreting tumors; (5.8 cm Vs. 4.5 cm, P value; 0.001). There was no significant difference between both groups regarding number of lesions and tumor location.

The recurrence of HCC was seen in 11(18.3%) patients out of 60 analyzed patients. Although patients with higher AFP secreting tumors showed higher rate of HCC recurrence in

comparison to those with lower AFP secreting tumors (8 patients vs. 3 patients, P value; 0.181), this was non-significant difference. To evaluate the relationship between AFP levels and the rate of HCC recurrence after locoregional therapy, we performed logistic regression and showed that odds ratio was 1 (confidence interval 0.99-1.00, p value 04), which was non-significant difference between both groups. This study also evaluated the accuracy of the high AFP in prediction of HCC recurrence, based on the cutoff value of the median (302.5ng/mL), values higher than 302.5ng/mL had higher sensitivity, specificity and positive predictive value compared to those with lower AFP values.

Table 1: Clinical, laboratory and pathological data of study population.

Variable	**N= 60**
Age (years)	55 (48-65)
Male sex n (%)	40 (66.7%)
Occupation:	
Farmer	20 (33.3%)
Housewife	20 (33.3%)
Teacher	8 (13.3%)
Worker	12 (20.0%)
Special habits:	
No special habits	38 (63.3%)
Cigarettes smoker	12 (20.0%)
Goza smoker	6 (10.0%)
Ex-smoker	4 (6.7%)
Splenomegaly	32 (53.3%)
Laboratory (mean ±SD)	
Hg (g/dl):	11.7 ± 1.5
RBCs ($x10^6$/ul):	3.9 ± 0.6
WBCs ($x10^3$/ul):	5.6 ± 1.8
Platelet count ($x10^3$/ul):	119.9 ± 42.4
INR (%):	1.4 ± 0.1
PT (sec.):	16.3 ± 1.5
ALT (IU/L):	58.0 ± 17.7
AST (IU/L):	50.0 ± 14.5
S. bilirubin (mg %):	1.2 ± 0.4
S. albumin (gm %):	3.2 ± 0.5
S. creatinine (mg %):	1.0 ± 0.2
AFP (median)	302.5 (205-1538)
PCR for AFP (median)	283024 (9685-8001757)
Occult HBV infection	8 (13.3%)
Pathology:	
Number of lesions:	
1	29 (48.3%)
2	16 (26.7%)
3	5 (8.3%)
>3	10 (16.7%)
Total tumor size	4.8 (3.3-7.3)
Tumor location:	
RT lobe	34 (56.7%)
LT lobe	8 (13.3%)
Multi-lobar	18 (30%)

Table 2: Demographic and clinical data of both groups.

Parameter	Group I (total; 30) mean±SD	Group II (total; 30) mean±SD	Test of significance	P value
Age	54.80±3.95	56.67±4.48	t -1.71	0.093
Sex Male (%)	24 (80%)	16 (53%)	**Fisher`s exact** 2.28	0.054
Smoking	8 (26.7%)	9 (30%)	**Fisher`s exact** -0.29	1.0
Splenomegaly	12 (40%)	20 (66.7%)	**Fisher`s exact** -2.15	0.069

Table 3: Laboratory characteristics between both groups.

Parameter	Group I (30) Mean ± SD	Group II (30) Mean ± SD	t.test	P value
WBC (x10³/ul)	5.52±1.18	5.77±2.35	-0.53	0.601
Hb. (gm/dl)	11.74±1.37	11.65±1.63	0.24	0.811
Plt. (x10³/ul)	125.7±42	114.1±42.8	1.07	0.291
Creat. (mg%)	0.981±0.207	0.950±0.183	0.61	0.546
Alb. (gm%)	3.265±0.500	3.153±0.434	0.93	0.358
ALT(IU/L)	58.6±15.5	57.4±19.9	0.26	0.795
AST(IU/L)	47.9±12.1	52.2±16.5	-1.16	0.251
Bilirubin total (mg%)	1.145±0.256	1.307±0.521	-1.52	0.135
PT (sec.)	16.17±1.64	16.51±1.36	-0.88	0.385
INR (%)	1.367±0.130	1.420±0.111	-1.69	0.097
AFP (median)	236 (205-300)	551 (305-2033)	-6.64	0.001
PCR for HCV (median)	123500 (9685-3490800)	345700 (36985-8001757)	-2.13	0.040
Occult HBV	3 (10%)	5 (16.7%)	Fisher`s exact	0.70

Table 4: Pathological characteristics between both groups.

Parameter	Group I (30)	Group II (30)	Test	P value
Number of lesions, n (%)			Mann-Whitney 850	0.308
1	16 (53%)	13 (43%)		
2	8 (27%)	8 (27%)		
3	3 (10%)	2 (6.7%)		
>3	3 (10%)	7 (23.3%)		
Total tumor size (cm)	4.5±1.2	5.8±1.5	T test -3.67	0.001
Tumor location			Mann-Whitney 0.57	0.570
RT lobe	16 (53.3%)	18 (60%)		
LT lobe	4 (13.3%)	4 (13.3%)		
Multi-lobar	10 (33.4%)	8 (26.7%)		

Table 5: Sensitivity, specificity, positive predictive value and negative predictive value according to cut off value 302.5 ng/mL.

AFP cut-off value (ng/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
<302.5	27	45	10	73
≥302.5	73	55	27	45

DISCUSSION

Diagnosis of HCC is determined after the characteristics of the dynamic imaging, while AFP cannot be relied on for diagnosis of HCC, since about 30% of patients with HCC don't secrete AFP and also, AFP may be elevated in tumours other than HCC **[9]**. However, it has a prognostic value, since those with lower AFP levels have better survival and lower recurrence rates than those with higher AFP levels **[10]**. Also, it is used for monitoring response to different modalities of treatment of HCC **[11]**. This study aimed to characterize the clinical, laboratory and pathological features of HCCs based on AFP levels.

The total size of the tumors was statistically larger in high AFP-secreting tumors compared with lower AFP-secreting tumors. This is consistent with Gurakar et al; 2018 who demonstrated that HCC that secrete higher AFP (>20ng/ml) has larger tumor size when compared to HCC with lower AFP secretion. In addition, higher AFP-secreting tumors had more aggressive behavior in the form of portal vein thrombosis, a finding that was evident in our study **[12]**. These findings were also evident in other studies, Agopian et al; 2017 classified HCC patients into AFP-secreting and non-secreting tumors and found that patients with non-AFP producing tumors had fewer (25% vs. 35% with>2lesions, p=0.03) and smaller lesions (4.2 vs. 5 cm, p= 0.02) compared to AFP-producing tumors **[13]**.

Regarding tumor recurrence following HCC treatment, it was evident from this study that high AFP-secreting tumors had higher recurrence rate compared to lower AFP-secreting tumors (26.7% vs. 10%, p=0.0181). In concordance with this, Hameed et al; 2014 found that pre-liver transplantation AFP >1000ng/ml was a strong predictor of tumor recurrence **[14]**. Also, Gurakar et al; 2018 showed that tumor recurrence following liver transplantation was higher among HCC patients with AFP higher than 20ng/ml compared to low AFP-secreting tumors (28% vs. 6%, p= 0.001) **[12]**. Also, Agopian et al; 2017 found that those with non AFP-secreting HCC had lower 5-year recurrence rates following liver transplantation compared to those with AFP-secreting tumors (8.8% vs. 22%, p=0.01) **[13]**.

From this study, it is apparent that high HCV viraemia was correlated with the occurrence of HCC, which is consistent with that reported by Ran Noh et al; 2016 who revealed that HCV viral load is considered risk factor for the development of HCC **[15]**. Also, in this study high HCV viraemia was associated with higher AFP-secreting HCC. In our cohort of patients, the underlying cause of HCC was cirrhosis secondary to chronic hepatitis C (HCV). However, although the prevalence of occult HBV infection among the patients was not significantly different but it seems higher in the group of the patients with higher AFP secreting HCC, this was consistent with that found in Gurakar et al; 2018 who found that chronic hepatitis B virus (HBV) was more common among patients with higher AFP levels compared to those with low AFP-secreting tumors (16.4% vs. 4.8%, p=0.068) **[13]**.

CONCLUSION

Hepatocellular carcinoma-secreting high levels of AFP are larger and aggressive in their behaviour when compared to HCC with low AFP-secretion.

Ethical approval: This study was reviewed and approved by the Institutional Review Board of the faculty of Medicine, Zagazig University, Egypt.

Conflict of Interest: None.

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