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Incidence of Hepatocellular Carcinoma in Patient with Elevated Alpha Fetoprotein before DAAS

Ahmed Mohamed Mansour Abd El Shafi, Abd El Wehab Fathi Ali, Mohammed Fakhery Mohamed Morsey
Department of Hepatology, Gastroenterology and Infectious Diseases, Faculty of Medicine,
Al-Azhar University (Assiut), Egypt.
***Corresponding author:** Ahmed Mohamed Mansour Abd El Shafi, **Mobile:** (+20) 01002159714,
E-Mail: ahmedserag932@yahoo.com

ABSTRACT
Background: Management of chronic infection with hepatitis C virus (HCV) with direct acting antivirals (DAAs) agents achieves high virological response. Recently, there is a matter of controversy about occurrence of hepatocellular carcinoma (HCC) following those agents. **Objective:** This study aimed to evaluate the efficacy of alpha fetoprotein (AFP) in development of HCC following DAAs therapy. **Patients and methods:** two hundred patients with chronic HCV infection who were eligible for DAAs therapy were recruited. Those patients were followed up for one year following therapy to detect development of HCC. Patients were grouped into either with normal AFP (100 patients) or high AFP (100 patients). **Results:** Majority (84%) of patients were males with overall mean age of 43.87±12.76 years. There were no significant differences between both groups of patients as regard baseline data. With exception of 4% of patients, all of them reached the sustained virological response (SVR). Frequency of HCC detection was higher among those patients with high AFP (9 (9%) vs 1 (1%); P< 0.001). We found that liver cirrhosis; FIB-4, APRI and AFP were predictors for HCC. AFP had the best diagnostic accuracy for prediction of HCC following DAAs
Conclusion: Patients who received DAAs for HCV infection should be regularly screened for development of HCC.
Keywords: Hepatocellular carcinoma, Alpha fetoprotein, Direct acting antivirals.

INTRODUCTION

Progression to liver cirrhosis and development of hepatocellular carcinoma (HCC) are the major complications of hepatitis C virus (HCV) infection. Pathophysiological mechanism to develop HCC and its progression till metastasis is considered a complex process. This process required long term interaction between the viral components and defense mechanism of the host [(1)]. Sofosbuvir (SOF) is one of the new direct acting antivirals (DAAs) that could be used with other agents belong to DAAs and produce high safety profile and efficacy. Its tolerability and efficacy was proofed whatever age, sex, HCV genotype and stage of hepatic fibrosis [(2)].

Alpha-fetoprotein (AFP) is released form gestational sac in the early embryonic life then liver synthesize it later on. Its high level in cirrhotic patients carries a risk for HCC [(3)]. This work was conducted to assess the predictive value of baseline AFP in prediction of HCC following DAAs therapy.

PATIENTS AND METHODS

A cross sectional study was conducted in period between 2020 and 2021 at Outpatients Clinics, Al-Azhar University Hospitals. Based on previously reported HCC following DAAs therapy that was 2.69% [(4)] with considering 3% alpha error and 95% confidence interval, a minimum of 112 patients receiving DAAs were required.

Two hundreds patients with HCV infection who were eligible for direct acting antivirals (DAAs) were enrolled. Any patient with one or more of the following criteria was excluded; patients aged <18 years, platelet count less than 5000 /mm^3, extra hepatic malignancy, pregnancy or inability to use effective contraception, inadequately controlled diabetes mellitus (glycosylated hemoglobin more than 9 %), and/or patient's refusal

Those enrolled patients were subdivided based on baseline level of alpha fetoprotein into [(5)]: One hundred patients with normal AFP (< 10 ng/ml), and one hundred patients with high AFP (≥ 10 ng/ml).

Complete history taking and clinical assessment were done. The following data were gathered; age, sex, body mass index, previous interferon therapy for HCV infection, residence, and comorbidities as diabetes mellitus, hypertension, ischemic heart disease and chronic kidney disease.

Laboratory tests and serum biomarkers:

The investigations that were ordered are complete blood picture, liver function tests, kidney function tests including blood urea and serum creation. Also, baseline AFP was assessed in all patients. Different markers of fibrosis were assessed at baseline during follow up; the AST to platelet ratio index (APRI) score and Fibrosis-4 (FIB-4) score. Abdominal ultrasonography was performed in all patients at baseline and SVR24 for the detection of focal lesions and hepatic decompensation. Screening for HCC development occurred every three months till one year after end of therapy by abdominal ultrasound. Any suspected lesion during follow up, a dynamic imaging either computed tomography and/or magnetic resonance image would be done.

Ethical consideration:

Approval of the study was obtained from the Research Ethics Committees in Faculty of Medicine,

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Al-Azhar University and it was conducted in accordance with the Code of Good Practice and the guidelines of Declaration of Helsinki, 7th revision, 2013. All patients were informed about the study and a written consent was obtained from each patient.

Statistical analysis

Data were collected and analyzed those using SPSS (Statistical Package for the Social Sciences, version 20, IBM, and Armonk, New York). Continuous data were expressed in form of mean ± SD and compared with Student t test while nominal data were expressed in form of frequency (percentage) and compared with Chi^2 test. Predictors for development of HCC following DAAs therapy were determined by multivariate regression tests, Level of confidence was kept at 95% and hence, *P* value was considered significant if < 0.05.

RESULTS

Baseline data of enrolled patients (Table 1):

Both groups of patients had insignificant differences as regard baseline data.

Table (1): Baseline data of enrolled patients

	High AFP (n= 100)	Normal AFP (n= 100)	*P* value
Age (year)	44.98 ± 12.87	42.22 ± 16.87	>0.05
Sex			>0.05
Male	89 (89%)	79 (79%)	
Female	11 (11%)	21 (21%)	
BMI (kg/m^2)	24.09 ± 4.87	25.89 ± 6.09	>0.05
Residence			>0.05
Rural	78 (78%)	81 (81%)	
Urban	22 (22%)	19 (19%)	
Occupation			>0.05
None	68 (68%)	65 (65%)	
Housewife	11 (11%)	15 (15%)	
Employee	12 (12%)	9 (9%)	
Student	9 (9%)	11 (11%)	
Diabetes mellitus	14 (14%)	16 (16%)	>0.05
Hypertension	5 (5%)	7 (7%)	>0.05
Ischemic heart disease	2 (2%)	1(1%)	>0.05
INF experienced	7 (7%)	10 (10%)	>0.05
Smoker	18 (18%)	21 (21%)	>0.05

Data expressed as frequency (percentage), mean (SD). AFP: alpha fetoprotein; INF: interferon

Baseline laboratory data among enrolled patients (Table 2):

There was a significantly higher platelets count among those with normal AFP. Patients with high AFP had significantly higher FIB-4 and APRI.

Table (2): Baseline laboratory data of enrolled patients

	High AFP (n= 100)	Normal AFP (n= 100)	*P* value
HCV RNA (10^6 u/l)	1.23 ± 0.10	1.11 ± 0.18	>0.05
Hemoglobin (gm/dl)	12.76 ± 1.87	12.99 ± 2.98	>0.05
Platelets (10^3/ul)	155.78 ± 33.87	216.98 ± 22.22	**<0.001**
Leucocytes (10^3/ul)	5.91 ± 1.33	6.01 ± 1.87	>0.05
Bilirubin (mg)dl	1.01 ± 0.11	0.99 ± 0.17	>0.05
Albumin (mg/dl)	4.01 ± 1.11	39.80 ± 2.01	>0.05
AST (u/l)	66.78 ± 10.14	69.01 ± 14.22	>0.05
ALT (u/l)	68.13 ± 5.66	70.01 ± 6.09	>0.05
Creatinine (mg/dl)	1.11 ± 0.10	1.09 ± 0.10	>0.05
Urea (mg/dl),	4.22 ± 0.54	4.89 ± 1.45	>0.05
INR	1.03 ± 0.03	1.04 ± 0.02	>0.05
FIB-4	4.64 ± 1.34	2.55 ± 0.98	**< 0.001**
APRI	6.65 ± 1.11	3.51 ± 0.12	**0.01**
U/S evaluation			
Liver cirrhosis	76 (76%)	12 (12%)	**< 0.001**
Splenomegaly	30 (30%)	10 (10%)	**<0.001**

Data expressed as frequency (percentage), mean (SD). APRI: AST to platelet ratio index; FIB-4: fibrosis-4; AST: aspartate transaminase; ALT; alanine transaminase; AFP: alpha fetoprotein; U/S: ultrasound; HCV RNA: hepatitis C virus ribonucleic acid.

Regimens of therapy and sustained virological response among enrolled patients:

As regard regimens of therapy majority (76%) of those patients with high AFP received triple therapy for three months in form of sofosbuvir, daclatasvir with weight based ribavirin while only 12 (12%) patients from those with normal AFP received triple therapy. Majority (88%) of those patients with normal AFP received dual therapy in form of sofosbuvir, and daclatasvir for three months while only 24 (24%) patients from those with high AFP received dual therapy.

Majority (95% of those with high AFP and 97% of those with normal AFP) achieved sustained virological response while only five patients with high AFP and three patients with normal AFP failed to achieve sustained virological response.

Frequency of hepatocellular carcinoma post-therapy among studied groups (Tables 3 and 4):

A total 10/200 (5%) patients of all enrolled patients developed HCC during follow up after therapy. It was found that frequency of HCC was significantly higher among those patients with high AFP in comparison to those with normal AFP.

Table (3): Frequency of hepatocellular carcinoma post-therapy among studied groups

	High AFP (n= 100)	**Normal AFP (n= 100)**	***P* value**
Development of HCC			
No	91 (91%)	99 (97%)	< 0.01
Yes	9 (9%)	1 (1%)	

Data expressed as frequency (percentage). AFP: alpha fetoprotein; HCC: hepatocellular carcinoma

Table (4): Characteristics of patients with hepatocellular carcinoma

Case	**Age**	**Sex**	**PVT**	**Baseline AFP**	**Time**	**No of HCC**	**Size (cm)**
1	51	Male	Yes	32	8	**2**	**2*4/ 2.5*4**
2	53	Male	No	12	4	1	2.5*5
3	51	Male	Yes	9	5	1	3.3*4
4	55	Male	No	22	6	1	1.8*2.5
5	55	Female	Yes	14	7	1	2*4
6	46	Male	No	12	8	1	2.5*4.5
7	65	Male	No	23	6	1	2.7*3.3
8	56	Male	No	21	11	1	1.5*3.5
9	48	Male	No	19	10	1	2*4.5
10	55	Male	No	15	11	1	3*5.5

PVT: portal vein thrombosis; AFP: alpha fetoprotein; HCC: hepatocellular carcinoma

Predictors of development of hepatocellular carcinoma following therapy (Table 5):

Based on the current study; the predictors for development of HCC following DAAs therapy were; liver cirrhosis, FIB-4, APRI, and AFP.

Table (5): Predictors of development of hepatocellular carcinoma following therapy

	Odds ratio	**95% confidence interval**	***P* value**
Age (> 60 years)	1.13	0.11-1.45	>0.05
INF experienced	0.98	0.44-1.40	>0.05
BMI (> 25 kg/m^2)	1.49	1.22-2.89	>0.05
Liver cirrhosis	4.56	2.34-6.87	< 0.001
FIB-4 (> 1.45)	1.45	1.34-3.03	< 0.001
APRI (> 3.25)	1.98	1.07-3.87	< 0.001
AFP (> 10 ng/ml)	3.87	2.01-4.98	< 0.001

APRI: AST to platelet ratio index; FIB-4: fibrosis-4; AFP: alpha fetoprotein; INF: interferon, BMI: body mass index

Performance of AFP, FIB-4 and APRI in prediction of HCC following DAAs therapy (Table 6 and figure 1):

The performance of AFP at cutoff point > 10 (ng/ml), of FIB-4 at cutoff point > 1.45; and of APRI at cutoff point > 3.25 are shown in table 6 and figure 1.

Table (6): Performance of different parameters in prediction of HCC

Indices	**AFP**	**FIB-4**	**APRI**
Sensitivity	87%	20%	20%
Specificity	70%	80%	93.3%
PPV	59%	87%	60%
NPV	91%	43%	70%
Accuracy	75.7%	60.1%	68.7%
Cutoff point	> 10 (ng/ml)	> 1.45	> 3.25
Area under curve	0.80	0.59	0.59

PPV: positive predictive value; NPV: negative predictive value; APRI: AST to platelet ratio index; FIB-4: fibrosis-4; AFP: alpha fetoprotein; HCC: hepatocellular carcinoma; DAAs: direct acting antivirals

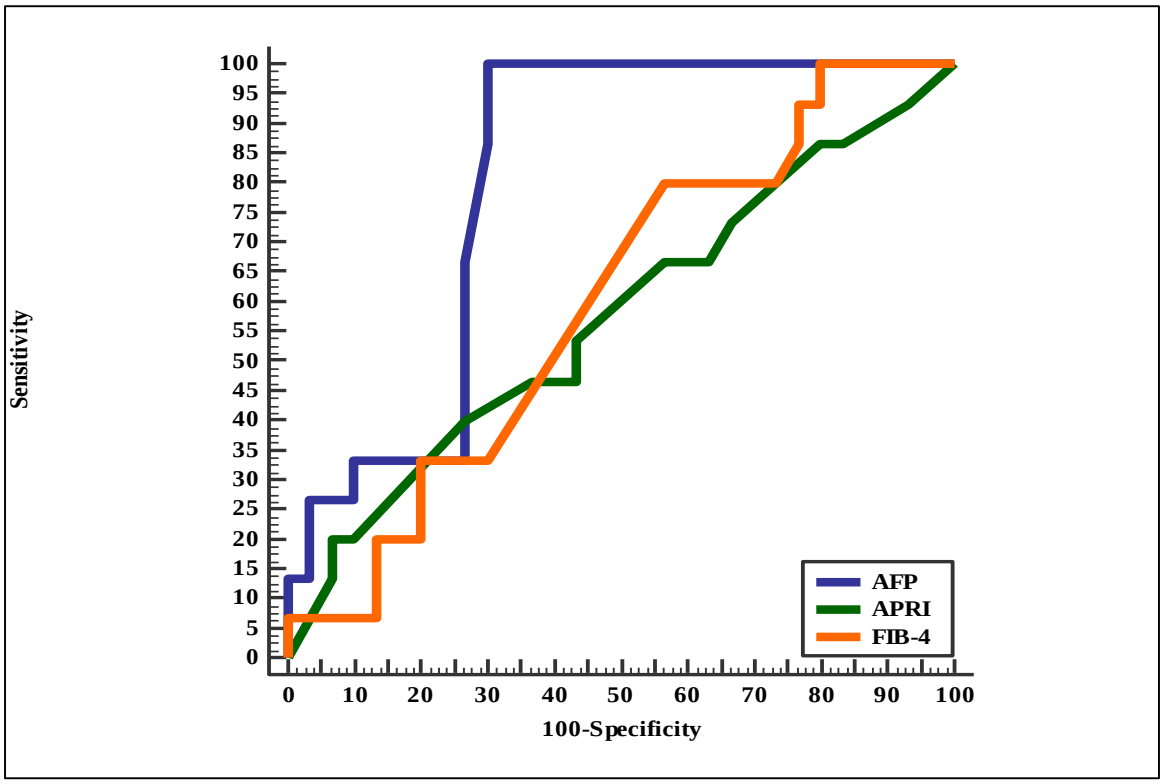

Figure (1): Diagnostic accuracy of AFP, FIB-4 and APRI in prediction of HCC following DAAs therapy. APRI: AST to platelet ratio index; FIB-4: fibrosis-4; AFP: alpha fetoprotein

DISCUSSION

In comparison to previous medications, SVR rates have increased to over 90% with very few documented side effects since the introduction of DAAs. DAA's introduction also permits individuals with decompensated cirrhosis to be treated. The long-term goal of DAA-induced SVR is to diminish fibrosis and chronic hepatitis C consequences, such as the development of HCC [6].

The current study enrolled 200 patients with chronic HCV infection. Those patients were followed for one year following DAAs therapy to assess the predictive value of baseline AFP in development of HCC following DAAs therapy. Those patients were divided into two groups either with high AFP (>10 ng/ml) or normal AFP (< 10 ng/ml).

Both groups had insignificant differences as regard baseline data. Based on the current study, we found that patients with high AFP had significantly lower platelets count and higher APRI and FIB-4. This could be explained by higher frequency of patients with liver cirrhosis among those with high AFP (78% vs. 12%).

Our study revealed that A total 10/200 (5%) patients of all enrolled patients developed HCC during follow up after therapy. It was found that frequency of HCC was significantly higher among those patients with high AFP in comparison to those with normal AFP (9% vs 1%). **Hamoir *et al.*** [7] also, studied 143 adult patients who have been treated with different regimens of DAA for HCV-related chronic liver disease. Out of their patients, 4.2% developed HCC during one year duration of follow up. This frequency is comparable with the current result. In a previous study with median duration of follow up was 17 months after end of therapy, a total of 95/1045 (9.9%) patients developed HCC. The discrepancy between this study and our study as regard frequency of development of HCC following DAAs may be because the authors of the latter study included cirrhotic patients and also, enrolled patients with previous history of HCC [8]. A review published by **Waziry *et al.*** [4] summarizing the evidence on HCC occurrence and recurrence following DAA or IFN based therapy over the last 17 years showed a similar annual occurrence rate of HCC (2.96%) in patients treated by DAA.

Based on the current study; the predictors for development of HCC following DAAs therapy were; liver cirrhosis, FIB-4, APRI and AFP. Also, we found that AFP had the best diagnostic accuracy for prediction of HCC following DAAs (75.5%) in comparison to APRI (68.7%) and FIB-4 (60.1%). In consistent with the current study, **Ogawa *et al.*** [9] showed that age > 70 (odds ratio= 2.39), male sex (odds ratio= 2.07), cirrhosis (odds ratio= 2.97), serum albumin < 3.5 g/dL (odds ratio= 2.72), and AFP > 7 ng/ml (odds ratio= 4.92) are considered risk factors for the development of HCC in patients received DAAs. In contrast to the current study, **Conti *et al.*** [10] stated that baseline AFP level wasn't a risk HCC following DAAs.

Additional risk factors for development of HCC following DAAs was reported by **Hamoir *et al.*** [7] who found that genotype 2 was associated with risk of HCC than patients with other genotypes (odds ratio = 2.8). The risk of HCC was also higher in patients with steatosis than in patients without (odds ratio=14.93).

Akuta *et al.* [11] concluded that although AFP levels at baseline > 10 ng/ml was higher among those with HCC, yet this was not approved with regression analysis.

In line with our findings, a previous study found that an AFP cut-off value > 6 ng/ml had the best sensitivity in prediction of HCC development [8].

The main limitations of the current study included; 1) short term of follow up that was only one year post-therapy, 2) Identifying the carcinogenic process in this situation would almost probably necessitate actual studies gathering pathological and immunological data to look into tumour biology and the immune system changes that occur after HCV is removed abruptly with DAAs.

CONCLUSION

DAAs are effective and safe agents in management of patients with HCV infection. But long term follow up of those patients is very important because those patients are still at risk of developing HCC even after viral clearance.

RECOMMENDATIONS

Future studies with large sample size in multiple centers are warranted to confirm these findings.

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