Significance of screening antibodies to hepatitis B core antigen among chronic hepatitis C patients before antiviral therapy

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Background and study aim: Anti-hepatitis B core (anti-HBc) sero-positivity in general population in Egypt is reported to be 10-13%. This study was performed to determine the prevalence of anti-HBc among chronic hepatitis C patients before antiviral therapy.

Patients and Methods: A total of 178 consenting patients with chronic HCV infection eligible for treatment with DAAs according to program of National Committee for Control of Hepatitis Infection in Portsaid, Egypt from April to October 2017. All of the patients were screened for anti-HBs and anti-HBc. Anti-HBc–positive patients were assayed for HBV DNA.

Results: Out of 178 chronic hepatitis C patients, Eighty four patients (47.2%) were treated with triple therapy (Sofosbuvir/ Daclatasvir/Ribavirin) and ninety four patients (52.8%) with dual therapy (Sofosbuvir/Daclatasvir). A65 patients (36.5%) were reactive for anti-HBc. Of 84 patients, 34 (40.5%) who treated with triple therapy were reactive for anti-HBc. Of 94 patients, 31 (33%) who treated with dual therapy were reactive for it. All patients were negative for anti-HBs and anti-HBc–positive patients were no detected HBV DNA at baseline and 12 weeks after DAAs.

Conclusion: Our results suggest including anti-HBc as an additional screening test for chronic hepatitis C patient in Egypt who are eligible for DAAs to reduce the risk of HBV reactivation and fulminant hepatitis after DAAs.

INTRODUCTION

Hepatitis B virus (HBV) and Hepatitis C virus infection are leading causes of chronic liver disease worldwide, affecting 350-400million and 170 million people, respectively **[1]**. HCV is currently the most significant public health problem in Egypt **[2]**. The recently published Egyptian Demographic and Health Survey (EDHS) in 2015 estimated an overall antibody to hepatitis C virus (anti-HCV) prevalence of 6.3% **[3]**. Anti-hepatitis B core (anti-HB_C) sero-positivity in general population in Egypt is reported to be 10-13% **[4]**.

HBV and HCV share common modes of transmission, thus simultaneous infection is quite frequent, particularly where both viruses are endemic as among people with a high risk for parenteral infections **[5]**. HCV infection has a suppressive effect on the replication of HBV, shown by the loss of replicative markers as HBV-DNA [6]. The extensive application of sensitive molecular tests such as polymerase chain reaction (PCR) and real-time PCR has enabled HBV-DNA to be detected in specimens from individuals without serological evidence of chronic HBV infection **[7]**.

Occult HBV infection (OBI) can be defined by the presence of HBV-DNA in the serum of patients who are negative for HB_S Ag **[8]**. In the last decade, OBI pattern has been documented and frequently identified in patients with chronic hepatitis C (CHC) infection **[9]**. The prevalence of OBI in chronic HCV patients was higher in subjects having either anti-HBs or anti-HB_C or both **[4]**. Sero-

logical findings in patients with OBI and HCV co-infection revealed that 35% of people were anti-HBs positive, 42% were anti-HB_C IgG positive and 22% were negative for both **[10]**.

Treatment of chronic hepatitis C virus (HCV) infection has been revolutionized in the last few years by the introduction of highly effective and well-tolerated DAAs able to achieve high rates of sustained virological response(SVR) in many groups of patients **[11]**. In past years, HBV reactivation occurring in HBV/HCV-co-infected patients treated with IFN-based therapy has been reported, probably as a consequence of an unbalanced HBV replication caused by treatment-related suppression of HCV, although a direct immune-modulatory effect of IFN might also be advocated for either on- or off-treatment HBV reactivation **[12].**

In contrast, DAAs have no effect on HBV replication, but such therapies may release HBV from HCV suppressive effects, resulting in HBV reactivation in CHC patients with a concomitant overt or occult HBV Infection, leading to acute hepatitis with the risk of liver failure both on- or off-treatment **[13,14]**. Despite this, up to 2015 EASL and AASLD guidelines on HCV treatment did not provide specific indications for the management of OBI during or after HCV clearance by DAAs **[11]**.

This study was performed to determine the prevalence of anti-HBc and frequencies of hepatitis B virus (HBV) DNA and antibodies to hepatitis B surface antigen (anti-HBs) among chronic hepatitis C patients before antiviral therapy.

PATIENTS AND METHODS

Type of Study: Follow up descriptive study.

Site of Study: Port-Said center for treatment of viral hepatitis in Port-Said Fever Hospital.

Study Population:
Chronic hepatitis C patients treated with Sofosbuvir- based regimens.

Criteria of selection:
All 178 patients enrolled in this study were previously diagnosed as chronic hepatitis C patients aged 18-70 years. All patients which had decompensated liver diseases, hepatocellular carcinoma, extra-hepatic malignancy and uncontrolled diabetes mellitus (HbA1c >8%) were excluded. All these criteria were according to the protocol provided by national committee for control of viral hepatitis in Egypt (NCCVH) in December 2016.

Study methods:
Patients who enrolled into the study assessed anti-HB_C in serum of CHC patients before starting Direct Acting Anti-viral(DAAs) treatment regimen if positive assess HBV-DNA at baseline, end of treatment and at SVR12weeks.

A- Data collected by personal interview included:

1- Personal interviewing: for age, sex, residence, special habits, education, job, marital status , duration of liver disease since diagnosis.
2- History suggestive of etiology (blood transfusion ,dental extraction).
3- Presence of any other chronic illness (e.g. diabetes mellitus, hypertension, thyroid disorder, cardiac patient,…etc).
4- History of encephalopathy or hepatocellular carcinoma HCC.
5- In treatment experience patients type of IFN and date of last dose taking.
6- History of schistosomal infection ,if present type of treatment taking.
7- Clinical examination of patients which include:

B- General examination: with special emphasis on vital signs and the presence of signs of chronic liver disease such as darking of the face, wasting of temporalis and maseter muscles and prominent zygomatic bone, bilateral parotid enlargement, jaundice, fetor hepaticus, palmar erythema, lower limb edema, flapping tremors and impaired level of consciousness.

C- Local examination**:** withspecial emphasis on liver examination and detection of ascites.

D- Laboratory investigations:

1. Fasting blood sugar and if diabetic HbA1c
2. complete blood count.
3. Aspartate aminotransferase(AST), alanine aminotransferase (ALT).
4. Serum creatinine.
5. Prothrombin concentration or INR.
6. Serum albumin.
7. Serum total and direct bilirubin.
8. AFP.
9. HBs Ag.
10. HCV Ab and PCR for HCV if positive.
11. Anti-HB_c and HBV-DNA if positive at baseline and at SVR12weeks.

E- Assay of Anti-HB_c: Antibody to Hepatitis B virus Core Antigen Elisa Kit was provided by *Wkea med supplies corp*.

1- Sample Preparation**:** 10ml blood taken from patients.2ml in EDTA tube for CBC and HbA1C and 8ml in a plain tube for all other tests. Samples are allowed to clot for 10-20 mins at room temperature before centrifugation for 20mins at the speed of 2000-3000 r.p.m. Remove serum in eppendorf centrifugal tubes and store it at -20C until used.

2- Principle of The Assay: This kit is based on solid phase, one step incubation competitive principle ELISA method. Anti-HBc if present in the sample, compete with monoclonal anti-HBc conjugated to horse radish peroxidase (HRP-Conjugate) for a fixed amount of purified HBcAg pre-coated in the wells. When no anti-HBc present in the sample, the HRP-conjugated anti-HBc will be bound with the antigens inside the wells and any unbound HRP-conjugate is removed during washing. Chromogen A and B solutions are added into the wells and during incubation, the colorless Chromogens are hydrolyzed by the bound HRP-Conjugate to a blue-colored product. The blue color turn yellow after stopping the reaction with sulfuric acid. No or low color developing suggests for presence of antibodies to HBcAg in the sample.

Statistical analysis

Statistical analyses were performed using the statistical software package SPSS for Windows, version 19 (SPSS, IBM Inc., NC, USA). Baseline demographic and clinical characteristics were analyzed descriptively for all patients. Categorical variables were expressed as frequency and percentage while the continuous variables were expressed as mean and standard deviation. The normality of distribution for all variables was tested by Shapiro- Wilk test. We used the Mann–Whitney *U*-test, the Fisher's exact test and student's t test where appropriate.

RESULTS

A Total of 178 chronic hepatitis C patients initiated treatment with DAAs. Eighty four patients (47.2%) were treated with difficult treatment (SOF/DAC/RBV) and ninety four patients (52.8%) with easy treatment (SOF/ DAC). one hundred and seventy patients were treated for 12 weeks and eight treated for 24 weeks (Table 1). There were no significant differences between chronic hepatitis C patients with positive HBcAb vs. those with negative HBcAb regarding sex, age, history of smoking, Hemoglobin, and HCV RNA. However, positive HBcAb patients had significantly diabetes mellitus (Table 2). There was no significant correlation between liver enzymes and HBcAb seropositivity. However ALT and AST had reasonable sensitivity around 70% at cut off points 29.5 and 32.5 respectively the specificity dropped to25.4%and 40.4%, respectively (Figure 1).

Table (1): Baseline demographics, clinical characteristics and laboratory values in all patients

Parameter	Overall patients (178)
Age (years)	54.3±8.3
Sex (No., %)	
Males	93(52.2%)
Females	85(47.8%)
Smokers (No., %)	53(29.8%)
Diabetes mellitus (No., %)	21(11.8%)
Previous treatment failure (No., %)	
NO	170 (95.5%)
SIM/SOF	1(0.6%)
SOF/RBV	7(3.9%)
Hemoglobin (g/dl)	13.4±1.6
White blood cell count (/mm^3)	6.1±2.0
Platelets (X 10^3/mm^3)	173.6±72.3
ALT (IU/ml)	49.8±35.7
AST (IU/ml)	51.0±36.6
Bilirubin (mg/dl)	0.8±0.4
Albumin (g/dl)	3.9±0.5
INR	1.1±0.1
AFP (ng/ml)	8.6±10.5
Fib 4 score	2.9±2.4
HBsAg (negative)	178(100.0%)
HbcIgG	
Negative	113(63.5%)
Positive	65(36.5%)
HCV RNA (log 10 IU/ml) (mean ± SD)	3.3±11.0
HBV DNA (log10iu/ml)	0.0±0.0
Splenomegaly (No., %)	36(20.2%)

(ALT) alanine amino-transferase, (AST) aspartate amino-transferase, (INR) international normalized ratio, (AFP) alpha-fetoprotein, (HCV) hepatitis C virus, (SD) standard deviation .

Table (2): Baseline data for patients with positive HBcAb vs. negative HBcAb

Sociodemographic data	HbcAb		P value
	Positive (64) Mean ± SD	Negative (114) Mean ± SD	
Age	54.6±7.8	53.9±8.7	0.644
Sex (No., %)			
Males	34(53.1%)	59(51.8%)	0.861
Females	30(46.9%)	55(48.2%)	
Smoking (No., %)			
Nonsmoker	86(75.4%)	45(70.3%)	0.457
Smoker	28(24.6%)	19(29.7%)	
Diabetes mellitus (No., %)	12(18.5%)	9(8.0%)	0.037*
Previous treatment failure (No., %)			
NO	62 (96.9%)	108 (94.7%)	
SIM/SOF	0(0.0%)	1(0.9%)	0.675
SOF/RBV	2(3.1%)	5(4.4%)	
Splenomegaly (No., %)	17(26.2%)	19(16.8%)	0.135
Platelets	161.2±63.1	180.6±76.3	0.146
Hb	13.2±1.5	13.4±1.6	0.417
WBC	6.0±2.1	6.1±1.9	0.532
AST	48.8±29.4	52.3±40.1	0.850
ALT	46.3±27.3	51.8±39.6	0.697
INR	1.1±0.1	1.1±0.1	0.244
T. bilirubin	0.8±0.3	0.8±0.4	0.107
S. albumin	3.9±0.5	3.9±0.5	0.534
AFP	7.9±5.5	9.0±12.4	0.130
Fib 4 score	3.2±2.7	2.7±2.2	0.250
HBV DNA (log 10 IU/ml)	0	0	
HCV RNA (log 10 IU/ml)	5.8±15.8	1.8±6.3	0.056

* Statistically significant at $p<0.05$

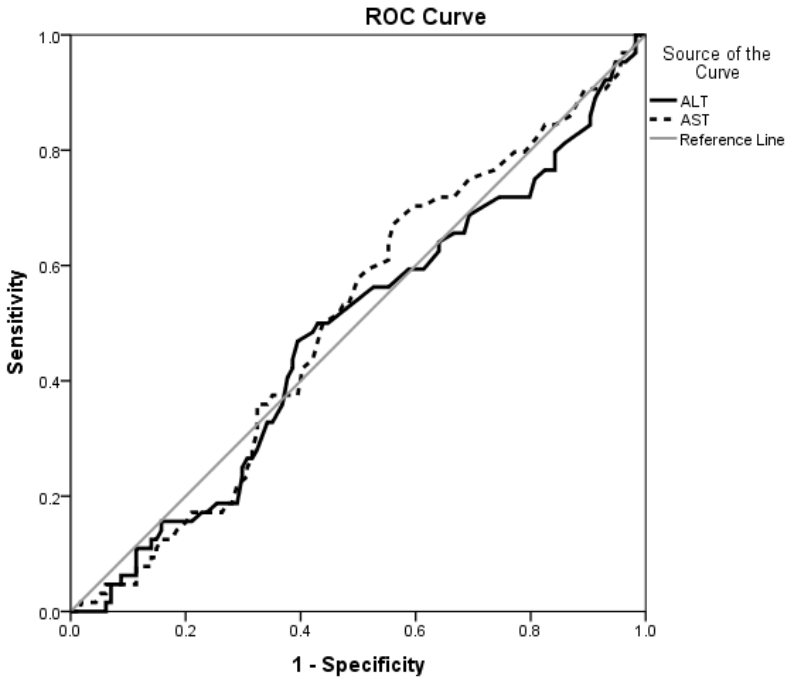

Figure (1): ROC curve and determine optimum cutoff value for ALT&AST to start screening for occult hepatitis B before DAAs

Area Under the Curve					
Test Result variables	Area	Std. Error	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
ALT	0.48	0.045	0.697	0.394	0.571
AST	0.51	0.044	0.850	0.421	0.596

	Cut off value	Sensitivity	Specificity
ALT	29.50	71.9%	25.4%
AST	32.50	70.3%	40.4%

DISCUSSION

In this study OBI seroprevalence tested by HBcIgG Elisa has positive for sixty four patients(35%)with mean age of 54.6years old ,more in males (53.1%) than females (46.9%), type 2 diabetes mellitus (18.5%), highly Fib 4 index >3.25 and highly HCVRNA($5.8x10^5$ IU/ml). The EDHS 2015 included tests for anti- hepatitis B core antibody (anti-HB) and hepatitis B surface antigen (HBsAg) in addition to testing for HCV. The age prevalence of anti-HBc (indicating exposure to hepatitis B virus [HBV] infection) mirrors the age prevalence of anti-HCV in both males and females.

The incidence of OBI in HCV patients varies greatly, ranging from 0% to 52**%** [15]**.** In this study; 35% of HCV patients were sero-positive for OBI. Our results were in agreement with those reported among Mediterranean countries **[16]**.

Furthermore, two similar studies Fukuda et al. and Liu et al., reported that the serum titer of HCV-RNA was visibly higher in patients with concurrent HBV and HCV infection than those with HCV mono-infection, this reports agreed with this study **[17,18]**. Additionally, Mrani et al., also found that HCV viral load was significantly higher in HBV-DNA positive than in negative patients **[1]**. Also, Emara et al. **[15]** reported that this seemed to be applicable to genotype 4, where HBV-DNA positive patients in their study showed higher baseline HCV viral load than HCV mono-infected patients.

Also, Chen et al.**[19]** reported that patients with both OBI and HCV infection had lower ALT

levels, liver histology activity index and fibrosis scores than those with HCV mono-infection. The clinical impact of OBI on anti-HCV therapy outcome is still controversial.

Few studies however evaluated the impact of occult hepatitis B infection on the current standard treatment of HCV infection and viral replication of HBV. European Medicines Agency (EMA, 2016) reported all the cases of OBI patients should be interpreted with caution before establishing a clear correlation between effective DAAs treatment and HBV reactivation, due to the presence of at least one possible confounding factor.

In recent study, De Monte et al., reported HBV reactivation in an HIV/HCV co-infected male who discontinued TDF 14 months before starting DAAs due to bone toxicity **[20]**. In this case the role of HIV infection and/or the immune reconstitution after effective HAART cannot be excluded as causes of the HBV reactivation, not to mention discontinuation of TDF that is effective on HBV.

Also, there is a recent Asian study in 124 HCV infections with OBI patients treated with DAAs showing no cases of HBV reactivation **[21]**.

Yeh et al., observed a minimal impact of anti-HBc seropositivity on HCV efficacy and safety, while the risk of reactivation was present for CHC patients with current infection **[22]**. Similarly, Belperio et al., and Sulkowski et al., affirmed the rarity of HBV reactivation after DAA, even in the setting of isolated anti-HBc **[23,24]**.

So, it is important to screen all CHC patients for HBV markers (HBsAg, anti-HBc and anti-HBs) before starting DAAs .OBI patients serum HBV DNA should be assessed with a very sensitive test at baseline and monitored during and after DAAs in those patients with positive baseline. Whereas no periodic monitoring of serum HBV DNA or HBsAg during and after DAAs treatment is recommended in patients with undetectable.

Baseline serum HBV DNA. In the latter patients periodic monitoring of ALT may be enough to detect hepatic flare reflecting HBV reactivation to be treated with anti-HBV therapy. Current EASL guidelines even suggest starting concurrent HBV nucleoside/nucleotide analogue therapy if HbsAg is present or HBV-DNA is detectable in OBI **[11]**.

However, further prospective studies in large cohorts of OBI patients better characterized from the virological point of view at baseline and during and after EOT are needed to quantify and stratify the risk of HBV reactivation in parallel with HCV eradication, and to standardize the management of such patients in order to avoid the risk of fatal complication.

CONCLUSION

In conclusion, occult hepatitis B Infection is highly prevalent in chronic hepatitis C infection and not depend on HBs Ag only in diagnosis for HBV infection. In future we need to check for HBsAg, anti HBc, and if positive screen for HBVDNA PCR in chronic hepatitis C infection treated with oral direct acting antiviral drug in initial visit, end of treatment and after 12 weeks of treatment for fear of reactivation of HBV infection.

Ethics:
The study confirmed to the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Suez Canal University Faculty of Medicine in February 2016. Written, informed consent was obtained from each patient included in this study.

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No Conflict of Interest.

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