Genotyping of occult hepatitis B virus infection in Egyptian hemodialysis patients without hepatitis C virus infection

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Summary

Background: Occult hepatitis B viral infection is the presence of hepatitis B viral nucleic acids in the serum and/or liver in the absence of hepatitis B surface antigen.

Aim: The study aimed to determine the prevalence of occult hepatitis B virus infection among hepatitis C virus-negative hemodialysis patients and to identify their genotypes.

Methods: of 144 patients on maintenance hemodialysis, 50 hepatitis B surface antigen and hepatitis C virus nucleic acid-negative patients were selected according to strict inclusion criteria to avoid the effect of confounding variables. The following investigations were done: serum AST and ALT; HBsAg; HBCAb; HCV-Ab; HCV-RNA; and HBV-DNA.

Results: Positive hepatitis B viral nucleic acid was confirmed in 12/144 (8.3%) hemodialysis patients and 12/50 (24%) in our study group (occult infection). Mean hemodialysis periods for negative patients and occult hepatitis B virus patients were 27.3 ± 18.8 and 38.4 ± 8.14 months, respectively, and this

Abbreviations: HBV, hepatitis B virus; OBI, occult HBV infection; HBsAg, hepatitis B surface antigen; PCR, polymerase chain reaction; ESRD, end-stage renal disease.

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difference was significant ($p$-value = 0.02). Mean alanine transaminase levels were 20.27 ± 5.5 IU/L and 25.3 ± 9.6 in negative patients and occult infection patients, respectively. This difference was non-significant. Aspartate transaminase levels were 21.4 ± 10.2 IU/L and 27.3 ± 4.6 IU/L, respectively, in negative patients and infected patients; this difference was significant ($p$-value = 0.03). Half (6/12) of the positive samples belonged to genotype 'B', 33.3% (4/12) to 'C', and 16.6% (2/12) to genotype 'D'.

**Conclusion**: OBI is likely among hemodialysis patients even without HCV coinfection (24%). Genotype D cannot be the only genotype distributed in Upper Egypt, as the current study reported relatively new results that 50% of the patients with occult B carry genotype B, 33.3% carry genotype C and only 16.6% carry genotype D.

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**Background**

Hepatitis B virus (HBV) infection is a global health concern with multiple clinical presentations, e.g., occult HBV infection (OBI) [1]. OBI is defined as the presence of a low amount of HBV DNA (less than $10^4$ copies/mL) in patients with negative hepatitis B surface antigen (HBsAg) serum/plasma [2]. The risk of infection transmission from OBI through blood transfusion could be avoided by utilization of highly sensitive polymerase chain reaction (PCR)-based techniques, which can identify HBV DNA in HBsAg-negative individuals [3].

OBI is highly prevalent among patients with end-stage renal disease (ESRD) on maintenance hemodialysis because of frequent blood transfusions [4]. ESRD is a serious problem globally; however, the prevalence is higher in developing countries, especially in the Middle East [5].

The exact cause of OBI is not understood. Several studies have suggested that OBI could be caused by mutant viruses that cannot be detected by current HBsAg assays. Other studies have suggested that OBI could be due to a failure of viral replication, gene expression, or virus release [6]. HBV has been classified into ten genotypes (labeled A–J) [7]; some genotypes are associated with different clinical outcomes. Genotypes C and D generally tend to be related to more severe prognosis (particularly hepatocellular carcinoma) than genotypes A and B [8]. Real-time PCR using genotype-specific primers is a very important technique because fluorescent probes allow for the highly sensitive detection and quantification of HBV DNA, while melting curve analysis allows the determination of genotypes, which aids in the prediction of therapeutic outcomes [9].

Different HBV genotypes have different geographic distributions. The HBV genotype D is common in Mediterranean area data [10]. Screening of OBI in hemodialysis patients in Egypt is limited [11], and little information exists about the relation between the HBV genotypes and OBI in Egypt [12]. Therefore, we investigated the prevalence of OBI occurrence without HCV-coinfection among Egyptian hemodialysis patients, and we identified their different genotypes using a highly sensitive and specific quantitative PCR assay.

**Materials and methods**

**Patients**

The study included all patients (144) on maintenance hemodialysis (more than six months) recruited from Minia University Hospital (MUH) at the time of the study. Serum samples were routinely collected for the detection of HCV antibodies and Hepatitis B surface Antigen (HBsAg) before the start of dialysis. Exclusion criteria for our study has included: 1 — acute or chronic HBV infection, as determined by positive Hepatitis B core antibodies (anti Hbc) and HBsAg, respectively; 2 — HBV vaccination history (to exclude vaccine-induced immunity); and 3 — HCV RNA and HCV antibodies, to exclude coinfection with HCV and other liver diseases.

A full history was collected from the selected group of patients (50/144 patients) with emphasis on their histories of blood transfusions, liver diseases and durations of hemodialysis. Clinical examinations were conducted with emphasis on signs of chronic liver disease, e.g., jaundice,
Detection of the HBV-DNA genotype

Genotyping was performed on patients diagnosed as having OBI using HBV genotyping real-time-PCR kits (Liferiver, Shanghai ZJ Bio-Tech Co., Ltd, China) according to the manufacturer’s protocol, and the results were compared with those of the kit’s positive controls.

Statistical analysis

Data were analyzed by SPSS version 10, using descriptive statistics (mean ± standard deviation) and the chi-squared test for comparison of qualitative variables. p-values <0.05 were considered statistically significant.

Results

Detection of OBI and HBV-DNA genotypes

The study samples (50/144) of patients (who were negative for all of the following tests, anti-HCV, HBsAg, HBCAg and HCV RNA), were examined for OBI by quantitative real-time-PCR. Positive HBV DNA was confirmed in 12 out of 144 (8.3%) of hemodialysis patients, as the definition of OBI includes the absence of HBsAg only. Incidence rates of OBI among HBsAg and Anti HCV negative patients would be 12 out of 50 (24%) in our group of patients.

The genotyping study

The genotyping study was conducted on samples of 12 HBV DNA-positive patients as described in the materials and methods section. Genotype B was predominant in HBV DNA-positive samples, which constituted 6/12 samples (50%). However, HBV genotypes C and D were observed in 33.33% and 16.66% of samples, respectively, as shown in Figs. 1 and 2 and Table 2.

As summarized in Table 1

As summarized in Table 1, the mean age of the negative patients was 46.96 ± 11.02 years, and the mean age of the OBI positive patients was 49.81 ± 13.15 years. Males constituted 23/38 (65.2%) of the negative patients and 66.6% (n = 8/12) of the OBI positive patients. Twenty-eight (73.67%) of the negative patients were rural residents, and eight (66.6%) of the OBI positive patients were rural residents. These results were statistically non-significant (p-value > 0.05). Mean hemodialysis periods for negative patients and occult hepatitis B virus patients were 27.3 ± 18.8 and 38.4 ± 8.14
Genotyping of occult hepatitis B virus

Figure 1 Gene typing of different amplification plots.

Table 1 Clinical and laboratory characteristics of the study patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>OBI negative patients</th>
<th>OBI patients</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>38 (76%)</td>
<td>12 (24%)</td>
<td></td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>46.96 ± 11.02</td>
<td>49.81 ± 13.15</td>
<td>0.5</td>
</tr>
<tr>
<td>Sex (male) (n/%)</td>
<td>23 (65.2%)</td>
<td>8 (66.6%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Residence (rural) (n/%)</td>
<td>28 (73.67%)</td>
<td>8 (66.6%)</td>
<td>0.1</td>
</tr>
<tr>
<td>Duration of hemodialysis (months, mean ± SD)</td>
<td>27.3 ± 18.8</td>
<td>38.4 ± 8.14</td>
<td>0.02*</td>
</tr>
<tr>
<td>Positive history of transfusion (%)</td>
<td>25 (65.67%)</td>
<td>2 (16.6%)</td>
<td>0.06</td>
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<tr>
<td>History of liver disease</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>20.27 ± 5.5</td>
<td>25.3 ± 9.6</td>
<td>0.07</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>21.4 ± 10.2</td>
<td>27.3 ± 4.6</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

p value; *significant (<0.05); **highly significant (<0.01). Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; OBI; occult hepatitis B virus infection; SD, standard deviation.

Figure 2 Genotypes of occult hepatitis B virus infection in hemodialysis patients.

Discussion

Blood-transmitted hepatitis viruses are considerable problems in developing countries. Egypt has been reported to be an endemic country for HCV infection and is considered as an intermediate area for HBV infection [14].

Our study aimed to investigate the prevalence of OBI occurrence without HCV coinfection among Egyptian hemodialysis patients. Exclusion criteria for our study included acute or chronic HBV infection, HBV vaccination history as determined by positive Hepatitis B core antibodies (anti HBc), HBsAg, HCV RNA and HCV antibodies to exclude coinfection with HCV and other liver diseases. We also excluded patients with an HBV vaccination history to avoid vaccine-induced immunity. We found that OBI was detected in 12/50 (24%) of our patients. Similar results have been reported by Sia-gris et al., who reported that HBV DNA was detected by PCR in 10/49 (20.4%) hemodialysis patients [15]. However previous studies in dialysis units have reported a lower prevalence, where OBI was identified in 2.7% (5/188) in Turkey [16] and 3.8%

months, respectively, which was a significant difference (p-value = 0.02). Mean ALT liver enzyme levels were 20.27 ± 5.5 IU/L and 25.3 ± 9.6 IU/L in negative patients and occult infection patients, respectively, which was non-significant. AST levels were 21.4 ± 10.2 IU/L and 27.3 ± 4.6 IU/L in negative patients and infected patients, respectively, which was a significant difference (p-value = 0.03) (Table 2).
(9/239) of HBs-Ag-negative North American adult hemodialysis patients using real-time PCR [17].

In Egypt, Abu El Makarem et al. [11] demonstrated that 3% of ESRD patients with HCV RNA-negative and HBsAg-negative reports from Upper Egypt had OBI. Other studies have revealed a higher prevalence of OBI in hemodialysis patient. For example, Besisik [18] reported that positive HBV DNA and HBsAg-negative (OBI) was detected in 36% (12/33) of patients. However, in this study, only 9.1% (3/33) of hemodialysis patients had a history of HBV vaccination. The fact that a higher prevalence of OBI is detected in hemodialysis patients with no history of HBV vaccination, suggests that the lack of past HBV vaccination could be an important risk factor. This difference may explain the high prevalence of OBI in hemodialysis patients in our study because we specifically excluded the vaccinated patients. These discrepancies in the rates of OBI in hemodialysis patients may reflect the diverse prevalence of HBV infection in hemodialysis patients, in different countries, and within different dialysis units in different clinical situations. Additionally, consequential differences in the composition of the study populations and sensitivity of the techniques used for HBV-DNA detection may lead to variations in prevalence [15]. In the current study, OBI was not significantly associated with age, sex, residence, history of hepatitis, history of blood transfusions, or ALT levels. However, there were significant associations between the presence of HB-VDNA and the length of hemodialysis periods, and HBV-DNA positivity and AST levels, which largely agreed with previous studies in Egypt [11]. The determination of HBV genotypes is important because HBV genotypes are associated with the course of the infection, the response to antiviral treatment regimens and the clinical prognosis [19]. In Egypt, there have been few reports about the predominant genotypes of HBV. The D genotype has been reported as the predominant type, e.g., Zaky et al., who studied clinicopathologic features and genotyping in patients with chronic HBV infection in the Upper Egypt and Ragheb et al., who studied intra-familial transmission patterns of the hepatitis B virus genotype [20,21]. However, our study revealed that the predominant genotypes of HBV were mainly B (50%), followed by C (33%) and D (17%) in our OBI patients. These findings suggest that genotype B is more likely to disseminate in hemodialysis environmentally. The differences between our findings and these others may have contributed to the different type of samples evaluated. While our samples were from a selected group (anti HBC, HBsAg, HCV RNA and HCV antibody-negative results), the majority of the previous studies’ published samples have been derived from patients with established chronic disease [20,21]. Another study in Egypt carried out on hemodialysis patients reported that the genotype D was not predominant. They reported that the C genotype was present in a plurality (44.4%), followed by A (27.8%) and B (22.2%), of hemodialysis patients [22]. Thus, a new pattern of genotypes may appear in Egypt or may be present in hemodialysis patients, which may be associated with genetic variations, mutations or other causes. Thus, further genotyping studies should be performed. It was difficult to demonstrate a clear association between HBV genotypes and the viral load because of the small number of genotypes.

**Conclusion**

We can conclude that OBI is a likely nosocomial infection among hemodialysis patients even in the absence of HCV coinfection (24%). The prolonged duration of hemodialysis is a major risk factor for acquiring the infection; thus, incorporation of sensitive molecular techniques beside serological analysis are required for earlier discovery and interference. Additionally, the study has illustrated that HBV genotype D cannot be the only genotype distributed in Upper Egypt, as our study found that 50% of the patients with occult B carry genotype B, 33.3% carry genotype C, and only 16.6% carry genotype D. Extensive epidemiological studies for genotyping should be performed to track the source of infections. OBI rates reported herein are very high compared to several other developed countries. These high rates are an important source of nosocomial HBV transmission and may induce the transmission of new genotypes. Given the frequency of dialysis and the uniquely high prevalence of HBV among Egyptian hemodialysis patients, we think that patient isolation
(particularly those known to have HBV infection) is very important in Egypt, in addition to the standard hygienic precautions.

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**Competing interests**

None declared.

**Ethical approval**

Not required.

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**References**


