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Occult Hepatitis (B) Infection in Hepatitis (C) Virus Infection Patients after the Treatment with Direct Acting Antiviral Drugs

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Abstract

BACKGROUND: The prevalence of occult hepatitis B ranges widely in patients with hepatitis C. This may have an impact on treatment of hepatitis C.

AIM: The present study aimed to evaluate the prevalence of occult hepatitis B infection in chronic hepatitis C patients who finished the course of treatment with direct acting antiviral drugs and its correlation with treatment failure.

SETTING: The study was conducted at Outpatient Clinic of Internal Medicine Department, Faculty of Medicine, Menoufia University, Egypt.

PATIENTS AND METHODS: This study was conducted on 900 Egyptian patients chronically infected with HCV. All patients tested positive for serum real-time polymerase chain reaction for HCV-RNA and received DAAs therapy for 12 weeks. Patients were categorized to: Group I: 450 patients with chronic hepatitis C after direct antiviral treatment who responded to treatment. Group II: 450 patients with chronic hepatitis C after direct antiviral treatment who did not respond to treatment. All patients were submitted to clinical examination, laboratory investigations, and abdominal ultrasonography. Detection of HBV-DNA and HCV-RNA was performed by PCR.

RESULTS: The prevalence of OBI detected in sera of HCV patients was 10.6% (96/900). The present study showed no significant correlation between prevalence of OBI and virologic failure (p: 0.084). There was no statistically significant difference (p > 0.05) between the studied groups as regards prevalence of OBI.

CONCLUSION: The prevalence of OBI was 10.6% in patients chronically infected with HCV. OBI does not affect the anti-HCV DAAs outcomes.

Introduction

Chronic hepatitis B (CHB) affects 240–400 million people around the world. Although Africa is considered to be a highly endemic region for HBV, it is considered to have an intermediate prevalence (2–6%) in Egypt [1]. It is estimated that 218,000 people in Australia live with CHB, a population prevalence of approximately 1% [2]. The infection is associated with a large spectrum of clinical manifestations ranging from acute or fulminant hepatitis to various forms of chronic infection, including asymptomatic carriers, chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [3]. The WHO estimates that the number of people exposed to this virus 2 billion, 240 million of whom are chronic carriers. In addition, the WHO estimates The number of HBV-related deaths from liver cirrhosis and HCC is 1.34 million deaths per year [4].

In general, HBV infection is diagnosed when the circulating HBV surface antigen (HBsAg) is serologically detected [5]. Occult HBV infection (OBI), a virological condition characterized by a low release of HBV from the liver cells and a low HBV-DNA level in the serum and/or liver tissue of hepatitis B surface antigen (HBsAg)-negative subjects [6]. OBI is defined as the presence of hepatitis B virus (HBV) DNA with negative results of HBsAg test with or without serological markers of previous viral exposure [7].

Occult hepatitis B is an independent risk factor in HCC development in anti-hepatitis C virus (HCV)-negative patients. A synergistic or additive role in the occurrence of HCC in HCV-confected patients is more problematic due to the HCC risk attributable to HCV alone, especially in patients with advanced fibrosis and cirrhosis [3].

Worldwide, the prevalence of OBI is quite variable. There is a growing evidence of a positive correlation between prevalence of OBI and the endemicity of HBV infection [8]. OBI has been found with a high prevalence in patients with chronic hepatitis C (CHC), probably because both HBV and HCV share the same parenteral way of transmission [9]. In particular, HBV-DNA is detectable in about one-third of HBsAg negative HCV carriers in the Mediterranean countries. In addition, it was suggested that OBI is highly prevalent in HCV-infected patients with the advanced liver disease even in areas with less HBV spread [10].

(CHC) is a major cause of end-stage liver disease, HCC, and liver-related death in the Western world [11]. Global epidemiology of HCV infection shows that the seroprevalence of anti-HCV antibody has increased over the last decade from 2.3% to 2.8%, corresponding to > 185 million infections worldwide [12]. Egypt has the highest prevalence of HCV infection in the world [13]. Before the advent of (DAAs) therapy, the mainstay of HCV therapy involved interferon (IFN)-based regimens that had frequent contraindications were poorly tolerated and achieved at best a 50% sustained virological response (SVR) rate [14]. The introduction of DAAs has since revolutionized the HCV treatment landscape. SVR rates for DAA therapy exceed 90% in registration trials and are better tolerated than IFN-based regimens [15].

The aim of our study was to evaluate the prevalence of OBI in CHC patients who finished the course of treatment with (DAAs) and its correlation with relapse after treatment.

Patients and Methods

This study was conducted on 900 Egyptian patients chronically infected with HCV and aged 18 years or older. All patients were positive for serum real-time PCR for HCV-RNA and received DAAs therapy for 12 weeks.

A written consent was obtained for each participant. The study was approved by the Local Ethics Committee, the patients were randomly selected by randomized and controlled trials (RCT) from those attending outpatients' clinic and inpatients of Internal Medicine Department, Menoufia University Hospital in the period from September 2019 to April 2020.

Setting

The study was conducted at Outpatient Clinic of Internal Medicine Department, Faculty of Medicine, Menoufia University.

Inclusion criteria

All inclusion criteria were abided according to the Egyptian National HCV Control Program**.** The age range was between 18 and 75 years. All patients were positive for serum real-time PCR for HCV- RNA.

Exclusion criteria

Application of exclusion criteria was taken in consideration the Egyptian National HCV Control Program guide lines. These criteria included the following:

Patients who are coinfected with HIV, patients who are <18 or >75 years old, pregnant females, HCC or other extrahepatic malignancy, total serum bilirubin more than 3 mg/dl, serum albumin <2.8 g/dl, INR more than 1.7, platelet count <50,000/ul, and renal impairment with glomerular filtration rate (GFR) <30 ml/min.

Patients were categorized to:

- Group I: 450 patients with chronic hepatitis C after direct antiviral treatment who responded to treatment.
- Group II: 450 patients with chronic hepatitis C after direct antiviral treatment who did not respond to treatment.

All patients enrolled in the study were subjected to clinical evaluation, laboratory investigations, and abdominal ultrasonography to evaluate hepatic echopattern of the liver, the patency of the portal vein, presence of splenomegaly, and to rule out presence of HCC.

All the studied patients were subjected to the following:

Laboratory investigations

1. Complete blood count using cell dyne-1800
2. Liver function and kidney functions tests, assessment of the levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase enzyme (AST), albumin level, total bilirubin and creatinine level (0.50−1.2 mg/dl); alpha-fetoprotein (AFP), prothrombin time, and concentration (PC) using STA compact Max Coagulation.
3. Hepatitis B markers by an enzyme immunoassay (EIA).
4. Hepatitis C virus RNA viral load evaluation was done by quantitative real-time (QT-PCR) test, using the Cobas Amplicor, TaqMan HCV test version 2.0 (the lower detection limit was 15 IU/ml).
5. HBV-DNA was detected using nested PCR (core fragment) technique. The method uses four primers from the X region of the HBV genome and has a lower limit of detection 100 copies/mL. Every sample with HBV-DNA detected by nested PCR was tested again using a commercially available real-time PCR kit (COBAS Taqman HBV Test; cutoff of detection: 6 IU/mL). The COBAS TaqMan HBV Test is an *in vitro* nucleic acid amplification test for quantitation of HBV in human serum or plasma, using the High Pure Viral Nucleic Acid kit for manual specimen preparation and the COBAS TaqMan 48 Analyzer for automated amplification and detection. The

highly conserved HBV pre-core/core region is amplified for this test. Only serum samples with repeatedly detectable HBV-DNA were considered positive for HBV-DNA.

Treatment regimen

Patients were submitted to direct acting antivirals therapy, depending on the guidelines of the European Association for Study of Liver [16].

Monitoring of anti-viral therapy efficacy

Virological assessment of HCV-RNA level was done three times: at the baseline (before beginning of the treatment), at the end of the treatment (EOT) (week 12), and 12 weeks after the end of treatment. The primary efficacy endpoint was established by a sustained virological response at 12 weeks after the end of therapy. Sustained virological response was considered when HCV-RNA is less than the lower limit of detection (LLOD) at week 12 after the end of treatment (sustained virological response-12) by quantitative HCV-PCR whereas treatment failure was established as confirmed HCV-RNA above the LLOD 12 weeks after the end of treatment.

Statistical analysis

Analyses were conducted using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Patients' demographic data are expressed as the mean ± SD or no. and %. The significance of the association between the two groups for qualitative variables was determined using Pearson's Chi-square (χ^2) test, Student's t test (t) test was used to compare between two groups for quantitative parametric data, while Mann–Whitney test was used for non-parametric data. A p-value was considered significant if <0.05.

Results

The prevalence of OBI in sera of HCV patients was 10.6% (96/900) (Figure 1).

Tables 1 and 2 show: Description of the demographic, clinical, and laboratory data of the studied patients. Among Group I, 73.3% were males, the mean age was 48.8 years, 8.9% were cirrhotic, and 13.3% were diabetic. Regarding pre-treatment laboratory profile, results showed that the mean levels of ALT, AST, AFP, platelets count, WBCs, and HB were 37.3 U/L, 32.8 U/L, 5.3 ng/dl, 211.3 × 10³/ul, 5.9 × 10³/ul, and 13.8 g/dl, respectively. Among Group II, 68.9% were males, the mean age was 50.7 years, 35.6% were cirrhotic, and

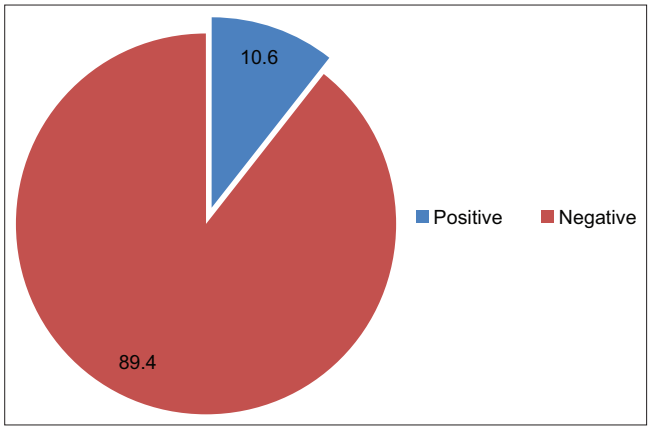

Figure 1: *Prevalence of Occult HBV infection among the studied patients*

20% were diabetic. Regarding pre-treatment laboratory profile, results showed that the mean levels of ALT, AST, AFP, platelets count, WBCs, and HB were 37.2 U/L, 34.8 U/L, 5.9 ng/dl, 130.2 × 10³/ul, 5.7 × 10³/ul, and 13.6 g/dl, respectively.

Table 1: Demographic data of the studied groups

Demographic data	Group I (N = 450)		Group II (N = 450)		p-value
Age (years)					
Mean ± SD	49.1 ± 11.3		50.6 ± 8.1		0.022*
BMI (kg/m²)					
Mean ± SD	26.8 ± 4.1		27.2 ± 5.1		0.515
Sex					
Males	330	73.3%	310	68.9%	0.141
Females	120	26.7%	140	31.1%	
Smoking					
Yes	130	28.9%	110	24.5%	0.132
No	320	71.1%	340	75.6%	
Other chronic diseases					
Non	340	75.6%	330	73.3%	0.004*
DM	60	13.3%	90	20.0%	
HTN	50	11.1%	30	6.7%	

*Significant.

There was no statistically significant difference ($p > 0.05$) between the studied groups as regard the prevalence of OBI (Table 3).

Table 2: Laboratory data of the studied groups

Group I (N = 450) Mean ± SD		Group II (N = 450) Mean ± SD	p-value
ALP (U/L)	39.2 ± 11.8	41.3 ± 16.7	0.608
Range	16–65	15–85	
Median	43	37	
ALT (U/L)	37.6 ± 27.6	43.9 ± 44.44	0.342
Range	5–126	5–205	
Median	30	36	
AST (U/L)	32.04 ± 17.3	38.1 ± 30.6	0.719
Range	8–74	7–138	
Median	31	30	
AFP (ng/ml)	5.3 ± 3.7	5.7 ± 3.5	0.040*
Range	1–16	1–18	
Median	4	6	
PLTs (×10³/ul)	211.4 ± 63.1	131.3 ± 12.2	< 0.001**
Range	153–461	101–149	
Median	193	134	
WBCs (×10³/ul)	5.9 ± 1.8	5.6 ± 1.8	0.068
Range	3–11.9	2.8–9.9	
Median	5.7	5.3	
Hb (g/dl)	13.7 ± 1.6	13.6 ± 1.6	0.373
Range	10.5–18.1	10.7–17	
Median	13.7	13.6	

*Significant, **Highly significant.

The findings of our study revealed a statistically significant variation between patients who responded to treatment and patients who did not respond to treatment as regard liver cirrhosis (LC). Table 4 reveals a statistically significant difference (p-value: 0.009)

Table 3: Prevalence of OBI among the studied groups

OBI	Group I (N = 450)		Group II (N = 450)		p-value
Yes	40	8.9%	56	12.4%	0.084
No	410	91.1%	394	877.6%	

between responders and non-responders as regard prevalence of cirrhosis.

Table 4: Abdominal US study of the studied groups

Abdominal US	Group I (N = 450)		Group II (N = 450)		p-value
Imaging results					
Normal liver	140	31.1%	110	24.4%	<0.001**
Abnormal echo pattern	270	60%	180	40%	
Liver cirrhosis	40	8.9%	160	35.6%	

**Highly significant.

The present study also showed significant correlation between thrombothytopenia and virologic failure (Figures 2 and 3).

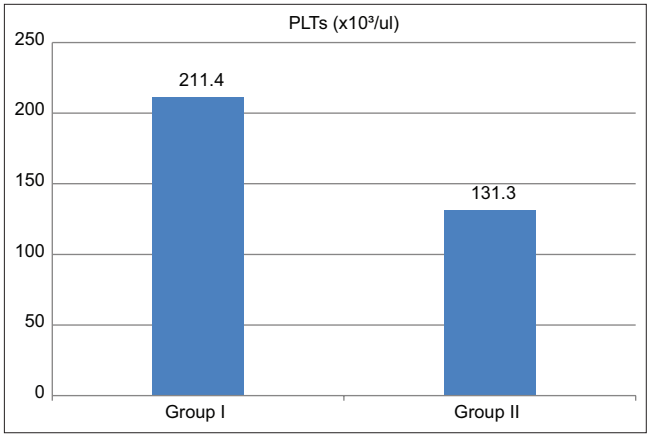

Figure 2: *Platelet count of the studied groups*

Discussion

The results of this study showed that the prevalence of occult hepatitis B in sera of HCV patients was 10.6% (96/900) Hassan *et al*. [17]. A similar result was previously reported. Evaluated 111 anti-HCV-positive patients presenting for liver biopsy before interferon (IFN)-based treatment with negative viral markers [18]. All of the 111 HCV-RNA-positive patients were serum HBsAg-negative. However, HBV-DNA was detected in the liver tissue of 13/111 (11.7%) among these patients Naga *et al*. [9]. The prevalence of occult hepatitis B among 210 chronic HCV-infected patients was 8.5% Mandour *et al*. [1]. In another Egyptian study, Mahmoud *et al*. [17] assessed 100 chronic HCV patients HBV-DNA, where 18 cases (18%) were positive for HBV-DNA and were considered OBI. Bhatia *et al*. [19] performed a retrospective and cohort study on 80 HBsAg-negative CHC patients who were initiated on DAA therapy. They reported that HBV-DNA was detected in only one patient by quantitative PCR and concluded that the prevalence of OBI was 1.25%. Intrahepatic HBV-DNA in this study among Abu El Makarem *et al*. [20] demonstrated that 5% of chronic HCV Egyptian patients with end-stage renal failure (ESRF) who were undergoing dialysis had OBI. On the other hand, Taha *et al*. [21] reported a higher rate of OBI among CHC patients. HBV-DNA (OBI) was detected in the sera of 9/40 patients (22.5%), 7 (77.8%) of whom belonged to Group II. OBI was detected in the liver tissue in 23/40 (57.5%) of chronic HCV-infected patients, 18 (78.3%) of whom belonged to Group II, conferring a 90% of prevalence among this group. The prevalence of the CHC patients was 57.5% while it was 22.5% in serum samples.

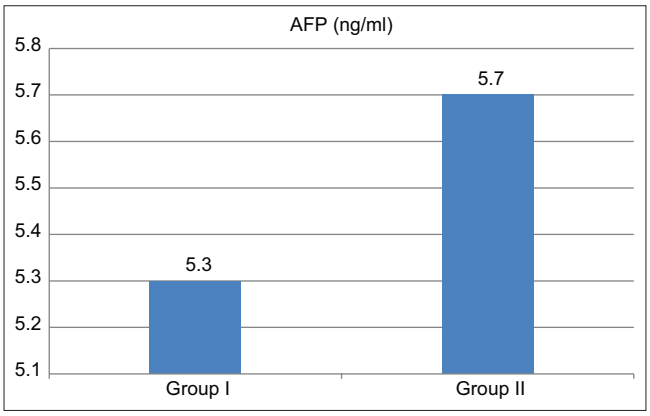

Figure 3: *AFP of studied groups*

Regarding the impact of OBI on sustained virological response (SVR), the present study showed no significant correlation between the prevalence of OBI and virologic failure. The results of our study are supported by Wang *et al*. [7] who evaluated 327 consecutive Chinese adults chronically infected with HCV and treated with oral DAAs therapy, where 124 of the 317 HBsAg-negative patients (39.1%) had OBI. DAAs included ledipasvir/sofosbuvir, daclatasvir/sofosbuvir, and ombitasvir/paraparesis/ritonavir plus dasabuvir. They reported no difference in HCV genotype, HCV viral load, and degree of liver fibrosis among patients with or without OBI. SVR at 12 weeks after the end of treatment was achieved in all patients.

In the era of IFN based therapy Naga *et al*. [9], 101 patients who received optimized peg-IFNa and Ribavirin therapy. Overall, a sustained virological response was observed in 59.4% of those patients. There was no significant difference in the response rate between those with and those without OBI (p = 0.27). Furthermore, Fabris *et al*. [22] studied 25 patients who were treated with Alfa IFN and ribavirin for at least 18 months. They found no significant difference in the SVR among patients with and without OBI. Hasegawa *et al*. [23] analyzed 140 HCV patients without HBsAg and found that 7.9% of their patients were positive for serum HBV-DNA; 4 of these 11 patients achieved SVR with IFN compared with 39 of 129 patients without HBV-DNA with no significant difference. On the other hand, Mrani *et al*. [24] reported that OBI affected sustained response to IFN and ribavirin. Among CHC patients, they observed that SVR was achieved in 11 (28%) of 40 HBV-DNA-positive

cases, compared with 65 (45%) of the 144 HBV-DNA-negative cases ($p < 0.05$).

Our study found a statistically significant association between virologic failure and degree of fibrosis, there was a highly significant difference between responders and non-responders as regard liver status (cirrhotic and non-cirrhotic patients) ($p < 0.001$). This result is supported by ALLY-3 Phase III study, conducted by Nelson *et al*. [25] where 100 treatment-naïve and 51 treatment-experienced genotype three-infected patients were enrolled to receive open- label daclatasvir 60 mg + sofosbuvir 400 mg once daily for 12 weeks. The majority of patients had baseline HCV-RNA levels of >800,000 IU/mL (71%). One hundred and nineteen (78%) patients had a fibrosis stage of F0-F3 and 30 (20%) had a fibrosis stage of F4; Fibro Test scores were not reported for three patients. SVR12 was achieved in 90% and 86% of the treatment-naïve and treatment experienced patients, respectively. SVR12 rates were higher in patients without cirrhosis (96%) than in patients with cirrhosis (63%). A similar trend was observed when SVR12 was analyzed by fibrosis stage, based on Fibro Test scores, of F0–F3 (93%) and F4 (70%). In accordance with the present study, Salama *et al*. [26] reported that the response rate was correlated with the fibrosis score. among 475 patients with chronic HCV infection. The treatment was given for 12 weeks with 12 week follow-up to assess SVR12. The exclusion criteria were patients with decompensated cirrhosis (Child Grade B and C), pregnant ladies, concomitant HBV infection, or HCC. The treatment was safe and effective and SVR12 in overall patients was 93.3% (433/475). In relation to fibrosis score, the response rate ranged from 95 to 100% in F1, F2 and it decreased to 80–93% in F3, F4. Non-responders were 32 (6.7%) patients out of 475 patients. In relation to fibrosis score, 29 out of the 32 non-responders were in F3, F4. Conti *et al*. [27] provided evidence about the impact of LC on DAAs outcome. In their study, a total of 556 HCV-IFN patients with advanced liver disease were treated with interferon-free regimens. Two hundred and eight-two (50.7%) were ≥65 years old (of whom 106 patients were ≥75 years) and 274 (49.3%) were <65 years old. The proportion of females was higher in patients aged ≥65 years than in patients aged <65 years. Liver cirrhosis was present in 86.5% of elderly and in 78.1% of younger ($p = 0.010$), but pre-treatment Child-Pugh-Turcotte (CTP) class distribution and MELD score were similar between the two groups. The SVR12 rate was 92.6% (92.6%) in the overall population. The presence of liver cirrhosis affected virologic response: SVR12 was achieved in 93.9% of cirrhotic patients, in comparison with 100% of the 38 patients with advanced fibrosis. In patients with cirrhosis, CTP class significantly affected SVR12: (80.8% in CTP-B vs. 95.4% in CTP-A; $p = 0.013$). Shiha *et al*. [28] studied 1168 patients treated in the Egyptian Liver Research Institute and Hospital, Mansoura, Egypt. They found sustained viral response after 12 weeks. At the end of treatment, SVR12 was achieved in 96.6% 95% CI 95.1–98.2% of the patients receiving 12 weeks of DCV/SOF treatment, in 95.7% 95% CI 93.6–97.8% of the patients receiving 12 weeks of DCV/SOF/RBV. The SVR12 rate was significantly higher in patients with no cirrhosis receiving DCV/SOF only for 12 weeks (97.4) than in patients with cirrhosis (91.7). Another large Egyptian study by Esmat *et al*. [29] who studied 300 patients with HCV infection. These patients were recruited in three groups: Treatment-naïve patients with or without compensated Child-A cirrhosis (Group1); IFN-experienced patients without cirrhosis (Group 2); and IFN-experienced patients with cirrhosis (Group 3). Groups 1 and 2 received ravidasvir 200 mg QD plus sofosbuvir 400 mg QD for 12 weeks and were randomized 1:1 to treatment with or without weight-based ribavirin. Group 3 patients received ravidasvir plus sofosbuvir with ribavirin. SVR12 was achieved in 95.3% of all patients who started the study, including 98% of patients without cirrhosis and 91% of patients with cirrhosis, in both treatment-naïve and IFN-experienced.

Regarding the impact of low platelet count on SVR, the present study showed significant correlation between thrombothytopenia and virologic failure. This result agrees with that reported by Elsharkawy *et al*. [30], who concluded that low platelet count was significantly associated with treatment failure as this factor might be associated with more advanced liver fibrosis. In accordance with the present study, Ahmed *et al*. [31] performed a prospective study on 300 Egyptian patients with chronic HCV genotype 4, who were treated with sofosbuvir plus daclatasvir with or without ribavirin for 12–24 weeks. A total of 92.67% of all patients achieved SVR12. SVR12 rates of 96.55% and 84.54% were found in non-cirrhotic and cirrhotic patients, respectively. These investigators concluded that liver cirrhosis, especially Child–Pugh class B, and low platelet count were the factors that were significantly associated with non-response to treatment. However, in contrast with our study, Zaghloul *et al*. [32] revealed that thrombocytopenia had no impact on sustained virological response or relapse with these regimens.

Conclusion

The prevalence of OBI was 10.6% in patients chronically infected with HCV. OBI did not affect the anti-HCV DAAs outcomes. There was a relation between the presence of liver cirrhosis and thrombocytopenia and the poor response to the therapy.

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