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Direct Acting Anti-Viral Therapy, and Potential Increase in the Incidence of Hepato-Cellular Carcinoma in Cirrhotic Patients With Hepatitis C Virus

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Abstract

Background: Direct-acting anti-viral drugs (DAAs) have become widely used for cirrhotic patients with hepatitis C infection. Virological responses are excellent with SVR rate in > 90% of cirrhotic patients. The association between the use of DAAs and the development of hepatocellular carcinoma (HCC) is debatable.

Aim of the work: investigate the incidence of HCC and characteristics of tumors after DAAs therapy.

Patients and methods: A prospective study of a cirrhotic patient with chronic hepatitis c virus who received 12 weeks of DAAs therapy (sofosbuvir, daclatasivir) in Mansoura specialized medical hospital and followed up for 6 months post-treatment. Untreated cirrhotic patients with hepatitis C infection for comparison.

Results: amongst (27/30) 90% of patients achieved sustained virological response 12 weeks post-treatment there were 3/30 (10%) not responded, and 7/30 from treated patients vs 11/30 untreated patients had de novo HCC.

Conclusion: DAAs reduced the incidence of HCC but with no significant difference with the untreated patient (refuse treatment) after six months, however, the pattern of tumor characteristics is very aggressive in the treated patient.

Key Words: Hepatitis C virus, Sustained virologic response, Direct-acting antiviral therapy, Hepatocellular carcinoma, Liver cirrhosis

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Introduction

Hepatocellular carcinoma (also known as HCC) is the sixth most common cancer-related incident case but the fourth most common cancer-related death. HCC is relatively common in Egypt, accounting for 33.63 percent of male cancer diagnoses and 13.54 percent of female cancer diagnoses [1]. The lack of early screening and effective surveillance programs contributes to the poor prognosis of HCC, even in developed nations. Only 12% of cases are still alive after five years.[2] Patients who have aggressive HCC is aggressive have a much lower chance of survival. [3].

It has been demonstrated that liver function parameters and tumor aggressiveness factors have independent effects on HCC patient survival. These variables are therefore incorporated into some classification schemes, such as the Okuda classification system, BCLC (Barcelona Clinic Liver Cancer), and CLIP (Cancer of the Liver Italian Program) [4, 5].

The Aggressiveness Index (AgI) is a scoring system for HCC tumor patterns that was recently developed. The maximum tumor diameter (MTD), the number of tumor nodules (NTN), the presence of portal vein thrombosis (PVT), and the serum alpha-fetoprotein (AFP) level are all considered. One study found that a higher AgI score was associated with a worse prognosis.[6, 7].

With the development of DAAs, there have been conflicted results between their use and the development of HCC.[8].

In addition to the virus's direct effect on the NS53 core protein, it is thought that indirect pathways involving cytokines, steatosis induction, and oxidative stress all contribute to the development of HCC with DAAs use [9]. This is in addition to the virus's direct effect on the virus on hepatocytes. After a 2-12 week incubation period, the virus enters an acute phase and is cleared from the body on its own. Chronic HCV infection develops from untreated acute HCV infection. Cirrhosis of the liver develops as a result of this CHC infection due to the persistent inflammation caused by the host's immune response to the HCV infection [10].

Therefore, the key to preventing HCC caused by HCV is to stop the progression of cirrhosis through antiviral therapy and subsequent monitoring under routine surveillance programs. This is the only way to stop HCC. Parenteral interferon (IFN) and ribavirin were the standard treatment for chronic hepatitis C. The success rate of this treatment was 40–50% [11].

It was the year 2013 when the FDA approved the oral use of DAAs as anti-HCV therapy. These agents have better treatment outcomes, such as a sustained virological response (SVR) rate that is higher than 90 percent of the time in most cases. [12] (DAAs) for oral anti-HCV therapy was approved by the FDA in 2013. These agents have better treatment outcomes, such as a sustained virological response (SVR) rate that is higher than 90 percent of the time in most cases [8].

Preliminary evidence suggests that HCCs formed after DAA therapy may exhibit a macroscopic aggressive pattern [9, 13]. The risk of HCC was greatly reduced, but not eliminated, when CHC was treated early on.

Patients and methods

Patients who suffer from both liver cirrhosis and chronic hepatitis C were recruited from specialized medical facilities in Mansoura for this study. When HCV viremia was confirmed for at least six months, it was considered chronic hepatitis C. Ultrasound liver morphological signs of cirrhosis and computer tomography (CT) results were the main indicators of the condition. If cirrhosis was not evident on imaging, it had to be confirmed by meeting additional clinical criteria, such as splenomegaly of less than 15 cm, moderate ascites, and esophageal varices that could

not be explained by any other medical condition. The aspartate/alanine ratio, platelets, coagulation parameters, and bilirubin levels were considered by a panel of hepatologists.

To begin the observation period, each patient had to have an ultrasound or CT scan to confirm that they did not have HCC. Non-eligible for this study were patients with a history of HCC or liver transplantation, as well as those who tested positive for both HIV and HBV. Throughout antiviral therapy and for six months after it ended, routine ultrasound imaging was carried out every three months. This study documents a result of typical clinical practice.

All patients were given sofosbuvir (400 mg daily), daclatasvir (60 mg daily, with dose adjustments to 30 mg or 90 mg daily as recommended in patients with relevant potential drug-drug interactions), and ribavirin for a maximum of 12 weeks (800-1200 mg daily).

Patient demographic information, pre-treatment Child-Pugh and MELD scores, and post-treatment laboratory results were recorded. These results included AFP, WBC, hemoglobin, platelets, INR, creatinine, AST, ALT, and albumin. In addition, we discovered that the patient was struggling with both high blood pressure and diabetes mellitus.

DAA-treated group:

Participants who received DAAs in a prospective, observational cohort study were considered to be the treatment or the study group. The aforementioned eligibility criteria were evaluated for a total of thirty patients who were receiving IFN-free DAAs treatment between June 2019 till February 2021. The treatment plans for DAAs patients included the use of ribavirin, daclatasvir, and sofosbuvir.

Control group:

The patients who (refused treatment) were referred to the hepatology outpatient clinic at the same Mansoura specialized medical hospital as the treated patients (n = 30). This was the prospective control group. Patients who had been diagnosed with HCC, HIV, or HBV coinfections before the start of the observation period were excluded. Furthermore, each patient's baseline liver imaging must have been completed between three and six months after the completion of antiviral treatment.

Ethical consideration

The Mansoura Faculty of Medicine Institutional Review Board (IRB) ethics committee approved the research project protocol (code number: MS 19.4.560). The study included all DAAs patients who gave informed consent. The perspective control data were analyzed according to institutional review board guidelines.

Statistical analysis of data

The software SPSS (version 27, distributed by IBM/SPSS Inc. of Chicago, Illinois) was utilized for analyzing the data. At the outset of the investigation, the characteristics of the population that was going to be the subject of the study were summarized using frequency and percentage (percent) distributions for categorical data, mean ± standard deviations, and median values (Range) for parametric and non-parametric quantitative data respectively.

It was decided whether to use Fischer's exact test, Monte-Carlo test, or Chi-Square test to compare two separate sets of categorical data. To compare two groups with parametric and non-parametric quantitative data, the independent samples t-test or Mann-Whitney u-test were used. The Wilcoxon-signed rank test was used to compare nonparametric data between two dependent groups. P values of less than 0.05 are considered significant.

Results

Regarding age, gender, residence, smoking, or a history of a chronic illness, demographic data between the control group and the treatment group show no differences that could be considered statistically significant (P> 0.05). (e.g., Diabetes Mellitus and hypertension) (Table 1).

Regarding the size of the liver and spleen, the prevalence of HCC, or any other abnormalities identified by radiologic imaging at baseline, three months after treatment, or six months after treatment (Figure, 2), there were no statistically significant differences (P>0.05) between the treatment and control groups. Comparing the control group to the treatment group at six months and three months, respectively, revealed a statistically significant increase in the control group's enlarged spleen and focal lesion. After six months, the treatment group's hepatocellular carcinoma (HCC) rates were lower than those of the control group, but this difference was not statistically significant. (Table 2).

Table (3) demonstrates that there were no statistically significant differences (P> 0.05) between the treatment group and control group as regards white blood cells, Hemoglobin, and Platelets at baseline. Whereas there was a statistically significant decrease (P< 0.05) in white blood cells, Hemoglobin, and Platelets in the treatment group at three months and six months in comparison to the control group with (pv o.oo1).

Table (1): Demographic data and medical disease among control group and treatment groups

Demographic data	**Treatment group (n=30)**	**Control group (n=30)**	***P-value***
	No. (%)	**No. (%)**	
Age (years)			
Mean ± SD	62.10±4.44	60.23±3.71	0.083
Gender			
Male **Female**	27 (90.0%) 3 (10.0%)	24 (80.0%) 6 (20.0%)	0.472
Residence			
Urban **Rural**	6 (20.0%) 24 (80.0%)	6 (20.0%) 24 (80.0%)	1.00
Smoking			
Smoker **Nonsmoker**	11 (36.7%) 19 (63.3%)	9 (30.0%) 21 (70.0%)	0.584
DM			
Positive **Negative**	10 (33.3%) 20 (66.7%)	7 (23.3%) 23 (76.7%)	0.390
Hypertension			
Positive **Negative**	11 (36.7%) 19 (63.3%)	8 (26.7%) 22 (73.3%)	0.405

Table (2): Radiological evaluation in the two studied groups at baseline and three months, six months after treatment;

Clinical examination	**Treatment group(n=30)**	**control group (n=30)**	***p-value***
	No. (%)	**No. (%)**	
Enlarged liver			
Baseline	4 (13.3 %)	8 (26.7%)	0.167
After 3 m	4 (13.3 %)	9 (30.0%)	0.105
After 6m	6 (20.0%)	12 (40.0%)	0.079
Enlarged spleen			
Baseline	5 (16.7 %)	9 (30.0%)	0.360
After 3 m	6 (20%)	9 (30.0%)	0.276
After 6m	6 (20%)	14 (46.7%)	0.027*
HCC			
Baseline	0 (0%)	0 (0%)	1
After 3 m	3 (10%)	10 (33.3%)	0.029*
After 6m	7 (23.3%)	11 (36.7%)	0.258

Table (3) CBC in the two studied groups: at baseline, three months, and six months

Laboratory investigations	**Treatment group (n=30)**	**control group (n=30)**	***p-value***
	Mean ± SD	**Mean ± SD**	
W.B.C (10^3/µL)			
Baseline	5.58± 1.89	5.27± 1.93	0.777
After 3 m	4.44 ± 1.31	5.33±1.37	0.013*
After 6m	4.10 ±1.18	5.05±1.19	0.001**
Hb (gm/dl)			
Baseline	11.22±0.66	11.50±0.70	0.120
After 3 m	7.90 ± 0.90	10.33±0.65	0.001**
After 6m	8.67±0.49	9.36±0.57	0.001**
Platelets (10^3/µL)			
Baseline	155.25 ±69.52	156.80±41.90	0.478
After 3 m	80.93 ±7.16	115.96 ± 15.11	0.001**
After 6m	87.33±8.04	94.53 ±7.06	0.001**

Table (4) shows that there were no statistically significant differences (P> 0.05) between the treatment group and control group in serum creatinine, serum bilirubin, serum albumin, INR, ALT, AST & AFP at baseline while there were statistically significant increases (P< 0.05) in serum creatinine, serum albumin, ALT, AST for the control group at 3 months and 6 months compared to a treated group, while for serum bilirubin it is a statistically significant increase for a treated group than the control group at three months but after six months it is a statistically significant increase for the control group than treated group, whereas for INR statistically significant increase for the control group at 6 months only compared to a treated group, while for AFP statistically significant increase at 3 months and 6 months for a treated group than the control group.

Table (4): Laboratory investigations in the two studied groups at baseline, three months, and six months after treatment.

Laboratory investigations	Treatment group (n=30)	control group (n=30)	*p-value*
	Mean ± SD	Mean ± SD	
Serum creatinine (mg/dl)			
Baseline	0.97±0.22	1.04 ± 0.24	0.264
After 3 m	1.24 ± 0.14	1.41 ± 0.17	0.001**
After 6m	1.43±0.12	1.78±0.094	0.001**
Serum bilirubin (mg/dl)			
Baseline	1.07±0.25	1.19±0.25	0.062
After 3 m	1.94±0.29	1.56±0.15	0.001**
After 6m	2.05±0.42	2.42±0.29	0.001**
Serum albumin (gm/dl)			
Baseline	3.56±0.33	3.66±0.25	0.0326
After 3 m	3.27±0.32	2.64±0.45	0.001**
After 6m	2.94±0.47	2.18±0.19	0.001**
INR			
Baseline	1.16±0.13	1.21 ±0.072	0.076
After 3 m	1.26±0.058	1.30±0.18	0.219
After 6m	1.28±0.052	1.43±0.096	0.001**
ALT (u/ml)			
Baseline	49.30 ±13.51	55.36 ±25.22	0.250
After 3 m	54.00 ±6.23	64.33 ±25.97	0.038*
After 6m	64.83 ±9.15	76.33 ±29.42	0.046*
AST (u/ml)			
Baseline	83.86 ±11.76	87.63 ±9.41	0.188
After 3 m	102.86 ±12.93	131.73 ±16.14	0.001**
After 6m	117.33 ±10.75	143.66 ±13.80	0.001**
AFP (ng/ml)			
Baseline	8.73±2.76	7.63±3.23	0.165
After 3 m	55.71±11.45	22.02±4.02	0.001**
After 6m	84.13±14.87	54.30±11.10	0.001**

Table (5) represents that there were no statistically significant differences (P> 0.05) between the treatment group and control group in FIB4, MELD score, and child Pugh in the baseline. Whereas there was a statistically significant increase in FIB-4, and Meld score at 3 months and 6 months for the control group compared to the treatment group. Regarding the Child-Pugh classification, there was no statistically significant difference between the two groups at baseline and after 3 months of treatment. However, after 6 months of treatment, the percentage of cases with Child-Pugh class C was statistically significantly higher in the control group compared to the treatment group.

Table (5) Scores of; Index for Liver Fibrosis (FIB-4), model end-stage liver disease (MELD), and Child pugh in the two studied groups at baseline, three months, and six months after treatment.

Laboratory investigations	Treatment group (n=30)	control group (n=30)	*p-value*
	Mean ± SD	**Mean ± SD**	
FIB-4			
Baseline	4.61±1.00	4.26±1.16	0.215
After 3 m	4.65±0.88	5.19±1.17	0.05*
After 6m	5.20±1.16	5.85±1.24	0.05*
MELD Score			
Baseline	15.40±097	16.29±2.29	0.058
After 3 m	17.34±2.19	19.44±1.02	0.005*
After 6m	17.09±2.36	21.98±3.54	0.001**
Child pugh			
Baseline	0 (0%)	0 (0%)	1
A	28 (93.3%)	25 (83.3%)	0.424
B	2 (6.6%)	5 (16.6%)	0.115
C			
Child pugh 3mon.			
A	0 (0%)	0 (0%)	1
B	27 (90%)	26 (867%)	0.500
C	3 (10%)	4 (13.3%)	0.508
Child pugh 6 mon.			
A	0 (0%)	0 (0%)	1
B	25 (83.3%)	20 (76.7%)	0.025*
C	5 (16.6%)	10 (23.3%)	0.029*

Table (6) represent SVR after three months of treatment was achieved in (27)90% and we had 3 patients don't achieve SVR (not responded to treatment.),thus SVR is highly statistically significant for the treatment group compared with the control group.

Table (7) reveals that in the two study groups, all of the HCC cases had enlarged livers. Comparing the treatment group to the control group, there were statistically more focal lesions in the treatment group. (2.71 ± 0.49 and 1.45 ± 0.69 respectively) (p=0.003).

The mean focal lesion size was statistically larger in the treatment group compared to the control group. (13.65 ± 7.54 and 8.47 ± 6.72 respectively) (p < 0.001).

71.4 percent of patients in the treatment group had a disease in both lobes of their livers, whereas in the

control group, no patients had a disease in both lobes of their livers. (p=0.022).

The condition was discovered in 71.4 percent of the patients in the treatment group, but only 27.3 percent of the patients in the control group. A statistically significant difference existed between the two groups. (p = 0.042).

Similar rates of abdominal LN metastasis were observed in the treatment and control groups. 42 percent of those in the treatment group and 9.9 percent of those in the control group had the condition. (p = 0.067).

Table (6): comparison of sustained virologic response (SVR) between the treatment group and control group after three months of treatment.

variable	treatment group(N=30)	Control group(N=30)	P value
SVR	27(90%)	0(0%)	**0.001****

Table (7): Comparison between HCC patients in the treatment group and HCC patients in the control group according to tumor characteristics:

	HCC treatment group(N=7)	**HCC Control group(N=11)**	**P value**
Size of the liver			
Enlarged liver	7 (100%)	11 (100%)	1
How many focal lesions there are	2.71 ± 0.49	1.45 ± 0.69	0.003*
Size of the lesions	13.65 ± 7.54	8.47 ± 6.72	< 0.001*
site of lesion			
Both	5 (71.4%)	0 (0%)	0.022*
Left lobe	0 (0%)	3 (27.3%)	
Right lobe	2 (28.6%)	8 (72.7%)	
Portal vein thrombosis			0.042*
Absent	2 (28.6%)	8 (72.7%)	
Present	5 (71.4%)	3 (27.3%)	
Abdominal Lymph Node metastasis			
Absent	4 (57.1%)	10 (90.1%)	0.067
Present	3 (42.9%)	1 (9.9%)	

Discussion

The second most common cause of cancer-related deaths worldwide is HCC, the most frequent primary liver neoplasia. People who already have HCV liver cirrhosis are at a 3 percent annual risk due to chronic HCV infection [14]. Concerns were raised, however, based on the findings of two 2016 studies on the high risk of HCC occurrence and recurrence following DAA therapies [15, 16]. Because of the existence of contradictory data, the link between DAA therapy and HCC has been called into question, prompting further debate.[17].

To take the first step toward resolving this controversy, the current study was carried out to determine the incidence rate of HCC in patients with hepatitis C virus-induced cirrhosis who had undergone treatment with DAAs (sofosbuvir, daclatasvir, and ribavirin). In the current study, male participants made up ninety percent of those assigned to the treatment group and eighty percent of those assigned to the control group. This was in line with the findings of some studies, including those conducted by Doss et al. (2015), Maan et al. (2016), and Fernandez Carrillo et al. (2017), all of which discovered that men held the majority of leadership positions in the field.[18-20].

The baseline levels of white blood cells, hemoglobin, and platelets in the treatment group and control group are not statistically significant (P> 0.05). At the end of the first three months of the study, those who had received the treatment had significantly lower counts of platelets, hemoglobin, and white blood cells than those who had received the control. (P 0.05).

In a study of cirrhotic patients who did not have HCC and were being treated with DAAs, Conti et al. (2016) discovered that a lower platelet count (p = 0.02) was significantly associated with the development of HCC. [16]. In a study of two groups of patients with decompensated cirrhosis, Foster et al. (2015) discovered that viral clearance with DAAs was associated with improvement in liver function within 6 months compared to untreated patients. The study included patients who had reached the end stage of their disease.. [21].

However, ***Quaranta et al. (2021)*** analyze that while the majority of patients with liver cirrhosis who underwent DAAs saw an improvement in their liver function tests after the viral infection had been eradicated, some of them still had a risk of liver disease progression. For ALT and AST as well [22].

Also, **Osinusi A et al. (2013)** showed that liver enzymes were also quickly normalized during interferon-free therapy of hepatitis C indicating reduced hepatic inflammation [23].

According to Abdelaziz et al., DDA-treated patients had higher AFP levels and more infiltrative tumor patterns, indicating significant lymphadenopathy and malignant PVT (2019). Treatment increased AFP significantly compared to the control group. [24].

In this study, three patients did not respond to the treatment, but twenty-seven percent of the patients achieved a sustained virologic response. These findings are very similar to those discovered by Conti et al. (2016), whose study's first encouraging result was that 344 cirrhotic patients achieved SVR in 91% of cases. These findings are strikingly similar to those of Conti et al (2016).[16].

A nearly identical percentage was reported in the study by El Raziky et al. (2022) which examined the data of 102 HCV patients who had completed treatment with DAAs. The results showed an SVR12 of 82.9 percent (81.1 percent in treatment-naive subjects and 87 percent in treatment-experienced subjects). [25].

In a 2018 study from Egypt, El-Khayat et al. reported a sustained virological response 12 (SVR12) rate of 94 percent, compared to 92 percent in cirrhotic patients who had no prior exposure to the virus. 25 uninformed patients. In the current study, the incidence of HCC development was 23.3 percent (7/30) in a group of treatment and 36.6 percent in a group of control (11/30). HCC still develops even after achieving SVR, but this incidence did not show a

statistically significant difference compared to the control group. [26].

Similar to Metteke et al. (2018), who found that patients with liver cirrhosis receiving IFN-free DAA therapy have a similar HCC incidence compared to a historical control cohort recruited without treatment [27].

Moreover, ***Deterding, et al. (2015)*** found that HCC still develops in a cirrhotic patient who was cured of HCV by IFN-free anti-viral therapy [28].

According to ROCHE et al. (2018), taking DAAs does not increase the risk of getting de novo HCC after SVR. Patients with HCV cirrhosis who receive DAA treatment and achieve an SVR still run the risk of developing de novo HCC. As a result, the reason why our results differ from those of other studies is that we had a smaller sample size and, typically, a shorter follow-up period, which made it harder to conclude. A significant number of later studies with better methodology have confirmed that antiviral therapy generally reduces the incidence of HCC**.** [29].

Our result showed that the HCC developed in the cases who received treatment with DAAs and ribavirin showed more aggressive tumor behavior and characteristics as compared to the control HCC group (who refuse treatment).

Renzulli et al. found that DAA therapy accelerated the development of HCC and microvascular invasion in patients with HCV-related cirrhosis.[30].

Additionally, DAAs were found to be associated with increased aggressiveness and tumor growth upon recurrence.[31]. Furthermore, Romano et al. found that after DAA, tumors exhibited aggressive behavior, had a higher number of nodules, and had extrahepatic metastases, suggesting that tumor growth is more rapid than usual in these patients.. [32],

Innate immunity may recover by downregulating type II and type III IFNs, their receptors, and IFN-stimulated genes. A decrease in IFN activation may promote malignant cell growth because DAAs cannot inhibit angiogenesis and cell proliferation. Furthermore, after HCV eradication, the immune system changes, with fewer natural killer cells with cytotoxic activity in the liver, promoting the rapid growth of HCC foci. This is a noticeable shift that has occurred.[8, 33].

Conclusion

DAAs reduced HCC incidence and sustained virologic response by (sofosbuvir and daclatasvir) achieved by 90% of decompensated cirrhotic patients with partial improvement in liver function within six months compared to the untreated group However, a more aggressive pattern of tumor characteristics was observed in patients who underwent treatment.

Conflict of interest

The authors have not disclosed any conflicts of interest.

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