Egyptian Journal of Medical Microbiology Volume 24 / No. 4 / October 2015 113-118

ORIGINAL ARTICLE

Occult Hepatitis B Virus Infection among Egyptian Hepatitis C Virus Seropositive and Seronegative Hemodialysis Patients in Sohag Government, Upper Egypt

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Key words:

Occult HBV, HCV, Hemodialysis

ABSTRACT

Background: Hepatitis B virus (HBV) and hepatitis C virus (HCV) Infections are important and common causes of liver disease in end-stage renal failure (ESRF) in patients on haemodialysis (HD). HBV is less endemic than HCV in Egypt (ranges from 2%–7%). Although, the prevalence of HBV in haemodialysis patients has decreased significantly due to HBV vaccine and screening of blood donors, the immunosuppressive nature of renal disease often leads to chronicity of the HBV infection and an opportunity for nosocomial spread of the infection among dialysis patients. Haemodialysis patients are more risky to develop occult hepatitis B infection (OBI) due to an increased number of blood transfusions, frequent invasive procedures, difficulty in diagnosis of occult hepatitis B infection (OBI) and immunosuppression. Occult hepatitis B infection (OBI) is defined by the presence of HBV DNA in serum or liver tissue in the absence of HBsAg. **Objective:** to study the prevalence of occult HBV infection in HCV-positive and HCV negative patients on regular hemodialysis from Upper Egypt. **Methodology:** One Hundred hemodialysis patients with negative HBsAg were included in the study. These patients were divided into two groups: HCV positive and HCV negative, based on the results of anti-HCV by ELISA and HCV-RNA by PCR. HBV-DNA was studied using the real-time PCR method in both groups. **Results:** HBV DNA was detected in 7 of the 100 patients (7%) and HBcAb was detected in 22 patients (22%). There were no statistically significant differences in the age, sex, duration of hemodialysis, biochemical parameters, HBcAb, or HBV DNA between patients with and without HCV infection. **Conclusion:** The prevalence of occult HBV infection (OBI) among Egyptian hemodialysis patients is 7 % with no significant difference in the prevalence of OBI between hemodialysis patients with or without HCV infection and we suggest screening of all HD patients for OBI by testing anti-HBc and HBV DNA

INTRODUCTION

Despite of vaccination against Hepatitis B virus (HBV) infection, it stills one of the major causes of acute and chronic liver disease. There are about 350-400 million chronically HBV infected people and 5.7 million HBV-related cases worldwide [1]. *Tabor et al.* [2] defined Occult HBV infection (OBI) by the presence of HBV DNA in blood or liver tissues in patients who test negative for HBsAg, with or without hepatitis B core (anti-HBc) or hepatitis B surface (anti-HBs) antibodies outside the pre seroconversion window period**.** [3,4]

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In patients with OBI HBV DNA is converted to covalently closed circular DNA (cccDNA), then binds to different proteins to become a stable and durable mini-chromosome that persists indefinitely within hepatocyte nuclei. [5]

According to detected serum markers of HBV, there are two different serological patterns have been defined. Seropositive pattern of OBI (about 80% of cases), which includes OBI patients who are positive for the anti-hepatitis B core antibody (anti-HBc) and/or for the anti-hepatitis B surface antibody (anti-HBs), yet lack detectable HBsAg in serum. This may be due to recovery from acute hepatitis B after months of HBsAg carriage. Seronegative OBI (about 20% of cases) represents patients who are negative for both antibodies (anti-HBc and anti HBs) with very low levels of HBV DNA. This serological pattern may reflect the time between infection and the detection of antibodies known

as the 'window period' (pre-seroconversion), or clearance of hepatitis B antibodies. This seronegative OBI pattern is very important as a source of infection since it is very difficult to diagnose due to absence of serum HBV antigens or antibodies. [27]

Occult HBV infection is worldwide distributed, and its prevalence is related closely to the endemicity of HBV infection. The prevalence of HBV is heterogenic worldwide; it is highly endemic in Africa and intermediately endemic in Egypt (range 2%–7%) [6,7] in contrast to HCV which is highly endemic in Egypt (17.5%). [8,9,10,11]

Many studies reported that occult HBV infection is highly prevalent in patients with chronic hepatitis C virus infection, patients with hepatic carcinoma, patients on haemodialysis machines, cryptogenic liver disease, drug injection misuse, HIV patients and in persons need for frequent blood transfusion. [3,12,13]

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are well-known and important causes of liver disease in end-stage renal failure (ESRF) patients on hemodialysis (HD) [14]**.** Although the adoption of preventive measures and extensive infection control guidelines, along with a decreased need for transfusions after the introduction of erythropoietin, and the development of an effective HBV vaccine, have significantly contributed to the progressive reduction of HCV and HBV prevalence in HD patients, but it still a distinct clinical problem, as the immunosuppressive nature of renal disease often leads to chronicity of the viral infection and results in an opportunity for nosocomial spread of the infection among dialysis patients [15,16]

Most of the studies that investigated occult HBV infection were done in the context of chronic HCV infection, so data on the prevalence of OBI among HD patients are scarce and heterogenic. In Egypt, *Abu El Makarem et al.* [17] and *Elgohry et al.* [18] reported that the OBI prevalence in Egyptian hemodialysis (HD)-patients range from 4.1% to 26.9%**.** In Spain *Cabrerizo et al* [19] reported the highest OBI prevalence (58%) but American investigators reported the lowest prevalence (3.8%) [22]. Results reported from Turkey varied from 0% to 27.5% [20,21]. Studies from Greece showed that 0.9%-20.4% of HD-patients suffered from OBI [23].

Despite the potential clinical importance of OBI, existing information regarding the prevalence of OBI among Egyptian patients undergoing long-term HD is limited. The aim of this survey is to study the prevalence of occult HBV infection in HCV-positive and HCV negative patients on regular hemodialysis from Sohag government, Upper Egypt.

METHODOLOGY

Patients:

This study was conducted on 100 patients (68 males and 32 females) with end-stage renal disease undergoing regular HD, 50 patients with chronic HCV infection and 50 patients without HCV infection from the dialysis unit of the Department of Internal Medicine, Sohag University, Egypt, during the period from March 2014 to October 2014. Their ages ranged between 18 to 72 years. The study protocol was approved by the local ethics committee and all patients gave their consent prior to the study.

Inclusion criteria:

Enrolled patients were:

a. On regular HD for at least 6 months
b. Group I: Positive for anti-HCV antibodies and HCV-RNA.
c. Group II: Negative for anti-HCV antibodies
d. Patients were negative for HBs-Ag and Antinuclear antibodies (ANA).

Exclusion criteria:

a. Overt HBV infection (HBs-Ag positive).
b. Patients received HBV vaccine
c. Patients with decompensated, alcoholic, autoimmune, malignant or metabolic liver diseases
d. Patients under HCV treatment.

All the studied patients were subjected to the following:

I. Clinical assessment: History taking, physical examination including age, residence, HBV vaccination history, blood transfusion history, duration of hemodialysis, etiology of renal disease, and history of schistosomiasis
II. Biochemical assessment: Liver functions, kidney functions, complete blood counts, ANA, Schistosomal antibodies and AFP

Methods:

Blood samples were collected before hemodialysis for serum separation from all patients and then stored at -80C° until tested.

1. Serodiagnosis of HCV infection

1a. Detection of anti-HCV AB by ELISA:

Antibodies to HCV were detected with commercially available ELISA kits (Sorin Biomedica, Italy) using the Stat fax 2600 micro-plate reader (Awareness Technologies, Palm City, USA) according to manufacturer's instructions

1b. Detection of HCV RNA by PCR:

RNA was extracted from patients' sera according to manufacturer's instructions by Qiagen kit (Germany). HCV RNA was detected using a quantitative PCR assay COBAS Amplicor HCV Test, v2.0; Roche Diagnostics; lower limit of detection; 50 IU/mL).

2. Serodiagnosis of Occult HBV infection (OBI)

2a. Serological markers of HBV infection

Serological markers of HBV infection (HBsAg, anti-HBc total) were determined using commercially available AviBion ELISA Kit, manufactured by orgenium Laboratories (Helsinki, Finland) for HBsAg and Dia. Pro. Diagnostic Bioprobes Srl. (Milano, Italy) for anti-HBc using the Stat fax 2600 microplate reader (Awareness Technologies, Palm City, USA) according to manufacturer's instructions

2b. Detection of HBV-DNA by real time PCR

HBV-DNA was extracted from serum samples using QIAxtractor®, and VX kit as recommended by the manufacturer (QIAGEN- Germany), PCR setup was automated via QIAgility (QIAGEN, Germany). HBV real-time assays were performed in combination of Artus HBV RG PCR Kit (Artus™ GmbH, Hamburg Germany) and the Real time PCR instrument, Rotor-Gene Q (QIAGEN, Germany). Thermal profile was set according to manufacturer's guideline. Detection limit of HBV-DNA in the current study assay is 3.8 IU/mL assessed by the World Health Organization (WHO) international standard (97/750) [24]**.** At least two negative controls, one non template control, and four standards (provided by the manufacturer) were added per run. Strict precautions were taken to avoid possible contamination.

Statistical Analysis

Data were analyzed using Sigma Plot software (SPSS version 2). We used Chi-square tests ($\chi 2$) and Student's t-test; Data are presented as percentages, means ± standard deviation (SD). *P* values <0.05 were considered significant.

RESULTS

Analysis of the patients data showed that their ages were 50.5 ± 11.2 years (range: 18–72 years), and 68 % of the patients were male. Two groups were studied according to HCV RNA results; Group I: HCV positive group (50 patients) and Group II: HCV negative group (50 patients) (Table 1).

The mean ages of the patients in groups I and II were 49.5 ± 10.0 and 50.0 ± 9.9 years, respectively. In group I; 76 % of the patients were males and 24 % were females, while 60 % of group II patients were males and 40 % were females. The mean hemodialysis duration of groups I and II were 39.3 ± 10.2 and 38.7 ± 11.0 months, respectively (Figure 1). 45 (90 %) and 37 (74 %) patients in groups I and II, respectively received blood transfusion. We found no statistically significant differences between the 2 groups based on age, sex, residence, hemodialysis duration or laboratory parameters (Table 1). HBV-DNA was detected in 7 of the 100 patients **(7%)** with ESRF (Table 2). These 7 patients were considered to have OBI. When these data were divided according to HCV RNA results; HBV-DNA was detected in 4 (8%) and 3 (6%) of group I and group II patients, respectively (*P* = 0.4). A total of 22 (22%) patients were anti-HBc positive, with significantly higher ratio in group I (14 patients (28%)) than in Group II (8 patients (16%)); (*P* = 0.04). The presence of anti-HBc, in the absence of HBV-DNA in 15 patients (15%), suggests recent recovery from an acute HBV infection (Table 2)

Table 1: Demographic and Laboratory Data of the HD patients

	All Patients (n = 100)	***HCV +ve (n = 50)***	***HCV -ve (n = 50)***	***P value***
Age(years), mean ± SD [a]	50.5 ± 11.2	49.5 ± 10.0	50.0 ± 9.9	0.4
Male No. (%)	68 (68)	38 (76)	30 (60)	
Female No. (%)	32 (32)	12 (24)	20 (40)	0.5
Rural No. (%)	75(75)	37(74)	38 (76)	
Urban No. (%)	25 (25)	13 (26)	12 (24)	0.3
Schistosoma antibodies Positive	65 (65)	35 (70)	30 (60)	
Schistosoma antibodies Negative	35 (35)	15 (30)	20 (40)	0.6
Duration of hemodialysis (Months)	40.2 ± 9.6	39.3 ± 10.2	38.7 ± 11.0	0.2
Positive history of transfusion, No. (%)	82 (82)	45 (90)	37 (74)	0.3
AST [a] **(IU/L)**	30.6 ± 4.3	31.0 ± 5.1	31.8 ± 4.8	0.1
ALT [a] **(IU/L)**	26.03 ± 6.1	27.2 ± 6.1	26.0 ± 1.1	0.1
Albumin (g/dl)	3.6 ± 0.9	3.4 ± 0.6	3.6 ± 0.8	0.3
Bilirubin (mg/dl)	1.7 ± 0.2	1.7 ± 0.6	1.7 ± 0.4	0.2

a Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; SD, standard deviation

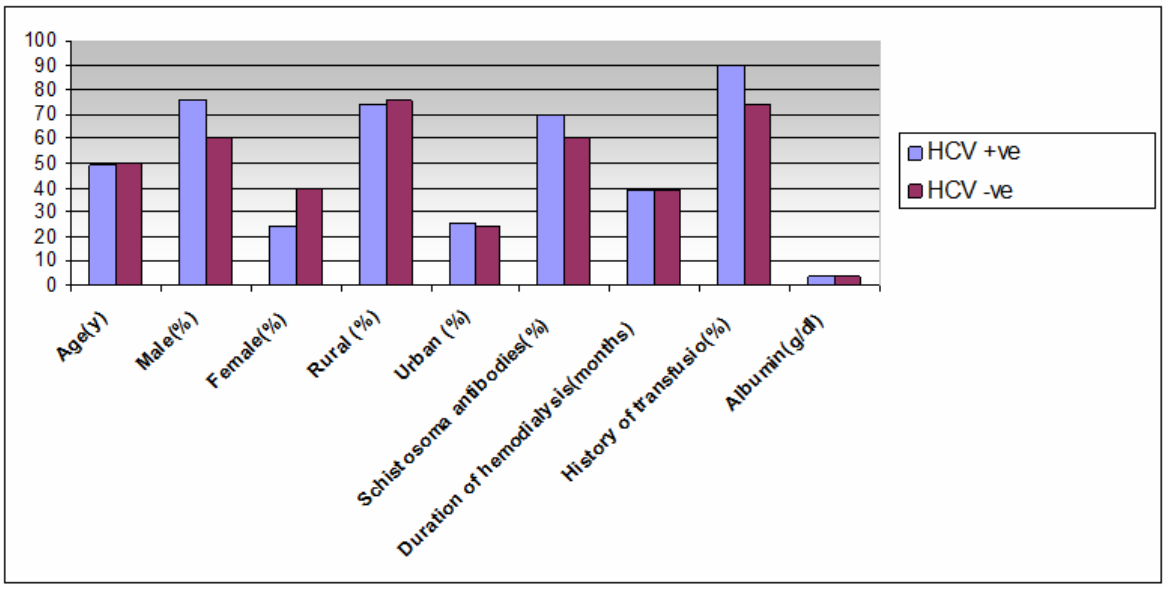

Figure 1: Comparison between HD patients with and without HCV infection

Table 2: Prevalence of anti HBc by ELISA and HBV DNA by PCR

Serological marker	*All Patients (n = 100)*	*HCV +ve (n = 50)*	*HCV -ve (n = 50)*	*P value*
Anti-HBc +ve	22 (22)	14 (28)	8 (16)	0.04
HBV DNA +ve	7 (7)	4 (8)	3 (6)	0.4
Anti-HBc +ve & HBV-DNA -ve	15 (15)	10 (20)	5 (10)	0.03

Abbreviations: Anti-HBc, hepatitis B core antibody; HBV DNA, hepatitis B virus DNA

DISCUSSION

Although the prevalence of HBV infection is decreasing in Egyptian children (0% to 1.6%) [25,26] due to universal vaccination, the prevalence in adults is relatively high (7.1% to 9.3%) [24].

It is not so easy to detect the real prevalence of OBI because it is diagnosed by detection of HBV DNA in the hepatocytes in patients who test negative for HBs-Ag, but this is not feasible because the performance of liver biopsies in patients undergoing HD is often very difficult, and is usually contraindicated, so the diagnosis of OBI is mostly based on the result of a blood test. However, blood sample tests may be negative for HBV DNA but liver sample is positive since HBV DNA occurs inside the nuclei of hepatocytes. [27,28,29,30]

It is very important to define the optimal methodology to quantify HBsAg and HBV DNA because HBsAg commercial assays may miss mutations in the S region causing false results of OBI. [29,31] Hepatologists should bear this in mind and we should use a highly sensitive and specific assay because OBI is usually associated with low levels of HBV DNA. Detection limit of HBV-DNA in the current study assay is 3.8 IU/mL which is very low level and clearly improves sensitivity. [24] When sensitive HBV DNA tests are not available, it is recommended to test for anti-HBc antibodies as a surrogate marker to identify potential seropositive OBI patients. It is the first antibody to appear and considered a sign of active or past infection depending on the other HBV serum markers but we should be aware that the absence of this antibody (anti-HBc) does not rule out OBI (seronegative-OBI). [28]

In our study only HBs-Ag negative HD patients were included and we found that 7 % of those patients had OBI. The prevalence in patients with HCV infection was 8% and in patients without HCV infection was 6% and this difference is not statistically significant.

The prevalence of OBI among HD-patients is difficult to assess because it is not region specific, difficult to diagnose and immunosuppressive nature of these patients. Different studies reported contradictory results from the same country. Previous reports from studies in dialysis units have indicated that the prevalence of OBI ranges from 0% to 58% among patients[15,20,21]. This discrepancy in results may attributable to differences in the sensitivity of the methods used for the diagnosis [22], the patients' sample investigated in each study and the endemicity of HBV different geographic areas.

OBI prevalence among Egyptian adults ranges from 0.48**%** to 58.3% [32], with lower prevalence among blood donors and higher prevalence among chronic liver disease patients. In Egyptian hemodialysis (HD) patients, the prevalence of OBI range from 4.1% to 26.8% [17,18]. Many studies on HD-patients have provided divergent results from different parts in the world; the highest OBI prevalence (58%) in HD-patients reported from Spain [19]**.** American investigators reported the lowest prevalence (3.8%) [22]. Results reported from Turkey varied from 0% to 27.5% [20,21]. Studies from Greece showed that 0.9%-20.4% of HD-patients suffered from OBI [23].

In HD patients; Positive anti-HBc Ab and negative HBs-Ag might suggest previous with HBV positive persons during adulthood. In our study, we found that 22% of patients were anti-HBc positive, with significantly higher ratio in patients with HCV infection (28%) than in patients without HCV infection (16%) and these results are closely similar to the results of a previous study that reported a significant associations between the presence of HBV- DNA and anti-HBc positivity, and reported that the prevalence of anti-HBc was 20 %. Also our results were similar to that reported by *Abu El Makarem et al* which also were reported in Upper Egypt. [17]

In this study we found no significant differences between patients with and without HCV infection as regard age, sex, and residence, and blood transfusion history, duration of hemodialysis, schistosomal antibodies, liver enzymes, and serum albumin level.

Few studies were done to compare the prevalence of HBV in Upper and Lower Egypt [25]. Also regarding the prevalence of OBI in Upper and Lower Egypt, few studied were done, only two studies addressed OBI prevalence in Upper Egypt and reported lower OBI rate (4.1%) among Upper Egypt (Minia and Assuit) hemodialysis (HD) patients [17] compared with HD patients in Lower Egypt (Alexandria) that reported higher rate (26.9%) [18].

HCV prevalence was significantly higher in patients from Lower Egypt according to *Lehman et al,* which in turn confirms the positive correlation between HCV and OBI frequencies [25].

Because prevention is the first line of defense against blood borne pathogens especially viral hepatitis infection and is much more cost-effective than treatment, prevention should be the main goal of current efforts to break the vicious cycle of this infection. In Egypt, we should provide more support for application of infection control policies regarding the vaccination of high-risk groups, such as HD patients and medical staff, as the cost of treatment is much higher than the cost of vaccination. The accurate prevalence of OBI in Egypt among high-risk groups, such as HD patients, is not well known and can only be definitively determined by giving more attention to large, national epidemiological studies.

CONCLUSION

The current study highlights the increase in OBI prevalence among Egyptian HD patients, so we suggest screening of HD patients for OBI by testing anti-HBc (in addition to HBs-Ag) and those positive for anti-HBc to be submitted to HBV DNA confirmation by the PCR method. Also in areas with financial support detection of HBV DNA by PCR should be done to all risk groups to diagnose ONI in seronegative group.

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