Tumor behavior of hepatocellular carcinoma after hepatitis C treatment by direct-acting antivirals: comparative analysis with non-direct-acting antivirals-treated patients


Introduction

Worldwide, hepatocellular carcinoma (HCC) is considered a drastic tumor with an estimated prevalence of 5.6%. It is considered the second most common cause of all cancer-related deaths [1]. There are several risk factors for HCC such as chronic hepatitis B, chronic aflatoxin exposure, alcoholism, and nonalcoholic steatohepatitis. However, chronic infection with hepatitis C virus (HCV) remains one of the most common risk factors for the development of HCC [2].

HCV is carcinogenic through several factors whether through direct viral oncogenic effects or mostly through chronic inflammation and fibrosis associated with chronic viral infection [3]. Forecasting models predicted that, without treatment, 14.4% of all patients with HCV would develop HCC [4]. This declares the importance of HCV treatment, as clearance of the virus [also known as sustained virologic response (SVR)] proved to be associated with dramatic reductions in all-cause mortalities, regression of HCV-related chronic liver disease, and an eventually decreased incidence of HCC [5].

Until 2011, the preferred treatment for chronic hepatitis C patients was the interferon (IFN)-based therapy with moderate SVR rates [6]. Furthermore, SVR rates varied with genotype and viral load and the regimen itself was poorly tolerated. Advances in understanding of the viral structure led to the development of the all-oral IFN-free regimens using two or more classes of direct acting antiviral drugs (DAAs) to achieve viral clearance and prevention of selection of resistant variants. DAAs showed potent activity with higher rates of SVR, even in difficult-to-treat patients [7]. Even more, this opened the gate to treat compensated and decompensated cirrhotic patients who were never eligible to be treated by IFN-based regimen. However, this led to a conflict that developed after the widespread use of DAAs regarding the fact of increased risk of HCC [8,9].

An alarming report was presented by Reig et al. [10], who demonstrated an unexpected high rate of de-novo or recurrent HCC after DAA treatment. This was paralleled by other papers that confirmed similar findings [11,12], and a conflict developed when a totally different opinion...
was proposed by the ANRS study [13] as well as many letters to the editors presenting variable studies [14–17] and did not find any significant risk. Otherwise, a minor focus was provided in this conflict on the tumor behavior in patients who had a history of DAA intake and reported a more aggressive behavior than usual [18]. So, the aim of this study was to assess the tumor behavior in patients who received DAA drugs and who subsequently developed HCC.

**Patients and methods**

**Study design**

This case–control study was conducted in a multidisciplinary HCC clinic, Kasr Al-Aini Hospital, Cairo University, Egypt. When our radiologist and hepatologists started to notice the aggressive behavior of HCC lesions developing in patients who received generic DAA drugs for HCV treatment, we started to recruit all patients who presented to our clinic during the period from October 2014 till December 2015 owing to HCV-related liver cirrhosis and HCC and took generic DAA and a comparative group of all non-DAA treated-HCC patients who presented during the same period and were age, sex, and cirrhotic status matched to the first group.

**Patients**

Group I included patients with HCV-related cirrhosis and HCC who were antecedently treated with generic DAA at the national treatment centers. According to the National Committee for Control of Viral Hepatitis guidelines, treated patients with HCV were proven to be chronically infected with HCV and confirmed by quantitative PCR results. They must be of accepted cirrhotic status so that their Child–Pugh score does not exceed Child B7 score and no evidence of hepatic decompensation. The patients previously treated for HCC waited for more than 6 months after confirmatory computed tomography or MRI evidence of complete response as rated using the modified Response Evaluation Criteria in Solid Tumors criteria [19].

A follow-up schedule was applied using triphasic computed tomography imaging 4 weeks after initial HCC treatment and then every 3 months during the first year. Patients returned to their routine surveillance program every 6 months if proved to achieve complete response. Thus, we discovered the new HCC lesions after DAA treatment during their routine follow-up.

All patients who presented at same period with HCC and who were age, sex and cirrhotic status matched were recruited as group II. Those patients had similarly HCV-related cirrhosis and HCC but did not receive HCV therapy. We excluded patients with combined HBV or HIV infections.

Lines of treatment for HCV, using DAA, ran through different phases. The first phase depended on using sofosbuvir-based therapy with either pegylated interferon (Peg–IFN)/ribavirin for 3 months or with ribavirin alone for 6 months. The first line was reserved for patients who were naive and had perfect laboratory test results (total bilirubin <1.2 mg/dl, albumin > 3.5 g/dl, international normalized ratio <1.2, and platelet count > 150 000/mm³).

The second era included combined sofosbuvir and simprevir, whereas the last era included sofosbuvir and daclatasvir with/without ribavirin.

**Methods**

All patients were diagnosed according to EASL guidelines, and AASLD updated practice guidelines for management of HCC and BCLC guidelines [20–22]. All patients signed informed consents for management of HCC and inclusion in the study and those who were HCV treated signed other informed consents before their therapy. The study was in compliance with ethics principles of good clinical practice and in respect to the 1975 Declaration of Helsinki. The study protocol as well as the suggested informed consent was approved by the Institutional Review Board of Endemic Medicine Department, Cairo University, Egypt, before the start of enrolment of the participants (number: 25/2014, date: 12 August 2014).

Baseline demographic, clinical and imaging criteria were studied. α-Fetoprotein levels and performance status are presented. Lines of management for both HCV and HCC are demonstrated as well as the response to management. Importantly, we performed a comparative study that clarifies the tumor behavior of HCC. This included the size, site and number of tumors and evidence of tumor aggressiveness such as presence of lymph node metastasis, vascular invasion and distant metastases.

At least two of the following three criteria were used to diagnose malignant portal vein thrombosis (PVT) [23]:

1. HCC size of more than 5 cm.
2. Distance from HCC to PVT of less than 2 cm.
3. Arterial enhancement of PVT.

**Statistical analysis**

Statistical analyses were performed using IBM SPSS advanced statistics, version 22 (SPSS Inc., Chicago, Illinois, USA). Numerical data were presented as mean ± SD and median (range), whereas categorical data were presented as number (percent). The Mann–Whitney U-test and the χ²-test are used when appropriate. Statistical significance is considered if P value is less than or equal 0.05.

**Results**

This study included 296 patients, divided into two groups. Group I included 89 patients with HCV-related cirrhosis and HCC who were antecedently treated with DAA, and all of them did not receive any previous antiviral therapy to DAA. Group II included 207 patients representing all patients who presented at same period with HCV-related HCC and who were age, sex and cirrhotic status matched with group I.

Most of the studied patients in group I were males (79.8%), with mean age of 57.9 years. The majority had compensated liver cirrhosis; 79.8% were Child A status whereas 20.2% only were Child B. A total of 45 (50.6%) patients had a previous history of ablated HCC before antiviral therapy, whereas the other 44 (49.4%) patients developed de novo HCC after therapy. The mean duration between ablation of the primary tumor and DAAs intake was 15.53 ± 7.2527 months in patients with previously
ablated lesions. Most of the studied patients had a good performance status in group I, 56 (62.9%) patients had a performance status score of 0 (P = 0.7; Table 1).

Multiple DAA regimens were used: 11 (12.4%) patients received sofosbuvir plus IFN and ribavirin, 20 (22.5%) patients received sofosbuvir plus simeprevir, 35 (39.3%) patients received sofosbuvir plus ribavirin, 13 (14.6%) patients received sofosbuvir and daclatasvir and only 10 (11.2%) patients received sofosbuvir plus daclatasvir and ribavirin (P = 0.4) according to the chronological change of the available treatment regimens in the national treatment centers in Egypt (Table 2).

SVR rate in the studied patients in group I was lower than usual. Overall, 60 patients showed complete viral clearance, showing SVR rate of 69%; 27 patients failed to treatment; and no data were available for two patients (P = 0.5).

Regarding the number of hepatic focal lesions, 67.4% of group I patients had a single lesion, 10.1% had two lesions and 22.5% had multiple lesions, whereas in group II, multiple lesions were a little bit more, observed in 23.6% of patients (P = 0.9). The mean size of the lesions was approximate among both the groups: 4.2 cm in group I versus 4.4 cm in group II (P = 0.5; Table 3). Group I showed a more infiltrative HCC pattern, whereas group II had more circumscribed and better delineated lesions.

AFP level was significantly higher in group I with a mean value of 40 ng/ml compared with a mean value of 19.6 ng/ml in group II (P = 0.02). Tumor aggression through the presence of significant lymphadenopathy and malignant PVT was shown in nine (10.1%) patients in group I compared with only 3.9% of group II patients, with a statistically significant difference among both groups (P = 0.03; Table 3). None of the studied patients developed other distant metastasis. We found no significant difference among the treatment regimens regarding focal lesion size, number, AFP, PVT or malignant lymphadenopathy (Table 4).

Tumor aggression had its reflection on treatment decision. Supportive treatment without ablation was more common in group I (30.4%) than group II (15.5%) (P = 0.03). Otherwise, trans-arterial chemoembolization was the commonest line of treatment followed by microwave ablation in both groups (Table 5). Incidence of complete responses (as assessed by modified Response Evaluation Criteria in Solid Tumors criteria) was 47.2 and 49.8%, for group I and group II, respectively.

**Discussion**

Chronic hepatitis C leads to liver cirrhosis in about 20–30% of patients who will carry a risk of HCC with an estimated annual incidence of 3.5% [24]. This incidence has reduced after treatment with Peg-IFN and ribavirin to less than 1.5% [25]. However, treatment with Peg-IFN and ribavirin had an intermediate SVR rate of about 50% [26].

The development of new DAA drugs with improved efficacy and safety profile raised expectations regarding a decline in HCC incidence as well as recurrence in those who had a prior history of HCC before antiviral treatment. However, unexpected data from new studies showed that DAAs might promote tumor occurrence in patients with cirrhosis, or recurrence in patients with presumed cure of HCC.

An Italian study done by Conti et al. [27] analyzed 344 consecutive non-HCC cirrhotic patients, who were treated with DAA. They were followed up for 24 weeks. A total of 59 patients had previously ablated HCC. They showed that incidence of de-novo HCC after DAA therapy was 3.16%, which is almost the same shown in the natural history without treatment. Surprisingly, recurrence rate in those who had a previous history of ablated or resected HCC was 28.81%, which is even more than the recurrence rate of untreated patients, which is estimated to be around 20% [27]. These findings matched with another Spanish study done by Reig et al. [10]. A total of 58 patients with a

**Table 1. Characteristics of the studied patients**

<table>
<thead>
<tr>
<th></th>
<th>HCC with DAA (group I) (N = 89)</th>
<th>HCC without DAA (group II) (N = 207)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.9 ± 8.6</td>
<td>58.5 ± 7.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Sex (male) [n (%)]</td>
<td>71 (79.8)</td>
<td>151 (73.3)</td>
<td>0.2</td>
</tr>
<tr>
<td>Child score [n (%)]</td>
<td>A 71 (79.8)</td>
<td>148 (71.5)</td>
<td>0.1</td>
</tr>
<tr>
<td>Performance status</td>
<td>B 18 (20.2)</td>
<td>59 (28.5)</td>
<td></td>
</tr>
<tr>
<td>HCC detection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before antiviral therapy</td>
<td>45 (50.6)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>After antiviral therapy</td>
<td>44 (49.4)</td>
<td></td>
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</table>

DAA, direct acting antiviral; HCC, hepatocellular carcinoma.

**Table 2. Antiviral regimens used**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>N (%)</th>
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</thead>
<tbody>
<tr>
<td>IFN/SOF/RBV</td>
<td>11 (12.36)</td>
</tr>
<tr>
<td>SOF/SIM</td>
<td>20 (22.47)</td>
</tr>
<tr>
<td>SOF/RBV</td>
<td>35 (39.32)</td>
</tr>
<tr>
<td>SOF/DACLA</td>
<td>13 (14.51)</td>
</tr>
<tr>
<td>SOF/DACLA/RBV</td>
<td>10 (11.23)</td>
</tr>
</tbody>
</table>

DACLA, daclatasvir; IFN, interferon; RBV, ribavirin; SIM, simeprevir; SOF, sofosbuvir.

**Table 3. Tumor characteristics in the studied patients**

<table>
<thead>
<tr>
<th></th>
<th>HCC with DAA (group I) (N = 89)</th>
<th>HCC without DAA (group II) (N = 207)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lesions [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>60 (67.4)</td>
<td>123 (59.4)</td>
<td>0.3</td>
</tr>
<tr>
<td>Two</td>
<td>9 (10.1)</td>
<td>31 (15)</td>
<td></td>
</tr>
<tr>
<td>Multiple</td>
<td>20 (22.5)</td>
<td>53 (25.5)</td>
<td></td>
</tr>
<tr>
<td>Site of lesions [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right lobe</td>
<td>62 (69.7)</td>
<td>145 (70)</td>
<td>0.2</td>
</tr>
<tr>
<td>Left lobe</td>
<td>17 (19.1)</td>
<td>26 (12.6)</td>
<td></td>
</tr>
<tr>
<td>Both lobes</td>
<td>10 (11.3)</td>
<td>3.8 (17.4)</td>
<td></td>
</tr>
<tr>
<td>Size of lesions [mean ± SD] (cm)</td>
<td>4.2 ± 2.5</td>
<td>4.4 ± 2.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Portal vein invasion [n (%)]</td>
<td>9 (10.1)</td>
<td>8 (3.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>Lymph node metastasis [n (%)]</td>
<td>9 (10.1)</td>
<td>8 (3.9)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

AFP, α-fetoprotein; DAA, direct acting antiviral; HCC, hepatocellular carcinoma. Bold values indicates statistically significant.
prior history of treated HCC received DAA therapy for HCV, and 16 (27.6%) patients developed radiologic tumor recurrence. The pattern of recurrence was intrahepatic growth (three patients), new intrahepatic lesion (one nodule in five patients, ≤ three nodules ≤ 3 cm in four cases and multifocal in one patient), and infiltrative ill-defined HCC and/or extrahepatic lesions in three patients [10].

Rising concerns in this issue led to more attention for this possible relation between DAA and HCC occurrence. However, they lacked a focus on the tumor characteristics that occur after DAA therapy, which is the main concern of our study. An observation of a more aggressive tumor behavior than usual was shown.

Romano et al. [18] demonstrated an aggressive behavior of tumors after DAA in the form of higher number of nodules and extrahepatic metastases, suggesting that the tumor growth in such patients is faster than usual. In our study, patterns of tumor occurrence or recurrence in patients who received DAA were in the form of higher α-fetoprotein levels, and higher incidence of metastasis in the form of vascular spread evidenced by malignant portal vein invasion or local spread through malignant lymphadenopathy. Advanced tumor stage had its effect on treatment decision, and 30.3% of patients who received DAA therapy were allocated for best supportive care.

Reig et al. [10] also showed that recurrence in patients who received DAA therapy was more aggressive compared with the initial tumor characteristics where four patients out of 16 (25%) had a deteriorated BCLC classification and they were not fit for any intervention. On the contrary, Conti et al. [27] showed no difference in recurrent tumor pattern, where of the 17 patients, five patients corresponded to BCLC stage 0, 11 patients to BCLC stage A, and only one patient to BCLC stage B.

Limited data are available till now regarding the mechanism by which DAA therapy might influence HCC occurrence and recurrence as well as the aggressive behavior by which tumor occurs. Theories suggest that rapid HCV clearance compared with a gradual clearance during the IFN era might lead to a sudden withdrawal of immune surveillance. In turn, this might favor the proliferation of isolated neoplastic cells that were dormant by the effect of the intrahepatic immune response stimulated by chronic HCV infection and lead to an increased expression of IFN-stimulated genes and activation of natural killer cells [28].

Conclusion

Despite the great advance in HCV treatment in the era of DAA drugs, HCC remains to be a great risk for cirrhotic patients. Moreover, patients with a history of HCC have to be closely monitored if they were advised to receive antiviral treatment, taking into consideration the ongoing reports of high recurrence rate of a more aggressive tumor.

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Conflicts of interest

There are no conflicts of interest.

References

HCV-related HCC behavior after DAAs Abdelaziz et al.


