Occult hepatitis B virus infection in patients with hepatitis C virus-related cirrhosis with or without hepatocellular carcinoma

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Introduction
Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide, with incidence ranging between 3 and 9% annually. Globally, it is the fifth leading cause of cancer and the third leading cause of cancer death. In Egypt, HCC prevalence about 4.7% of patients with chronic liver disease. Hospital-based studies from Egypt have reported an overall increase in the relative frequency of HCC cancers in Egypt from ~ 4% in 1993 to 7.3% in 2003.

Patients and methods
This study was carried out in Tropical Medicine and Gastroenterology Department at Assiut University Hospital in Faculty of Medicine, Assiut University, Egypt, in the period between November 2016 and November 2017 on 200 patients, who were divided into two groups: the first group was formed of 100 patients with hepatitis C virus (HCV)-related liver cirrhosis (LC) and the second group was formed of 100 patients with patients with HCV-related LC and well-established diagnosis of HCC based on their medical profile, hepatitis B core antibody total, hepatitis B surface antigen, hepatitis B virus DNA PCR, HCV antibody, liver profile, complete blood count, ultrasound, triphasic computed tomography, and alpha-fetoprotein.

Results
The frequency of seropositive occult HBV infection among the studied patients was 11 (11%) patients in those with LC and 15 (15%) patients in those with HCC, which showed a statistically significant relation with development of HCC in general in comparison between HCC and non-HCC patients, but its role in the development of HCC in HCV-coinfected patients was less related, with a statistically insignificant relation.

Conclusion
Occult HBV infection leads to significant liver disease, and it is an important cause of HCC and may accelerate the underlying liver disease.

Keywords:
hepatitis B core antibody total, hepatitis C virus, hepatocellular carcinoma, liver cirrhosis, occult hepatitis B virus

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Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are considered as the major risk factors that contribute to the development of hepatocellular carcinoma (HCC). The relationship between occult hepatitis B virus infection (OBI) and HCV-related HCC has been extensively reviewed but remains controversial [1].

The serological assay for the HBV core antigen [anti-hepatitis B core (HBc)] represents a qualified candidate as a surrogate for DNA amplification, or for increasing overall sensitivity when assessing the risk of occult hepatitis in peripheral blood. The risk of occult hepatitis associated with anti-HBc seropositivity has been demonstrated extensively, and the presence of antibody response to HBc can be considered a sentinel marker of OBI [2].

Aim of the work

The aim is to determine the prevalence of seropositive OBI in patient with HCV-related cirrhosis with or without HCC.

Patients and methods

This study was carried out in Tropical Medicine and Gastroenterology Department at Assiut University Hospital, Assiut University, Egypt, from November 2016 to November 2017 on 200 patients, who were selected based on the following inclusion and exclusion criteria.

(1) Inclusion criteria:
   (a) Adult patients with liver cirrhosis (LC) and HCV with or without HCC with age range from 18 to 70 years of both sexes.
(2) Exclusion criteria:
   (a) The patients who refused to be entitled in the study.
   (b) Patients with positive hepatitis B surface antigen (HBsAg).
   (c) Patients with negative HCV antibody markers.

All patients were subjected to the following:
(1) Informed consent was obtained from all patients before participation in the study.
(2) History taking with special emphasis on history of alcohol intake and history of hepatitis, HCC, previous treatment, and drug history.
(3) Thorough clinical examination with special emphasis on local examination of liver and spleen and detection of ascites.
(4) Laboratory and radiological investigations:
   (a) HCV antibody was assessed by using ELISA technique.
   (b) HBsAg was determined by using commercial enzyme immunoassay kits (as a perquisite to the study entry).
   (c) Anti-hepatitis B core antibody (HBcAb; total antibodies) was assessed by using competitive enzyme immunoassay (ELISA) for the determination of antibodies to hepatitis B core antigen in human plasma and serum.
   (d) HBV DNA 'quantitative' was assessed by real-time PCR, which has a detection limit of assay 12 IU/ml of HBV DNA.
   (e) Complete blood picture.
   (f) Serum albumin, total bilirubin, prothrombin time, and liver enzymes to asses liver functions.
   (g) Alpha-fetoprotein was assessed for all patients.
   (h) Abdominal ultrasonography with special comment on liver echogenicity, size, hepatic focal lesions, spleen size, and presence of ascites.
   (i) Serum creatinine and BUN to assess kidney function for whom we did triphasic contrast-enhanced computed tomography.
   (j) Triphasic contrast-enhanced computed tomography of the liver data for patients with HCC diagnosis were included with special comment on number and size of hepatic focal lesions and patency of portal vein.

Recording of data

Results are calculated as a normalized signal, relative to the cutoff value (signal/cutoff). During the calibration process, a lot-specific parameter is used to determine a valid stored cutoff value for the VITROS Immunodiagnostic and VITROS Integrated Systems (Table 1).

$$\text{Cut-off} = \frac{\text{Negative control} + \text{Positive control}}{5}$$

$$\text{Result} = \frac{\text{Signal for test sample}}{\text{Cutoff value}}$$

Hepatitis B quantitative (hepatitis B virus DNA) PCR

Test indications

It is indicated for confirmation of chronic HBV infection, quantification of HBV DNA in serum of patients with chronic HBV infection, and monitoring disease progression in chronic HBV infection and/or response to antiviral therapy.

Assay

Aseptically centrifuge specimen and separate serum from the clot within 6 h. Serum aliquot should be placed in a screw-capped, round-bottom plastic vial, and stored and shipped at frozen temperatures. Sterility should be maintained, and it should be forwarded to the laboratory promptly.

Interpretation

The quantification range of this assay is 12–170 000 000 IU/ml (Table 2).

Table 1 Interpretation of hepatitis B core antibody results

Interpretation	Conclusion from testing algorithm	Initial VITROS anti-HBcAb test result (s/c)
Specimen is presumed to be reactive for anti-HBc	Reactive	<0.90
If 2 of 3 results are <1.00, then specimen is presumed to be reactive for anti-HBc	Retest in duplicate	≥0.90 and ≤1.10
If 2 of 3 results are >1.00 and <4.80, then the specimen is negative for anti-HBc	Retest in duplicate	≥0.90 and ≤1.10
Specimen is negative for anti-HBc	Negative	>1.10 and<4.80
If 1 : 20 dilution and retest result is ≤1.00, then the specimen is presumed to be reactive for anti-HBc	Dilute 1 : 20 and retest	≥4.80
If 1 : 20 dilution and retest result is >1.00 and <4.80, then the specimen is negative for anti-HBc	Dilute 1 : 20 and retest	≥4.80

HBcAb, hepatitis B core antibody; s/c, signal/cutoff.

Limitations

This test is not licensed by the FDA as a screening test for HBV infections or a diagnostic test to confirm the presence of HBV infection. An 'undetected' HBV DNA test result in conjugation with a positive anti-HBV status does not exclude the possibility of a resolved HBV infection. When clinically indicated, patients should be retested for HBV DNA in 1–2 months, to distinguish between past/resolved HBV infection and chronic HBV infection with episodic viral replication.

Occult hepatitis B virus infection

The gold standard test for detection of OBI is the amplification of HBV DNA. However, the serological assay for the long-lasting antibody response to the highly immunogenic HBV core antigen (anti-HBc) represents a qualified candidate as a surrogate for DNA amplification, or for increasing overall sensitivity when assessing the risk of occult hepatitis in peripheral blood. The risk of occult hepatitis associated with anti-HBc seropositivity has been demonstrated extensively, and the presence of antibody response to HBc can be considered a sentinel marker of OBI.

In our study, we depended on HBcAb as a sentinel marker for OBI for all patients, confirmed by HBV DNA for HBcAb-positive patients only for diagnosis of OBI.

Ethical consideration

Informed consent was obtained from all participants; it was explained to all participants that the collected data will be kept confidential and used for the purpose of the scientific research only. All investigations were free of cost without any financial burden on the participants.

Statistical analysis

Data were collected and analyzed those using SPSS (Statistical Package for the Social Science, version 20; IBM, Armonk, New York, USA). Continuous data were expressed in the form of mean ± SD, whereas nominal data were expressed in the form of frequency (percentage).

χ^2 test was used to compare the nominal data of different groups in the study, whereas Student *t* test was used to compare mean of two different groups and analysis of variance test for more than two groups.

Results

Demographic data of the studied patients

Table 3 shows the demographic data of both groups, where male predominance presented in both groups (54 vs. 58% patients were males in LC group and HCC group, respectively). Mean age of patients with LC was 53.2 ± 9.25 years, whereas in case of HCC group was 57.17 ± 8.67 years.

Table 2 Interpretation of hepatitis B virus DNA results

Results	Interpretation
Undetected	This assay indicates that HBV was not detected in the specimen
Detected, with the comment 'HBV DNA level is a number between 12 to 170 000 000 IU/ml'	This assay indicates that HBV was detected in the specimen with quantification of viral load
Detected, with the comment 'HBV DNA level is >170 000 000 IU/ml'	This assay cannot accurately quantify HBV DNA above this level and indicates that the HBV DNA level is above the upper limit of quantification for this assay

HBV, hepatitis B virus.

Table 3 Demographic data of the studied patients

Variables	LC group (*n*=100)	HCC group (*n*=100)	*P*
Age (years)	53.2±9.25	57.17±8.67	**0.00**
Sex			0.09
Male	54 (54)	58 (58)	
Female	46 (46)	42 (42)	
Smoking	22 (22)	24 (24)	0.43
Comorbidities			
Diabetes mellitus	37 (37)	40 (40)	0.87
Hypertension	10 (10)	13 (13)	0.11
Cardiac disease	5 (5)	3 (3)	0.34

Data were expressed in the form of frequency (%) or mean±SD as appropriate. HCC, hepatocellular carcinoma; LC, liver cirrhosis. Bold, *P* value was significant if less than 0.05.

Twenty-two (22%) patients with LC were smokers, whereas 24 (24%) patients in HCC group were smoker. Comorbidities such as diabetes mellitus, hypertension, and cardiac disease presented in 37 (37%), 10 (10%), and five (5%) patients with LC, respectively, whereas in 40 (40%), 13 (13%), and three (3%) patients with HCC, respectively.

It was noticed that there was a significant difference between both groups regarding age ($P = 0.00$), whereas sex, smoking, and comorbidities had no significant differences between both groups, where *P* value more than 0.05.

Laboratory data of the studied patients

Table 4 shows the laboratory data of both studied groups. Regarding the complete blood picture, patients with LC had significantly higher hemoglobin level and platelets count in comparison with those with HCC, with *P* value of 0.01 and 0.00, respectively, whereas white blood cells had no significant differences between both groups ($P = 0.09$).

Moreover, patients with LC had significantly higher serum albumin level (P = 0.02), lower aspartate aminotransferase (P = 0.02), and lower total bilirubin level (P = 0.00) than those with HCC. Other parameters of liver function tests had no significant differences between both groups (P > 0.05).

Prothrombin time, prothrombin concentration, and international normalized ratio were significantly prolonged in those with HCC in comparison with those with LC (P < 0.05) and also alpha-fetoprotein was significantly higher in the HCC group (462.26 ± 111.19 vs. 6.46 ± 3.07 ng/l in LC and HCC groups, respectively; P = 0.00). Kidney function tests had no significant differences between both.

A total of 70 (70%), 15 (15%), and 15 (15%) patients with LC were Child A, B, and C, respectively, whereas in case of HCC group, 20 (20%), 38 (38%), and 42 (42%) patients were Child A, B, and C, respectively. It was noticed that there was a significant difference between both groups regarding Child classification (P = 0.00). Patients with HCC had higher MELD score in comparison with those with LC (17.11 ± 4.52 vs. 14.09 ± 2.11; P = 0.01).

Overall, 22.8% of the studied patients had very low fat mass, whereas 13.9% had high water content, but there was no statistically significant difference in body composition analysis between groups of Child score except in water percentage between Child A and B and between Child A and C (Table 2).

Occult hepatitis B virus infection in each group

In the current study, all patients were HBsAg negative. HBcAb was positive in 19 (19%) and 21 (21%) patients with LC and HCC, respectively (P = 0.43), whereas HBV DNA was positive in 11 (11%) and 15 (15%) patients with LC and HCC, respectively (P = 0.01).

So, in the current study, frequency of OBI was 11 (11%) patients in those with LC and 15 (15%) patients in those with HCC. So, of 200 patients included in this study, 26/200 (13%) patients had OBI.

Relation between presence of hepatitis B core antibody and number of hepatic focal lesions

The current study showed that the number of hepatic focal lesions in patients with HCC was significantly increased in those with positive HBcAb, with P = 0.00.

Risk analysis of relation between hepatocellular carcinoma and occult hepatitis B virus infection

It was noticed that patients with OBI had a risk for HCC four times higher than those without OBI (odds ratio = 4, 95% confidence interval = 1.62–9.4, P = 0.00), but there was insignificant correlation between HBcAb and HCC, with P = 0.43.

Alpha-fetoprotein level in the studied patients based on hepatitis B core antibody

Table 5 showed that patients with positive HBcAb had significantly more level of alpha-fetoprotein in comparison with those with negative HBcAb in HCC group (813.07 ± 80.87 vs. 369.17 ± 66.32 ng/ml, with P = 0.01).

Relation between presence of hepatitis B core antibody and presence of portal vein thrombosis

The current study showed that portal vein thrombosis (PVT) was significantly higher in patients with HCC and positive HBcAb (47.6%) in comparison with those with HCC and negative HBcAb (12.7%) with P = 0.01 (Tables 6–9).

Table 4 Laboratory data of the studied patients

Variables	LC group (n=100)	HCC group (n=100)	P
Complete blood count			
Hemoglobin (g %)	11.81±2.95	10.76±2.75	**0.01**
Platelets (×10^3/ml)	160.34±22.89	100.56±21.09	**0.00**
WBCs (×10^3/ml)	5.79±1.22	5.43±2.09	0.09
Liver function tests			
Total bilirubin (mg/dl)	1.36±0.77	2.57±0.65	**0.00**
AST (U/l)	44.05±11.34	56.23±16.78	**0.02**
ALT (U/l)	36.78±6.78	40.56±8.97	0.34
Albumin (g %)	3.37±0.76	2.26±0.51	**0.02**
ALP (U/l)	102.11±23.01	111.09±21.04	0.45
PT (s)	14.23±2.09	16.05±4.87	**0.00**
PC (%)	65.98±13.28	60.09±9.08	**0.00**
INR	1.12±0.24	1.15±0.33	**0.00**
Kidney function tests			
Urea (mg/dl)	18.5±4.29	20.61±7.76	0.08
Creatinine (mg/dl)	0.96±0.26	10.3±0.38	0.07
α-fetoprotein (ng/l)	6.46±3.07	462.26±111.19	**0.00**
Child score			**0.00**
A	70 (70)	20 (20)	
B	15 (15)	38 (38)	
C	15 (15)	42 (42)	
MELD score	14.09±2.11	17.11±4.52	**0.01**

Data were expressed in form of frequency (%) or mean±SD as appropriate. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; INR, international normalized ratio; LC, liver cirrhosis; n, number; PC, prothrombin concentration; PT, prothrombin time; WBCs, white blood cells. Bold, P value was significant if less than 0.05.

Table 5 Alpha-fetoprotein level in the studied patients based on hepatitis B core antibody

	Positive HBcAb (n=21)	Negative HBcAb (n=79)	P
Alpha-fetoprotein (ng/ml)	813.07±80.87	369.17±66.32	0.01

Data were expressed in the form of mean±SD. HBcAb, hepatitis B core antibody. P value was significant if less than 0.05.

Table 6 Occult hepatitis B virus infection in each group

Variables	LC group (*n*=100)	HCC group (*n*=100)	*P*
Negative HBsAg	100 (100)	100 (100)	-
Positive HBcAb	19 (19)	21 (21)	0.43
Positive HBV DNA (OBI)	11 (11)	15 (15)	**0.01**

Data were expressed in the form of frequency (%). HBcAb, hepatitis B core antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LC, liver cirrhosis; OBI, occult hepatitis B virus infection. Bold, *P* value was significant if less than 0.05.

Table 7 Risk analysis of relation between hepatocellular carcinoma and occult hepatitis B virus infection

Variables	Odds ratio	95% confidence interval	*P*
Positive HBcAb	1.16	0.55-2.23	0.43
Positive HBV DNA (OBI)	4	1.62-9.4	**0.00**

HBcAb, hepatitis B core antibody; HBV, hepatitis B virus; OBI, occult hepatitis B virus infection. Bold, *P* value was significant if less than 0.05.

Table 8 Relation between presence of hepatitis B core antibody and number of hepatic focal lesions

Number of hepatic focal lesions	Positive HBcAb (*n*=21)	Negative HBcAb (*n*=79)	*P*
One	9 (43)	63 (80)	0.00
Two	5 (24)	7 (9)	
Multiple	7 (33)	9 (11)	

Data were expressed in the form of frequency (%).HBcAb, hepatitis B core antibody.*P* value was significant if less than 0.05.

Table 9 Relation between presence of hepatitis B core antibody and presence of portal vein thrombosis

Portal vein thrombosis	Positive HBcAb (*n*=21)	Negative HBcAb (*n*=79)	*P*
Yes	10 (47.6)	10 (12.7)	0.01
No	11 (52.4)	69 (87.3)	

Data were expressed in the form of frequency (%). HBcAb, hepatitis B core antibody. *P* value was significant if less than 0.05.

Discussion

OBI is one of the most challenging topics in the field of viral hepatitis, with its virological and clinical relevance being debated for more than 30 years [3].

One Egyptian study observed that anti-HBc-positive/HBV DNA negative patients showed a similar prevalence of severe liver disease to anti-HBc positive/HBV DNA positive patients and a significantly higher prevalence than anti-HBc negative cases. This notion raises the clinical significance of isolated positive anti-HBc antibody in relation to liver disease [4], although in other studies no association was found between OBI and the degree of liver necroinflammation and fibrosis [5,6].

Patients with HCC living in areas endemic for HBV were frequently found positive for HBsAg and/or anti-HBc antibodies, and this strong relationship was the first epidemiological evidence of HBV-related oncogenic transformation [7]. Persistent HBV infection may have a critical role in the development of HCC even in HBsAg-negative patients [2]. Development of HCC in patients with OBI seems to be related in most cases to the associated co-infection with HCV and to the presence of cirrhosis, although OBI mono-infection still bears an oncogenic potential [2,8].

The highest prevalence of OBI in Egypt was reported among patients with HCC. A difference in OBI prevalence rates as per the biological matter tested is reflected in an Egyptian study where intrahepatic occult HBV DNA was detected in 62.5% cases, whereas serum occult HBV DNA was detected in only 22.5% of the same HCC patient group [2].

The present study was designed to assess the indirect but important role of the traditional anti-HBc assay for the identification of patients previously exposed to HBV in the context of the modern definition of OBI and determine the influence of OBI on the risk of HCC in HCV-infected patients.

In the present study, the mean age among patients with HCV plus HCC (HCC group) was 57.17 ± 8.67 years, which was higher than the mean age among HCV cirrhotic patients (LC group) (53.2 ± 9.25 years), with statistically significant difference between the groups.

In accordance with this finding, Moucari *et al*. [9] reported that most of HCC patients were older than 57 years, and the mean age in this age group showed significant difference when compared with patients with chronic hepatitis C.

The current study revealed that detection of anti-HBc antibody positivity was higher in patients with HCC (HCC group) (21%) than cirrhotic patients (LC group) (19%). However, these differences revealed in our study were statistically insignificant. In accordance with our study, several studies [10–12] showed that anti-HBc antibody positivity was not found to be associated with the development of HCC in patients with HCV-associated chronic liver disease.

In the present study, HBV DNA on HBc antibody-positive patients was detected in patients with HCC (15/100 patients, 15%) 'HCC group' and (11/100 patients, 11%) in HCV cirrhotic patient 'LC group', so of 200 patients included in this study, 26/200 (13%) had OBI, that is, not detected in all HBc antibody-positive patients. Comparable to our study, others showed that HBV DNA sequences could be detected in some of the liver or serum from anti-HBc-positive patients [10,13], and the presence of anti-HBc did not entirely exclude the possibility of chronic HBV infection. Although the presence of

anti-HBc had been used as a marker of past HBV infection, the integration of HBV DNA in hepatocytes might cause carcinogenesis. That is, anti-HBc positivity might represent OBI. The presence of anti-HBc alone, in the absence of HBV DNA testing, had been used in some studies as a marker of occult hepatitis B.

In our study, OBI showed statistically significant relation with development of HCC in general in comparison between HCC and non-HCC patients, but its role in the development of HCC in HCV-confected patients was less related; the statistically insignificant relation might be owing to the risk attributable to HCV alone. In accordance with our study, Pollicino *et al*. [14] provided clear evidence that occult HBV was a risk factor for the development of HCC and showed that the potential mechanisms whereby HBV might induce tumor formation occur in most cases of occult infection. Ikeda *et al*. [15] demonstrated in a multicenter prospective study that in patients with HCV-related LC (n = 270), HCC developed in 85 (60.3%) of 141 patients with anti-HBc and 58 (45.0%) of 129 patients without anti-HBc. Their multivariate analysis of factors contributing to HCC occurrence identified that anti-HBc positivity was an independent risk factor with a hazard ratio of 1.58.

In our study, there was a significant relation between OBI and progression and advancement of HCC with risk of HCC four times higher than those without OBI (odds ratio = 4, 95% confidence interval = 1.62–9.4) regarding the number of hepatic focal lesions, size of largest one, and invasion of PVT. In accordance with our study, it showed statistically significant more hepatic focal lesions and malignant PVT through radiological diagnosis in group I (OBI/HCV dual infection) as compared with group II (HCV mono-infection). Similar results were obtained in an Indian study by Kumar *et al*. [16] who found that the incidence of gastrointestinal bleed was high in their study (22%).

Conclusion

In conclusion, this study revealed also statistically significant relation between OBI and development of HCC in general in comparison between HCC and non-HCC patients as well as progression and advancement of HCC regarding number of hepatic focal lesions, size of largest one, and invasion of PVT, but its role in the development of HCC in HCV-confected patients was less related, with statistically insignificant relation might be owing to the risk attributable to HCV alone.

Finally, we recommend carrying out further large-scale studies to confirm the findings of the current study. Larger studies including a large number of patients should be done to assess the status of OBI in Egypt. OBI should be included in the study of any patient with chronic liver disease or HCC. Other studies should include testing for intrahepatic DNA by liver biopsy besides testing for serum HBV DNA. Moreover, studies should include testing for serum HBV DNA for all participants including HBcAb negatives for more accurate assessment of sensitivity and specificity of HBcAb as a marker for identification of OBI. Evaluation and management of patients with HBV and HCV without HCC should be done.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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