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Long-term Follow-up of Chronic Hepatitis C Patients Treated by Direct- Acting Antivirals

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ABSTRACT

Background: Hepatitis C virus HCV infection is one of the main causes of chronic liver disease worldwide, Egypt has the highest national-level HCV prevalence in the world, and direct Acting Antivirals are widely used to treat HCV infection in Egypt. **Aim:** To perform a long-term follow-up of patients with chronic hepatitis C who received IFN free regimen of direct-acting antivirals (DAAs). The follow-up aimed to study the incidence of hepatocellular carcinoma, viral recurrence in this cohort of patients and improvement of liver biochemicals or liver decompensation.

Methodology: We recruited 1041 Egyptian HCV patients, who finished treatment more than 1 year before recruitment and then we assessed the improvement of the biochemical profile and the liver condition, incidence of occurrence or recurrence hepatocellular carcinoma after treatment.

Results: The follow-up values of ALT, AST, INR, Bilirubin, Platelets and fibrosis-4 (Fib-4) showed a statistically significant improvement more than 1 year after treatment, while the improvement in albumin and AFP was noted without statistical significancy. By comparing the different groups of the study, patients with advanced liver disease showed a greater improvement in those parameters. However, they carried an increased risk of developing hepatocellular carcinoma.

Conclusion: Achieving SVR using DAAs can improve liver functions tests, AFP, platelet count and FIB-4 value and persons with severe liver disease should be advised to undergo HCC surveillance after achieving sustained virological response (SVR).

Keywords: HCV, DAAs, Long-term, Hepatitis C virus.

INTRODUCTION

Infection with the hepatitis C virus (HCV) is a major contributor to chronic liver disease worldwide. Hepatocellular carcinoma (HCC) and minor histologic alterations to cirrhosis are possible long-term effects of HCV infection. The proportion of persons aged 15 to 59 who tested positive for HCV RNA represent 7% of the Egyptian population, making Egypt the country with the greatest national incidence of HCV in the whole globe [(1)].

An important health problem and financial burden is the morbidity of HCV in untreated individuals with cirrhosis of the liver, liver cell failure, and hepatocellular cancer [(2)].

Recent developments in pharmacological research have led to the emergence of several direct antiviral medicines (DAAs) with high levels of SVR and dramatically better side-effect patterns [(3)].

Beginning in November 2015, a new SOF and daclatasvir (DAC) combination, with or without RBV, was added to the treatment regimens. This new formulation became the primary form of therapy in the the national program, due to a cost saving of more than 80% of the reduced cost of the brand medications that were being used in the program [(4,5,6)].

There is still disagreement on how to handle people with decompensated cirrhosis.

Cheung and colleagues [(7)] discovered advantages of utilizing DAAs to treat individuals with Child-Pugh C cirrhosis. They asserted that viral eradication may enhance liver function. The risk of a late relapse is extremely rare, according to **Simmons and colleagues [(8)]**, systematic review and meta-analysis. Additionally, DAAs have demonstrated real and substantial impacts on all health risks associated with HCV, with proof coming from the regression of liver fibrosis, which is thought to be the endpoint of chronic liver disease and its aftereffects [(9)]. It was anticipated that as time went on, more significant fibrosis-related changes would manifest. On the other hand, DAAs have proven to be quite accurate in the early diagnosis of dynamic fibrosis regression changes [(10)].

Cardoso and colleagues [(11)] reported a significant post-DAA incidence of HCC among SVR patients in a letter to the editor in 2016 with a proportion of 7.41%. Additional studies showed that the reappearance of HCC following DAA therapy was more aggressive than it was before to the treatment [(12)].

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PATIENTS AND METHODS

Patients

Table (1): Inclusion and exclusion criteria

Inclusion Criteria	Exclusion criteria
Patients with chronic hepatitis C who received a full course (12 weeks or 24 weeks) of any IFN free regimen DAAs, and finished treatment more than 1 year before being enrolled in the study.	Patients with chronic hepatitis C who received IFN based therapy
Adults 18-65 years old of both sexes	Other causes of chronic liver diseases e.g. Co-infection with HBV, hemochromatosis, alpha 1-antitrypsin deficiency, Wilson's disease, autoimmune disease, alcoholic liver disease

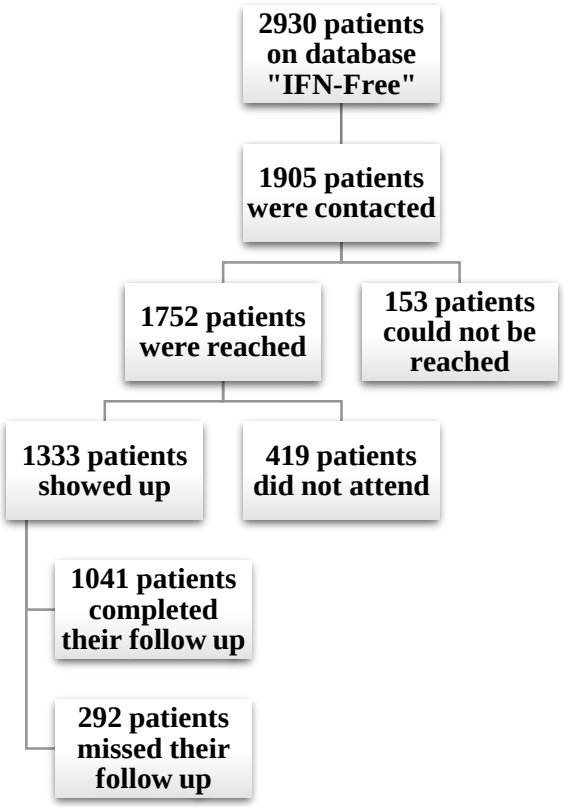

Figure (1): Flowchart of our cohort.

Table (2): Classification of our patients

	Group A Non-cirrhotic	Group B Cirrhotic compensated	Group C Cirrhotic Decompensated
Number of Patients	635 (60.9%)	343 (32.9%)	63 (6.09%)

Table (3): Baseline workup for our subjects

No	Baseline workup for our subjects
1	Full history taking including manifestations of hepatic decompensation and clinical assessment.
2	laboratory investigations:
	Complete blood count
	AST, ALT
	Total bilirubin
	Serum Albumin and INR
	Alpha-fetoprotein
	Quantitative HCV RNA polymerase chain reaction
3	Child-Pugh score
4	Fib-4 calculation
5	Abdominal and pelvic ultrasonography was done to assess liver condition, portal hypertension signs and presence of any hepatic focal lesions. - Triphasic CT scan was performed for patients with detected focal lesions or elevated Alpha-fetoprotein > 100 ng/ml to confirm its nature.

Ethical Approval:

Ethical approval was obtained from the Research Ethical Committee-Helwan Faculty of Medicine (9-2019). The study was under the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Statistical analysis

The statistical analysis was carried out in three phases, including the following steps:

1. Making a comparison between the data collected before and at least 1 year after therapy for the entire cohort.
2. Dividing the cohort into three groups:
    a. Non-cirrhotic subjects
    b. Cirrhotic compensated subjects
    c. Cirrhotic decompensated subjects
3. Comparing the different groups' response in order to identify which group will gain the most from therapy.
4. The comparison between two groups with qualitative data were done by using Chi-square test.
5. The comparison between two independent groups with quantitative data and parametric distribution was done by using Independent t-test.
6. The comparison between more than two groups with parametric distribution were done by using One Way Analysis of Variance (ANOVA).

RESULTS

This study was conducted to prospectively evaluate the long term follow-up of patients with chronic hepatitis C who received IFN free regimen of DAAs, including the incidence of Hepatocellular carcinoma, viral recurrence, and liver decompensation in this cohort of patients in addition to changes in liver biochemical profile. A total of 1041 patients who had been treated with an IFN-free regimen of DAAs at the New Cairo Viral Hepatitis Treatment Center and had completed their follow-up for at least one year prior enrolling in the study and their data has been analyzed.

Follow-up intervals:

The median follow-up interval for our patients from the end of treatment till the presence of follow-up visit was 28.42 months (ranged from 12.1-57.5 months).

Basic variables (measured once):

The backbone of our study was classifying the patients into 3 main categories according to their ultrasound results. There were 635 non-cirrhotic patients (60.9%) (Group A), 343 patients with compensated cirrhosis (32.9%) (Group B) and 63 patients with decompensated cirrhosis (6.09%) (Group C) in the total number of subjects recruited.

Subjects who received SOF/DAC regimen with or without ribavirin represented the majority of our subjects with a percentage of (86.7%) reflecting a total number of 903 subjects. The rest of subjects received different regimens including SOF/LED with or without ribavirin, SOF/SIM, OMB/PAR/RIT, SOF/SIM/DAC or SOF/OMB/PAR/RIT.

PCR: The pretreatment PCR value was 1504896.8 ± 2306147.34 before all of our subjects achieve SVR 12 weeks after end of treatment. Except for four patients, all subjects maintained their PCR below the detection limit in our long-term follow up for at least 1 year after treatment, which is significant for the efficacy of the

treatment regardless of the prescribed regimen, with a percentage of 99.61%.

Table (4): Baseline variables for different groups

Variable	Groups			P-Value
	A	B	C	
ALT	52.53±6.48	58.85±6.25	62.06±5.54	0.057
AST	45.71±3.31	58.08±4.49	79.86±5.37	**<0.001**
Albumin	4.12±0.43	4.11±1.12	3.21±0.44	**<0.001**
Bilirubin	0.74±0.12	0.81±0.19	1.63±0.17	**<0.001**
PLT	224.72±6.55	179.7±7.74	108.68±8.92	**<0.001**
Fib-4	1.59±0.08	3.1±0.78	6.73±1.06	**<0.001**

Table (5): Comparison between baseline and follow-up.

Variable		Mean ± SD (%) baseline	Mean ± SD (%) 1 y after FU	P
Gender	Male	534 (51.3%)		
Age		50.9±12.91		
Duration of TTT	3 months	1016 (97.6%)		
	6 months	25 (2.4%)		
Previous TTT	Yes	58 (5.57%)		
	No	983 (94.43%)		
Maintained SVR	No		2 (0.2%)	
	Yes		1039 (99.8%)	
ALT		55.18±5.91	21.23±4.07	**<0.001**
AST		51.84±7.79	24.23±5.02	**<0.001**
Serum Albumin		4.06±1.28	4.08±0.45	0.436
INR		1.13±0.36	1.09±0.2	**<0.001**
Bilirubin		0.82±0.13	0.69±0.14	**<0.001**
Hemoglobin		13.47±2.74	13.38±2.02	0.494
TLC		6.37±1.29	6.36±1.23	0.741
Platelets		202.91±7.85	204.46±7.42	**0.019**
AFP	Median e IQR	5 with range of 7	3.15 with range of 3.13	0.055.
Fib-4		2.39 ± 0.81	1.85 ± 0.68	**<0.001**

Follow-up Results:

The follow up values of ALT, AST, INR, Bilirubin, Platelets and Fib-4 showed a statistically significant improvement more than 1 year after treatment, while the improvement in albumin and AFP was noted without statistical significance as shown in table (5).

Comparing the different groups of the study, patients with advanced liver disease showed a greater improvement in those parameters especially (AST, with a p-value of <0.001 for albumin, platelets, and FIB-4, while the p-value for improvement of AFP and AST was 0.001 as shown in tables (6&7). However, those patients with advanced liver disease carried an increased risk of developing hepatocellular carcinoma.

Table (6): Follow up variables for different groups

Variable	Groups			P-Value
	A	B	C	
ALT	19.13±4.08	23.4±1.35	29±2.77	**<0.001**
AST	21.12±3.88	26.08±4.22	41.876.85	**<0.001**
Albumin	4.19±0.39	4±0.41	3.47±0.54	**<0.001**
Bilirubin	0.6±0.16	0.72±0.13	1.29±0.17	**<0.001**
PLT	223.32±6.68	188.59±7.15	124.38±6.51	**<0.001**
Fib-4	1.39±0.79	2.14±0.85	4.44±0.66	**<0.001**

Table (7): Comparison of changes between different groups

Variable	Groups			P-Value
	A	B	C	
ALT	33.41±5.55	35.45±5.33	33.06±3.7	0.783
AST	24.6±3.37	32±3.98	37.98±6.3	**0.001**
Albumin	0.73±0.17	0.94±0.1	-0.1±0.01	**<0.001**
Bilirubin	0.21±0.09	0.16±0.09	0.34±0.09	**0.01**
PLT	19.66±4.61	-8.33±1.97	-15.7±3.09	**<0.001**
alpha-otein (AFP)	2.56±0.237	5.25±0.95	13.08 ±1.1	**0.001**
Fib-4	1.59±0.08	3.1±0.78	6.73±1.06	**<0.001**

Hepatocellular Carcinoma:

In our study, 16 patients developed HCC during follow-up following therapy among the 1041 people who participated in our study, representing 1.5% incidence rate in a median follow up of 28.42 months as shown in table (8). As illustrated in table (4) out of 635 patients without cirrhosis, there were 2 patients who developed HCC within two years or more of follow-up with a percentage of 0.3%. In patients with cirrhosis, a total number of 14 patients (3.4%) were found to have HCC during the long-term follow up in our cohort. Only 2.9% of patients with compensated cirrhosis developed HCC (10 patients).

The highest reported risk was associated with decompensated cirrhosis with percentage of 6.3% (4 patients). By comparing our 3 groups, chi-square test revealed that the disease stage is highly significantly associated with the increased risk of developing de novo HCC in patients who achieved SVR following DAAs with a p value <0.001.

Table (8): Comparison between incidences of HCC in different groups

			HCC		Total
			No HCC	HCC	
group	Non Cirrhotic	Count	633	2	635
		% within group	99.7%	0.3%	100.0%
	Compensated Cirrhosis	Count	333	10	343
		% within group	97.1%	2.9%	100.0%
	Decompensated Cirrhosis	Count	59	4	63
		% within group	93.7%	6.3%	100.0%
	Total cirrhotic	Count	392	14	406
		% within group	96.6%	3.4%	100.0%
Total		Count	1025	16	1041
		% within group	98.5%	1.5%	100.0%

DISCUSSION

According to current estimates, the probability of developing cirrhosis within 20 years is between 10 and 20%, and in certain situations, it can reach as high as 50% [13]. Egypt implemented a comprehensive HCV screening campaign that reached more than 50 million citizens and resulted in the treatment of more than 4 million people with the virus. It is on the verge of becoming the first country in the world to completely eradicate HCV within its borders [14].

Recently published studies investigated the long-term follow-up of real-life cohorts of HCV patients who achieved SVR using IFN-free DAAs. Nearly all of these studies reported very low HCV recurrence rates after SVR. More than 99.5% of the included patients in different studies preserved their SVR status for more than 1 year after treatment. One of the recently published reports in which the median follow-up period was similar to our study (123 weeks) discussed the SVR status durability, and showed that only 3 out of 842 patients lost their SVR status with a percentage of 0.4%, which were nearly the same in our study in which only 2 patients out of the total cohort (0.2%) have lost their SVR status [15]. Another study that showed interesting results, and found no late relapses among patients during follow up [16]. The largest cohort was performed by **Sarrazin and colleagues [17]** and revealed late relapse in only 0.2% in 3004 patients.

Chronic HCV patients with severe fibrosis who achieved SVR with DAAs were found to have ongoing liver inflammation, according to a Chinese study. Steatosis and poor liver function have been linked to it. However, at the end of treatment, only 45 patients out of 461 had elevated liver enzymes [18]. In our research, achieving SVR after treatment with DAAs improved liver enzymes and this improvement sustained throughout the period of the follow-up. Another study found similar results in Portuguese patients with advanced liver disease [19]. However, our findings disagree with those of another Egyptian study where, greater improvement in ALT levels was shown to be associated with the absence of advanced fibrosis/cirrhosis [20].

Serum albumin levels were observed to be slightly elevated in the majority of cases following therapy and throughout follow-up, but this was statistically insignificant. When this elevation was compared between the three groups, it was discovered that patients with decompensated liver cirrhosis following HCV who were treated with DAAs had statistically significant improvement when compared to patients who did not have cirrhosis or who had compensated cirrhosis after more than one year of treatment completion.

Talking about AFP levels, although the improvement in AFP levels was notable for the whole cohort, statistically it was not highly significant enough. However, by comparing the different groups of our cohort, it was shown that patients with advanced liver disease had the most statistically significant improvement in AFP. This goes in alignment with the findings of an Egyptian cohort that recruited 456 patients and assessed the effect of treatment with DAAs on AFP levels and concluded that for individuals with HCV, DAA-based regimens are effective antiviral medication that improves serum AFP levels [21].

HCV eradication resulted in a statistically significant elevation in platelet count. This elevation was more significant in both patients with compensated and decompensated cirrhosis than those who did not have cirrhosis. This can be explained by the fact that most of patients without cirrhosis had initial normal count of platelet before the start of treatment. A study recruited 2186 patients and detected a significant increase in the platelets count that was detected at the end of treatment in comparison with the pretreatment levels after achieving SVR [22]. Another study with a longer follow-up period, in which changes in platelet count and its relation to SVR were analyzed. The platelet count showed statistically significant improvement from baseline but remained unchanged thereafter to SVR24 [23].

The FIB4 score is one of the serum-based fibrosis markers that we evaluated in our cohort of patients. The mean value for FIB4 in the whole cohort decreased from 2.39 pre-treatment to 1.85 in our long-term follow-up. In patients with advanced liver disease, the FIB4 mean value drop was more significant than patients with no cirrhosis or patients with compensated cirrhosis, in which the improvement mean value reached up to 2.29 in those patients. Another study agrees with these results by reporting improvement of FIB4 levels 24 weeks after treatment [24]. Although numerous studies found a statistically significant drop in the mean value when comparing baseline and post-SVR FIB-4 scores [25,26]. An Egyptian study found that the percentage change in liver stiffness is unrelated to that of liver enzymes or Fib4 [27].

In the present study, only 16 patients developed HCC during our follow up representing 1.5% incidence rate. However, when comparing the different groups, the incidence of de novo HCC post-treatment with DAAs in non-cirrhotic patients was 0.3%, while for patients with

cirrhosis the incidence rate was 3.4%, which was statistically significant that the disease stage can increase the risk of developing HCC after treatment. According to one of the largest cohort studies of HCC risk and incidence in patients treated with DAAs, people with cirrhosis had a 1.82 % yearly incidence of HCC, compared to 0.34 % in patients without cirrhosis. Using a systematic review and meta-analysis, **Waziry *et al.*** [(28)] also observed no increased risk of developing HCC in individuals with cirrhosis who had DAA treatment. The risk of HCC was assessed in 285 individuals with cirrhosis who had no prior history of HCC and were treated with DAA. A total of nine patients were found to have HCC during the 24-week post-treatment follow-up (3.16%) [(29)]. As per **Kozbial and colleagues** [(30)], the incidence rate of HCC of patients with cirrhosis who received DAA within 2 years of follow-up was 6.6% compared to 3.4% in our cohort in a median follow-up period of 28.4 months. Cardoso and colleagues noted that the HCC incidence (7.4% in the first year) among cirrhotic patients obtaining SVR was greater than previously reported for IFN-containing regimens (1.2–1.4%). There were no significant variations in baseline factors between the affected patients that could be related with an increased risk of HCC. However, they agree with **Reig and her colleagues** [(31)] that although a direct carcinogenic effect of antivirals is highly improbable, given the coincidence with viral clearance, the underlying mechanisms are likely to be comparable.

CONCLUSION

Based on the results of our research we can conclude that DAAs are highly effective and safe in the treatment of HCV infection especially in patients without cirrhosis and patients with compensated cirrhosis. According to our results, patients who obtain SVR can be evaluated for HCV recurrence or reinfection if they are at high risk of HCV infection or have unexplained hepatic impairment, otherwise HCV recurrence is extremely rare. Achieving SVR using DAAs can improve liver functions tests, AFP, platelet count and FIB-4 value, and these improvements were also obvious in patients with more advanced liver disease. Due to the increased risk of developing HCC in patients with decompensated cirrhosis, persons with severe liver disease should be advised to undergo HCC surveillance after achieving SVR in accordance with guidelines.

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