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# The Prevalence of Occult HBV Infection Among Hemodialysis Patients of Tehran, Iran

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## Abstract

**Background:** The presence of HBV DNA in the absence of hepatitis B surface antigen (HBsAg) in the serum of patients is called occult HBV infection (OBI). Because of the risk of hepatitis B transmission, OBI is an important subject for haemodialysis (HD) patients and centres.
**Objectives:** The present study was conducted to determine the prevalence of OBI among haemodialysis patients.
**Methods:** In this cross-sectional study, serum specimens were obtained from 200 haemodialysis patients who referred to HD centres of Iran University of Medical Sciences in Tehran (Iran) and were tested for HBsAg. They were then tested for HBV viral load in plasma and the presence of the HBV genome in peripheral blood mononuclear cell specimens using real-time PCR. Demographic data (age, sex, history of HBV vaccination, and blood transfusion), liver enzymes (AST, ALT), and duration of HD were recorded.
**Results:** Among the 200 HD patients, 109 (54.5%) were male and 91 (45.5%) were female. The mean age of the patients was 56.09 $\pm$ 15.5 years (range: 22 to 88 years). A total of 38 patients (19%) had a history of blood transfusion and all patients had received 3 doses of hepatitis B vaccine (34 patients also received a booster dose after recording a low serum level of HBsAb.). The prevalence of OBI was 0.5% (1/200).
**Conclusions:** Considering the moderate prevalence of hepatitis B virus infection in Iran, the prevalence of OBI in HD patients was very low, which could be due to regular and on-time vaccination for hepatitis B and the good preventive care in HD centres.

## 1. Background

Hepatitis B virus is the most common disease transmitted through blood (1). Current evidence indicates that occult HBV infection is a common and long-term consequence of the resolution of acute hepatitis B. This form of residual infection is called a secondary occult infection (2). Data from the woodchuck model of HBV infection indicate that exposure to small amounts of hepadnavirus can also cause primary occult infection, where the virus genome, but not the serological makers of exposure to the virus, is detectable and the liver is not involved. The virus replicates at low levels in the lymphatic system in both these forms (2).

The presence of HBV DNA without HBsAg is called occult hepatitis B infection (OBI) (3-5). To date, OBI has been reported in subgroups of patients with chronic HCV infection, HIV, hepatocellular carcinoma (HCC), intravenous drug users, advanced cryptogenic liver fibrosis and comorbidities (2, 4, 6), and in patients who require permanent blood transfusion, such as those with thalassemia, haemophilia, and those requiring HD (1). Studies on the prevalence of OBI in HD patients are highly important because it is a high-risk factor for post transfusion hepatitis, HCC, and cirrhosis (1).

The prevalence of OBI in HD patients has been reported to be between 0% and 58% in several studies in different countries (7). The current study was conducted to study the prevalence of OBI in HD patients. It is hoped that sufficient information in this area could facilitate the diagnosis of infection promptly to allow for rapid treatment. If the prevalence of OBI is high, planning can be undertaken to reduce its incidence.

## 2. Methods

### 2.1. Study Population

This was a cross-sectional study conducted on 200 HD patients in 3 HD centres of Iran University of Medical Sciences in Tehran in 2011. Patients with a history of liver disease, such as viral hepatitis and HBsAg positive, were excluded. Demographic information of patients (age, sex, history of transfusion, and history of hepatitis B vaccination) and laboratory findings (AST and ALT) were collected from the renal database and entered into a questionnaire.

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Ethical approval for the present study was obtained from the ethics committee of Iran University of Medical Sciences under the ethical code of IR.IUMS.FMD.REC.1390.10389. Informed consent was obtained from all patients and the purpose of the research was explained to all.

### 2.2. Collection and Preparation of the Participants

All dialysis units monitor the patients for viral infection at the time of admission and repeat it every 3 months. Blood samples were collected before dialysis on the day of HD. About 6 mL of peripheral blood was collected from each patient into sterile EDTA-containing vacutainer tubes. After separation of plasma from the whole blood by centrifugation, the peripheral blood mononuclear cells (PBMCs) of the samples were isolated using the standard method of Ficol gradient centrifugation (Lympholyte HTM; Cedarlane and Hornby; Canada). The PBMC pellet was washed with phosphate-buffered saline (pH = 7.3 $\pm$ 0.1), resuspended in 200 $\mu$L of RNA later solution (Ambion; USA) and was stored at -80°C for HBV-DNA extraction. For positive and negative control, the plasma and PBMC samples of 10 patients with HBV infection and 10 healthy blood donors were used, respectively.

### 2.3. Serologic Tests Using Enzyme Immunoassay

Main HBV serological marker (HBsAg) was tested using the commercial enzyme immunoassay (EIA) kit (Dia.Pro; Italy) according to manufacturer protocol.

### 2.4. DNA Quantitation (Viral Load) of Hepatitis B Virus

The viral load of HBV in the plasma specimens (500 $\mu$L) of the patients was tested using the COBAS TaqMan kit (Roche Diagnostics; USA). High pure DNA isolation was used according to manufacturer recommendations. This method is a real-time PCR assay based on a dual-labelled hybridisation probe that targets the HBV core and pre-core regions. The detection limit of this kit is about $20 > 1 \times 10^8$ IU/mL (8).

### 2.5. DNA Extraction and Real-Time PCR

The viral DNA was isolated from a pellet of PBMC specimens (approximately 4- 6 $\times$ $10^6$ cells) using a QIAam DNA Mini Kit (Qiagen; Germany), with a procedure adapted from the manufacturer instructions. DNA was eluted using 50 $\mu$L of elution buffer and stored at -20°C for HBV-DNA detection.

The real-time PCR was performed to detect HBV-DNA in the PBMC samples using Rotor-Gene Q (Qiagen; Germany) as described previously. A pair of primers (sense and antisense), which can amplify a conserved region of the HBV-S gene, and a TaqMan probe were used. To amplify the HBV-S gene, 10 pmol of each primer (HBVTAQ1, HBVTAQ2) and 5 pmol of the TaqMan probe (HBVTAQPR) were used. The real-time PCR was performed using a 25 $\mu$L mixture containing the following materials: 12.5 $\mu$L Maxima Probe qPCR Master Mix (Fermentas), primers, the TaqMan probe, and 5 $\mu$L of the DNA template.

The human ß-globin gene was used as an internal control for DNA extraction and PCR amplification. To amplify the human $\beta$-globin gene in the PBMC samples, a pair of primers (300 nM of each primer) and a TaqMan probe (200 nM) were added to the master mix of real-time PCR (9). The reaction continued for 2 minutes at 50°C (to destroy the activated uracil-N glycosylase and potential carry-over amplicons) and then for 10 minutes at 95°C (for activation of the thermostable DNA polymerase), followed by 45 cycles of amplification including 15 rounds at 95°C and 1 minute at 60°C.

### 2.6. Statistical Analysis

Statistical analysis was performed using SPSS Version 17 (SPSS; USA).

## 3. Results

A total of 200 HD patients were investigated in this study; 28 patients (14%) at Firoozgar hospital, 21 (10.5%) at Rasoul-Akram hospital, and 151 (75.5%) at Hashemi Nejad hospital were dialyzed. The mean age of the patients was 56.09 $\pm$ 15.5 years, and their ages ranged from 22 to 88 years. Other demographic data of the patients are demonstrated in Table 1.

**Table 1.** The Demographic Information of Patients

| Parameters/Catagorized | No. (%) |
|---|---|
| **Age, y** | |
| Under 40 | 33 (16.5) |
| 40 to 60 | 87 (43.5) |
| Above 60 | 80 (40) |
| **Gender** | |
| Male | 109 (54.5) |
| Female | 91 (45.5) |
| **Transfusion history** | |
| None | 162 (81) |
| Once | 26 (13) |
| Twice | 10 (5) |
| Thrice | 2 (1) |

The patients in this study were divided into 3 age groups: under 40, 60 to 40 years, and over 60 years. The average duration of dialysis in the patients was 51.2 ± 60 months, and the range was 7 to 365 months. The mean ALT was 14.9 ± 6.8 mg/mL, with a range of 8 to 53 mg/mL, and the AST was 16.8 ± 6.9 mg/mL, with a range of 9 to 56 mg/mL. All HD patients were vaccinated for hepatitis B, and 34 patients (17%) received an extra booster dose.

One patient had a positive PCR test for both serum and PBMC samples, and the prevalence of OBI was 0.5%. Because the prevalence of OBI cases was low, it was not comparable to the PCR negative group in other factors such as age and sex.

## 4. Discussion

Hepatitis B virus (HBV) infection is a significant problem in the health care system in developing countries such as Iran (10). WHO reported in 2001 and the CDC reported in 2005 that Iran is an intermediate region with a prevalence of 2% to 7% HBV (11). To avoid HBV transmission, most blood transfusion services use ELISA techniques to detect HBsAg, but they have not been 100% effective, and cases of post transfusion hepatitis (PTH) have occurred.

OBI is HBV DNA detection in the serum or liver by sensitive diagnostic tests in HBsAg-negative patients with or without serologic markers of previous viral exposure (10). OBI appears to be the most likely mechanism for PTH in permanent blood recipients including in patients with haemophilia and thalassemia and those requiring HD (1). The prevalence of occult HBV infection varies in different populations and runs parallel to the general prevalence of HBV infection in the region (12).

The prevalence of occult HBV infection in dialysis patients has ranged from 0% to 58% in different surveys (12). In the current study, the prevalence of OBI was 0.5%. This was confirmed by positive PCR testing of both the serum and PBMC. However, it should be considered that it is for the first time in Iran that both plasma and PBMC HBV DNA have been used in HD patients to diagnose OBI.

In a literature review of Iranian patients in 2014, Aghakhani et al. (13) reported that 3.11% of HD patients with isolated anti-HBc in Tehran were HBsAg negative and HBV DNA positive. In 2014, Ramezani et al. (12) in Tehran reported that 1% (1/100) of HBsAg negative HD patients were HBV DNA positive, which is in line with the results of the current study. Another study in 2014 found that 2.4% (1/126) of HBsAg negative HD patients were HBV DNA positive in Yazd, Iran (14), which is slightly more than the results of the current study.

Minul et al. (5) reported an incidence of 3.8% OBI in 241 HD patients in 2004. A high rate of occurrence of OBI was reported by Altandis et al. (4), with a prevalence of OBI of 12.4% in HD patients and 6.8% in the control group in 2007. Abu et al. (15) evaluated 145 HD patients from Egypt and reported that 4.13% of their patients suffered from OBI. In contrast, Cabrerizo et al. reported the highest OBI prevalence among HD patients (58%) in Spain in 1997 (16).

### 4.1. Conclusions

In the present study, the incidence of OBI infection was lower than that of similar studies and was negligible compared to the prevalence of hepatitis B in Iran, which is located in the mid-endemic area of hepatitis B (11). This could be due to timely vaccination of HD patients and implementation of health principles at HD centres. We suggest conducting another study to illustrate the prevalence of OBI more precisely in private, primary, and secondary haemodialysis units and in some hospitals in Tehran using healthy individuals as a control group.

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