RESEARCH ARTICLE

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Detection of Occult Hepatitis C Virus Infection in Egyptian Patients Who Achieved a Sustained Virologic Response to Direct-Acting Antiviral Agents

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Abstract

Background: Occult hepatitis C virus (HCV) infection (OCI) is diagnosed based on the detection of HCV-RNA in non-serum reservoirs, such as peripheral blood mononuclear cells (PBMCs) and/or hepatocytes with undetectable HCV-RNA in the serum. The current study was designed to shed more light on the presence of occult HCV in a population of cases who achieved an SVR after receiving treatments for HCV-infection and its significance. **Methods:** This cross-sectional study evaluated 111 chronic HCV patients treated at Theodor Bilharz Research Institute, Egypt and achieved a sustained virological response (SVR) 12 -24 weeks after treatment with Direct acting antiviral drugs (DAAs). The treatment lasted 12 or 24 weeks using generic medications including Sofosbuvir (SOF) 400 mg/day and Daclatasvir (DCV) 60 mg/day ± weight-based Ribavirin (RBV) 600-1000 mg/day. After achieving the SVR 12 -24 weeks, all patients were subjected to clinical examination and full laboratory investigations. All the candidates were assessed for fibrosis pre/post-treatment by transient elastography (Fibroscan©). Eighty-seven patients (78.4%) received dual therapy (SOF/DCV) and 24 patients (21.6%) received triple therapy (SOF/DCV/RBV). One hundred and seven patients received the regimen for 12 weeks (96.4%) and only four patients received the regimen for 24 weeks (3.6%). All patients were examined in terms of HCV RNA in plasma and PBMCs. **Results:** Nine patients (8.1%) were positive for PBMCs HCV RNA. The presence of Occult HCV infection (OCI) was significantly correlated with age, level of AFP, and the degree of liver stiffness. **Conclusion:** The OCI was present in 8.1% of the patients who achieved an SVR 12 – 24 weeks. These patients were mostly aged and with elevated AFP and advanced fibrosis. Monitoring and follow-up of those patients may help to assess the outcomes.

Keywords: Hepatitis C virus (HCV)- Occult Hepatitis C Virus Infection (OCI)-Direct-Acting Antiviral Agents (DAAs)

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Introduction

In 2004, Pham et al. reported occult HCV infections (OCIs) in anti-HCV IgG antibody-positive naive patients who had spontaneous recovery from HCV-infections and in experienced cases with a sustained virologic response (SVR) after treatment using interferon (Pham et al., 2004).

During the same year, Castillo et al. reported HCV-RNA existence in anti-HCV IgG antibody- negative naive patients with active hepatocellular disease (Castillo et al., 2004).

To give a further refined definition, OCIs include a) Secondary naive OCIs (SNOCIs): Patients with spontaneous negative serum real time PCR (SRT-PCR) with positive intracellular RNA manifesting as secondary naive OCIs. b) Secondary experienced OCIs (SEOCIs): Patients with negative SRT-PCR after receiving treatment with positive intracellular RNA. And c) Cryptogenic occult HCV-infection (COCIs), which is diagnosed in IgG-seronegative population through detecting the intracellular RNA strands. The prevalence is around 3.5% in asymptomatic populations (Abd Alla et al., 2017).

The HCV might have an active replication inside PBMCs, which is identified by detection of either the viral NS3-protein or antisense-strand (Pawełczyk et al., 2013). In 1999, El-Awady et al. predicted that checking viral replication in PBMCs can enhance the sensitivity of diagnosing and monitoring post HCV hepatitis (El-Awady et al., 1999).

The immune system is not only a part of OCI patients' reaction to the infection but it also serves as a host to the viral RNA. Thereby it facilitates its replication, as indicated by antisense HCV-RNA strands found in the

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PBMCs in these patients (de Marco et al., 2012). Patients with cryptogenic liver diseases can potentially develop cirrhosis and hepatocellular carcinoma. Investigating the prevalence of HCV and developing more accurate techniques to diagnose OCIs in these patients is critically important (Keyvani et al., 2013).

Some researchers have expressed doubts about using SRT-PCR in post-treatment follow-up (Attar et al., 2015). They have concluded that HCV treatment in individuals achieving a state of undetectable viremia after an eight to twelve-week course of direct-acting antiviral (DAA) therapy may not be totally valid. The same researchers recommended careful longitudinal follow-up utilizing highly sensitive assays and unique approaches for viral detection. Because PBMCs present a convenient extrahepatic home for HCV adoption, translation, transcription, assembly, and finally release into the serum and/or other adjacent cells, intra-PBMCs PCR can be used to further refine the definitions of spontaneous HCV clearance.

In addition, the post-treatment RNA genomic seroconversion is attributed to either treatment failure or new infection and it is known as HCV-relapse (Hanno et al., 2014), which has not been described in naive subjects with spontaneous viral clearance because no temporal follow-up is recommended. Eradication of intra-PBMC HCV-infection is recommended for naive cirrhotic and experienced post-treatment patients given its association with cirrhotic changes and the probability of relapse in more than 18% of the cases (Abd Alla et al., 2018).

Considering the high cure rate of DAA therapy, physicians are looking for effective treatments for COCIs and SNOCIs. Patients with OCIs should be offered the option of getting rid of the intracellular HCV genomic materials to prevent the subsequent hepatic fibrosis (Keyvani et al., 2013). For this reason, the current study was designed to shed more light on the OCI problem in a population of cases from one of the most endemic areas of HCV-infection in the world to promote attention to this unmet challenge and satisfy clinical practice demands.

Materials and Methods

This cross-sectional study was applied on 111 chronic HCV patients treated at Theodor Bilharz Research Institute between 18 and 70 years' old who achieved an SVR 12 -24 weeks after receiving DAAs treatment. The treatment was designed for 12 or 24 weeks using generic medications including Sofosbuvir SOF 400 mg/day and Daclatasvir DCV 60 mg/day ± Ribavirin RBV. The dose of RBV was 600 mg /day with the trial for escalating the dose to 1000-1200 mg /day. The treatment was given as a part of the national hepatitis C treatment program in Egypt. After achieving the SVR 12-24 weeks, all patients were reexamined through full laboratory tests. According to the Helsinki Declaration 1975 (amendment 2008), the experiments were authorized by the Theodor Bilharz Research Institute 's Ethical Review Board (FWA00010609). Each subject was given an informed consent form before collecting blood samples.

Patient with Hepatocellular carcinoma (HCC) and patients who refused to participate in this study were excluded.

The following were conducted for all patients

1. Full medical history: With an emphasis on the history of chronic liver disease, history of viral hepatitis, exposure to risk factors (such as tarter emetic injections, blood transfusion, and operations), and receiving treatment for viral hepatitis and its type, upper and lower gastrointestinal bleeding and symptoms of liver cell failure such as jaundice, lower limb edema, abdominal enlargement, and bleeding tendency, and so on.

2. Clinical examination: With an emphasis on liver and splenic size, presence or absence of ascites, and signs of liver cell failure as jaundice, palmar erythema, lower limb edema, encephalopathy and so on.

3. Laboratory investigations (around 15cc blood sample was taken from each patient) to carry out liver function tests, Aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum bilirubin (conjugated and unconjugated), and serum albumin, renal function tests (serum creatinine), and complete blood count and international normalized ratio (INR) through standard lab tests.

HCV real time RNA Quantification

All patients were subject twice to quantitative reverse transcription PCR (qRTPCR) in plasma before and after the treatment (EOT) and 12 weeks after EOT. All the patients also had nested RT-PCR in PBMCs, which was performed once and 12 weeks after EOT.

Extraction of total cellular RNA from PBMC

Briefly, peripheral blood samples were diluted with five volumes of a freshly prepared RBC lysis buffer (38.8 mmol/L NH4Cl, 2.5 mmol/L K2HCO3, 1 mmol/L EDTA, pH 8.0), incubated at room temperature for 10 min. The nucleated cells were precipitated before lysis in 4 mol/L guanidinium isothiocyanate containing 25 mmol/L sodium citrate, 0.5% sarcosyl, and 0.1 mol/L mercaptoethanol. Cellular RNA was extracted using the single-step method described originally by (Chomczynski and Sacchi,1987; Attar et al., 2015).

Amplification of HCV RNA by nested RT-PCR in PBMC

Qualitative detection of HCV RNA by reverse transcription- polymerase chain reaction was performed according to Lohr et al. (1995) with a few modifications. Retro-transcription was performed in 25 µL reaction mixture containing 20U of AMV reverse transcriptase (Clontech, Mountain View CA, USA) with 400mg of total PBMCs RNA as a template, 40U of RNAsin (Clontech, Mountain View CA, USA), a final concentration of 0.2 mmol/L from each dNTP (Promega, Madison, WI, USA), and 50pmoL of the reverse primer 1CH. The reaction was incubated at 42°C for 60min and denatured at 98°C for 10min. Amplification of the highly conserved 5'-UTR sequences was performed using two rounds of PCR with two pairs of nested primers. The first round amplification was performed in 50µL reaction mixture, containing 50pmol of each 2CH forward primer and P2 reverse

primer, 0.2mmol/L of each dNTP, 10µL from RT reaction mixture as template, and 2U of Taq DNA polymerase (Promega, USA) in a 1× buffer supplied with the enzyme. The thermal cycling protocol included 1min at 94°C, 1min at 55°C and 1min at 72°C for 30 cycles. The second-round amplification was done similar to the first round, except for using the nested reverse primer D2 and forward primer F2 at 50pmol each. A fragment of 179bp was identified in the positive samples (Hajarizadeh et al., 2013).

Primer sequences were as follows

1CH	5'-ggtgcacggtctacgagacctc-3'
2CH	5'-aactactgtcttcacgcagaa-3'
P2	5'-tgctcatggtgcacggtcta-3'
D2	5'-actcggctagcagtctcgcg-3'
F2	5'-gtgcagcctccaggaccc-3'

Quantification of HCV RNA in sera using Real- time PCR amplification

The concentration of HCV RNA in patients' sera was quantified using real-time PCR. Briefly, viral RNA was extracted using the QIAamp Viral RNA Extraction Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. Purified RNAs were quantified using Abbott Real Time HCV Amplification reagent Kit (Abbott Molecular Inc., Abbott Park, IL, USA) on ABI 7500 Analyzer following the manufacturer's protocol.

LiPA 2.0.

The purified HCV-RNA was amplified using the Versant HCV amplification 2.0 kit (manufactured by Innogenetics, Ghent, Belgium, for Siemens, Tarrytown, NY, USA). The 240-bp 5′ UTR and 270-bp core fragments were amplified. Subsequently, amplicon denaturation, hybridization, washing, and color development of the genotyping strips were carried out on an Auto-LiPA system (Innogenetics) with a Versant HCV genotype 2.0 kit (Siemens). The results were interpreted according to the LiPA 2.0 interpretation chart, where the line patterns and the corresponding genotyping results are listed.

Abdominal Ultrasonography
Equipment: Hitachi, EUB-5500

Measurements were performed after overnight fasting for the patients in supine position with an emphasis on the liver size, criteria suggestive of chronic liver disease and cirrhosis {increased liver echogenicity, irregular liver margins, attenuation of the intrahepatic portal and hepatic veins, relative enlargement of caudate lobe and atrophy of right lobe (ratio of caudate/ right lobe in cirrhosis >0.65)}, portal vein diameter and presence or absence of ascites, and hepatic focal lesions and periportal thickening.

All patients were classified according to Child-Pugh's criteria (Pugh et al., 1973), as listed in Table 1.

Transient elastography (Fibroscan©)

Echosens Fibro Scan 502 was performed on patients in supine position with their right arm in maximal abduction. All measurements were performed on the right lobe of the liver through the coastal space. The tip of the probe transducer was covered with coupling gel and placed on the skin between the ribs at the right liver lobe level. The operator, assisted by ultrasound time motion and A-mode images provided by the system, located the apportion of the liver that was at least 6cm thick and free of any large vascular structures. The measurement depth ranged from 25mm to 45mm and 10 valid measurements were obtained for each patient. The results were expressed in units of kilopascal (kPa). All the non-viremic subjects underwent the examination with a normal probe and XL probe was used for BMI>35. The liver stiffness corresponds to the median value of the validated measurements. The inter-quartile range (IQR) was used as the interval around the median that contained 50% of the valid measurements.

To be considered interpretable and valid, the examination must have included at least 10 measurements with a SR of at least 66% and the IQR must not exceed 33% of the results of the examination.

According to the manufacturer's guidance the patients were classified as:

F0: when reading is from 0 to 2.9 kPa
F1: when reading is from 3 to 5.9 kPa
F2: when reading is from 6 to 8.9 kPa
F3: when reading is from 9 to 16.9 kPa
F4: when reading is from 17 to 75 kPa

The patients were further classified as early fibrosis (F0-F2) and advanced fibrosis (F3-F4).

Upper GI endoscopy was done for all patients: using Pentax EG 2940 scope to evaluate the presence of esophageal varices and its grade.

Statistical Analysis

The results are expressed as mean ± standard deviation or number (%). A comparison between categorical data [number (%)] was performed using Chi square test or Fisher exact test whenever it was appropriate. The comparison between numerical variables in the two groups was performed using Mann Whitney test. Statistical Package for Social Sciences (SPSS) (version 19 windows) was used for data analysis (P value ≤ 0.05).

Results

In total, 111 patients with age an range from 18 to 70 years took part in the study. Fifty-one patients were men and 60 of them were women. All patients were treated as naive patients and achieved SVR with the following regimens: sofosbuvir / Daclatasvir ± ribavirin (SOF/

Table 1. Child-Pugh's Score for Classification of Patients with Liver Cirrhosis:

Parameters	1	2	3
Bilirubin (mg/dl)	<2	2_3	>3
Albumin(g/dl)	>3.5	2.2-3.5	<2.8
Prothrombin time in seconds	1_3	4_6	>6
Ascites	None	Slight	Moderate
History of encephalopathy	None	1_2	2_4

Table 2. Number of Patients with Occult HCV Infection

	Number	Percent (%)
Total number of patients	111/111	100
Total negative patients for pos. or minus RNA strand in PMNs	102/111	91.90
Total positive patients for pos. or minus RNA strand in PMNs	9/111	8.10
Positive for both pos. & minus RNA strand in PMNs	2/111	1.80
Positive for pos. RNA strand only	2/111	1.80
Positive for minus RNA strand only	5/111	4.50

DCV±RBV) for 12 weeks.

As demonstrated in Table 2, the number of OCI in the studied patients was nine patients (8.1%). Two patients (1.8%) were positive for both positive and minus strand in PBMCs; two patients (1.8%) were positive only for the positive RNA strand in PBMCs; and five patients (4.5%) were positive for the minus RNA strand in PBMCs.

The prevalence of OCI was significantly higher in the male patients than the female patients and higher in aged ones regarding baseline demographic features. Regarding the baseline laboratory studies, OCI was significantly correlated with the level of AFP, prothrombin time (PC), international normalized ratio (INR) (but still PC & INR within the normal range) and pretreatment viral load. In addition, the liver stiffness OCI was positively correlated with the degree of liver stuffiness by fibroscan (Table 3). There was no statistically significant association between the Child score and incidence of OCI (Table 4).

Based on radiology and ultrasonography results,

Table 3. Comparison of Baseline Demographic, Clinical and Laboratory Data, and Liver Stiffness by Fibroscan between Patients without and Those with OCI

	Negative for OCI (n= 102)	Positive for OCI (n= 9)	p value
Age (yrs.)	51.97 ±10.34	58.89 ±4.91	0.034*
Gender			
Male	43 (42.2%)	8 (88.9%)	0.011*
Female	59 (57.8%)	1 (11.1%)	
Smoking (yes)	30 (29.4%)	4 (44.4%)	0.452
DM	29 (28.4%)	2 (22.2%)	0.691
HTN	23 (22.5%)	1 (11.1%)	0.681
HCV load by iu (x10 ^{6})	2.20 ± 9.38	2.55 ± 1.59	0.019*
AST(IU/L)	70.96 ± 54.92	73.88 ± 46.53	0.37
ALT(IU/L)	70.25 ± 75.32	70.56 ± 43.21	0.549
Albumin(g/L)	3.92 ± 0.61	3.97 ± 0.55	0.991
Bilirubin(mg/dL)	0.87 ± 0.44	0.86 ± 0.55	0.965
PC (%)	87.82 ± 15.76	77.78 ± 12.38	0.024*
INR	1.13 ± 0.23	1.21 ± 0.17	0.026*
Hb (g/dL)	13.22 ± 1.81	13.53 ± 2.66	0.493
TLC (x10 3)	6.54 ± 2.19	6.07 ± 3.46	0.305
PLTs (x10 5)	2.05 ± 073	1.70 ± 0.74	0.069
FBS (mg/dL)	126.38 ± 66.96	146.67 ± 57.75	0.126
Creatinine (mg/dL)	0.85 ± 0.19	0.80 ± 0.14	0.349
AFP (ng/mL)	6.58 ± 5.75	16.40 ± 19.90	0.021*
Fibro.Stiff (mean ± SD)	15.37 ± 15.59	31.43 ± 22.22	0.016*
Fibro.Stiff Median (IQR)	8.4 (14.0)	25.1 (35.4)	0.016*
Fibrosis stage			
Early (F0 -2)	56 (54.9%)	1 (11.1%)	0.015*
Advanced (F 3-4)	46 (45.1%)	8 (88.9%)	

DM, Diabetes mellitus; HTN, Hypertension; ALT, Alanine transaminase; PC, prothrombin concentration; Hb, Hemoglobin level; TLC, total leucocytic count; PLTs, platelets; FBS, fasting blood sugar; AFP, Alpha fetoprotein; Data are expressed as mean ± SD, median (interquartile range) or number (%); p> 0.05= not significant; p≤ 0.05= significant.

Table 4. Comparison of Child Score between Patients without and those with OCI

	Negative (n= 102)	Positive (n= 9)	p value
Not cirrhotic	68 (66.7%)	3 (33.3%)	0.142
Child Score A5	17 (16.7%)	5 (55.6%)	
Child Score A6	11 (10.8%)	1 (11.1%)	
Child Score B7	4 (3.9%)	0 (0.0%)	
Child Score B8	1 (1.0%)	0 (0.0%)	
Child Score C10	1 (1.0%)	0 (0.0%)	

Data are expressed as number (%); p> 0.05, not significant.

Table 5. Comparison between Ultrasonography and Esophageal Varices Findings in Patients without and with OCI.

	Negative (n= 102)	Positive (n= 9)	p value
Liver size			
Normal	87 (85.3%)	7 (77.8%)	0.626
Enlarged	15 (14.7%)	2 (22.8%)	
Liver parenchyma			
Normal	42 (41.2%)	2 (22.2%)	0.741
Coarse	27 (26.5%)	3 (33.3%)	
Bright	12 (11.8%)	2 (22.2%)	
Bilharzial	3 (2.9%)	0 (0.0%)	
Cirrhotic	18 (17.6%)	2 (22.2%)	
Portal vein			
Normal	91 (89.2%)	7 (77.8%)	0.284
Dilated	11 (10.8%)	2 (22.2%)	
Esophageal varices (yes)	6 (5.9%)	2 (22.2%)	0.127

Data are expressed as number (%); p> 0.05, not significant.

there were no significant changes in the liver size, liver parenchyma, and portal vein diameter in patients with OCI. In addition, there was no significant increase in esophageal varices prevalence in patients with OCI compared to other patients (Table 5).

Discussion

From a clinical perspective, Egypt has conducted a successful screening program and treated more than four million people using effective DAAs, paving the way to reach the WHO elimination target (Hassanin et al., 2021). Despite the great progress achieved in HCV treatment during the last few years, HCV related complications still are a major global health problem, particularly in Egypt (Kandeel et al., 2017). Since oral DAAs therapy have been used for HCV infection, more than 90% of treated patients achieved SVR. However, a few patients experienced relapses later (Simmons et al., 2016).

The term OCI is now a challenging entity in the field of post hepatitis management and follow-up. It is diagnosed when HCV-RNA is detectable in the liver and/or in the case of PBMCs in patients with undetectable serum HCV-RNA (Nicot et al., 2010). As to the level of molecular pathology, it is well known that exposure of the intracellular HCV-RNA strands of antiviral therapy is associated with the disappearance of the antisense strand that leads to interruption of the virus life cycle (Idrees et al., 2011; Zaghloul et al., 2010). Concordant clearance of intracellular HCV-RNA strands, and hence, the whole virus particles from serum are a promising sign of a cure. On the contrary, the persistence of the intra-PBMCs strands and its reappearance or the presence of non-responding antisense strand to antiviral therapy is a predictor of non-responders to therapy or relapse (Zaghloul et al., 2010 ; Carreño et al., 2012). Therefore, OCI may be considered as a hidden source for HCV recurrence or a remote source of re-infection in patients with SVR. This is a debatable point of discussion, especially in localities with a high endemicity of HCV infection. From this point of view, we tried to investigate the frequency of OCI in the form of HCV persistence in PBMCs.

Some of cirrhotic patients exhibit continuous liver injury after SVR with the progression of decompensation and the possible development of HCC. Studies focusing on long term follow up of OCI patients are few. The OCI may be related to liver fibrosis and progression of liver affection, indicating the need for more research to clarify its clinical implications (Abdalla et al., 2018). Post-treatment persistence of intracellular HCV infection (occult HCV infection) possibly acts as a reservoir and hence might be related to later serologic relapse, and even to the ongoing hepatocellular damage, fibrosis, and cirrhosis (Yousif et al., 2018).

The OCI still is a debatable point of discussion, especially in localities with a high endemicity of HCV infection. In this regard, it was tried to investigate the frequency of OCI in the form of HCV persistence in the PBMCs in a relatively large well-defined cohort.

The OCI was diagnosed in nine out of 111 patients (8.1%) who achieved SVR based on negative serum HCV viremia. Mekky et al. reported that the OCI after SVR post-treatment with DAA was diagnosed in 50 out of 1280 patients (3.9%) (Mekky et al., 2019). In addition, Yousif et al. tested OCI in different regimens of all-oral treatments and found a relatively higher prevalence of OCI, 17 out of 150 patients (11.33%) (Yousif et al., 2018). Moreover, Khadr et al., found secondary OCI in about 25% of cases treated with SOF/DCV. However, this study was criticized for the small sample size (40 patients) (Abu Khadr et al., 2018). Abd Alla et al. reported a high prevalence of OCI in treatment-experienced and naive cases (49 out of 215 non-viremia patients (22.7%)) (Abd Alla et al., 2017).

These studies have shown positive results for OCI in patients who achieved SVR for DAA. The different percentages could be explained by the number of cirrhotic patients in the studied group, as it was evident in our study that a degree of liver stiffness positively correlates with the incidence of OCI; and secondly, the number of patients who had experienced prior IFN treatment failure. Mekky et al., noted that all OCI cases had experienced IFN treatment failure (Mekky et al., 2019). Lastly, the number of patients who use ribavirin in their treatment regimen contribute to the different findings by studies. Ibarra et al. documented the impaired cellular uptake of RBV into PBMCs over time and this may also explain why PBMCs could become a reservoir of HCV (Ibarra et al., 2011).

The prevalence of OCI was statistically and significantly higher in the male patients, which is related to the AFP level and degree of liver stiffness. Moreover, Mekky et al. found a positive correlation between the degree of liver stiffness and the incidence of OCI, based on fibroscan results and pretreatment bilirubin level (Mekky et al., 2019). In their recent study Wang et al. linked OCI to the degree of fibrosis and active inflammation at post-SVR and reported that both severity and frequency of fibrosis regression were lower in patients with OCI compared to those without OCI (Wang et al., 2019). These results are in contrast to Yousif et al., who found that OCI prevalence had no statistically significant relation to any of the baseline demographic, clinical, or laboratory characteristics of the study population (Yousif et al., 2018).

Persistence of HCV as occult infection following natural clearance or clinically successful antiviral therapy has a substantial pathogenic and epidemiologic importance to the patient and the community. This warrants a comprehensive investigation and consideration of new therapeutic practices against HCV infection (Pham et al., 2010). Therefore, it is probably useful to do dual testing of HCV RNA in both serum and PBMCs at the end of treatment with DAAs and during validation of SVR, especially in aged male patient with elevated AFP and those with advanced fibrosis to assess the outcomes in future. Regarding the possibility of treating patients with post antiviral therapy OCI and the expected outcome, there are no available trials involving those who finished oral DAAs, and there are fewstudies showiung significant reduction of late relapse rate (Hanno et al., 2014). Therefore, an extended treatment course or a consolidation course of DAAs in those who achieve SVR after the standard course of DAAs therapy but still have OCI could be an option.

One of the limitations to this work was the small number of the patients in the study. Our study confirmed that OCI prevalence was correlated to the degree of liver fibrosis but there was no long term follow-up to clarify its clinical implications. In addition, testing for HCV RNA in PBMC was not evaluated at the baseline. This issue should be considered in further studies for a better understanding of the dynamics of HCV infection.

In conclusion, our results shed light on the presence of OCI in patients who achieved SVR. The OCI was found in 8.1% of the patients, especially the male and aged patients with an elevated AFP and advanced fibrosis. Therefore, it is probably useful to do dual testing of HCV RNA in both serum and PBMCs at the end of treatment with DAAs and during validation of SVR to assess the outcomes in the patients. Further trials are needed to find out the possible predictors for the persistence of OCI after DAAs. Long-term observational studies using a larger number of patients are warranted to confirm our conclusions. Modulation of treatment protocols to prevent OCI needs further work, especially with the new and more potent DAA generations. The need for retreatment in such patients is another issue that must be evaluated.

List of abbreviations

AFP: Alpha fetoprotein.
ALT: Alanine aminotransferase.
AST: Aspartate aminotransferase.
BMI: Body mass index.
COCIs: Cryptogenic occult HCV-infection.
DAAs: Direct acting antiviral agents.
DCV: Daclatasvir.
EOT: End of treatment.
HCV: Hepatitis C virus.
INR: International normalized ratio.
IQR: The inter quartile range.
kPa: kilopascal.
OCI: Occult hepatitis C virus infection.
PBMCs: Peripheral blood mononuclear cells.
PCR: Polymerase chain reaction.
qRT-PCR: Quantitative reverse transcription PCR.
RBV: Ribavirin.
SCSD: Serum HCV spontaneous disappearance.
SEOCIs: Secondary experienced OCIs.
SNOCIs: Secondary naïve OCIs.
SOF: Sofosbuvir.
SRT-PCR: Standard reverse transcription PCR.
SVR: Sustained virological response.

Author Contribution Statement

Moataz Seyam, Ahmed R. Mashaal, Mohamed A. Shemis: Conceptualization, Project administration, Data curation, Funding acquisition, Tarek Mahmoud Diab: Data curation, Formal analysis, Validation. Reham M. Dawood, Mohamed A. Shemis: Investigation, Methodology, Supervision, Validation, and Writing – original draft. Ahmed R. Mashaal, Mohamed Abd El-Hameed, Ahmed A El Ray: Resources, Validation and supervision. All the authors read and approved the final manuscript.

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Author Agreement/Declaration

All authors have seen and approved the final version of the manuscript being submitted. All authors confirm that this work is original and all data, tables, etc. used in the manuscript are prepared originally by authors, and has not been published elsewhere nor is it currently under consideration elsewhere.

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Ethical approval

This research was authorized by the Theodor Bilharz Research institute's Ethical Review Board (FWA00010609).

Availability of the data

All the data are included within the article.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

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