



# Occult Hepatitis B in Hemodialysis Patients

## Hemodiyaliz Hastalarında Okült Hepatit B

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### ABSTRACT

**Objectives:** We aimed to detect occult hepatitis B (OHB) in hemodialysis patients at a higher-risk for OHB.

**Materials and Methods:** The study included 567 patients with chronic renal failure aged 18 years and older who underwent hemodialysis in 10 dialysis centers in İzmir province between May 2013 and July 2013. Hepatitis B surface-antigen (HBsAg), anti-hepatitis B core (HBc) immunoglobulin G (IgG) and anti-HBs were detected by ELISA and HBV-DNA levels with polymerase chain reaction (PCR). Detection of HBsAg negativity with HBV-DNA positivity was considered as OHB.

**Results:** Of 567 patients, 49% were male and the mean age was 62.2 years. All the patients were HBsAg-negative. Isolated anti-HBc IgG positivity was detected in 8 patients while HBV-DNA was negative. Serum HBV-DNA level was 270 IU/mL in only one patient (0.2%) who was anti-HBc IgG-negative.

**Conclusion:** HBsAg alone is not an adequate serological test to detect HBV infection. HBV-DNA should be tested using molecular diagnostic methods in patients with suspected OHB. Further studies investigating cost-effectiveness and the role of PCR in diagnosis are warranted.

**Keywords:** Chronic viral hepatitis, hemodialysis, occult hepatitis B

### ÖZ

**Amaç:** Risk grubunda yer alan hemodiyaliz hastalarında okült hepatit B'yi (OHB) saptamayı amaçladık.

**Gereç ve Yöntemler:** Mayıs 2013-Temmuz 2013 arasında, İzmir ilinde 10 farklı diyaliz merkezinde hemodiyalize giren, 18 yaş ve üzeri, 567 kronik böbrek yetmezliği olan hastalar çalışmaya dahil edildi. Hepatit B yüzey antijeni (HBsAg), anti-hepatit B çekirdeği (HBc) immünoglobulin (IgG) ve anti-HBs ELISA yöntemiyle, HBV-DNA ise polimeraz zincirleme reaksiyonu (PCR) yöntemiyle çalışıldı. HBsAg negatifliği ve HBV-DNA pozitifliği OHB olarak değerlendirildi.

**Bulgular:** Beş yüz altmış yedi hastanın %49'u erkek, ortalama yaş 62,2 idi. HBsAg tüm hastalarda negatif saptandı. İzole anti-HBc IgG pozitifliği 8 hastada gözlenmiş olup hiçbirinde HBV-DNA saptanamadı. Sadece bir hastada (%0,2) serum HBV-DNA düzeyi 270 IU/mL olarak ölçüldü.

**Sonuç:** HBsAg tek başına HBV enfeksiyonunu saptamada yeterli bir serolojik test değildir. OHB şüphesi olan hastalarda HBV-DNA düzeyi moleküler tanı yöntemleriyle test edilmelidir. Maliyet etkinliği ve tanıda PCR'nin rolü için daha fazla çalışmalara ihtiyaç olduğu kanaatine varıldı.

**Anahtar Kelimeler:** Kronik viral hepatit, hemodiyaliz, okült hepatit B

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### Introduction

Being one of the most important causes of chronic liver disease, hepatitis B virus (HBV) is a significant cause of morbidity and mortality all over the world. HBV infection is a major health problem with 400-500 million people chronically infected worldwide. It is a known fact that 5% of people with acute hepatitis B develop chronic infection and a substantial number of these cases develop

cirrhosis associated with higher risk of, hepatocellular carcinoma (HCC) (1).

HBV infection is diagnosed by detection of various antigens belonging to this virus or the antibodies developed by the host against these antigens with specific serological tests. Hepatitis B surface antigen (HBsAg) is one of the most important markers of HBV infection. HBsAg positivity in serum for more than six months indicates chronic HBV infection (2,3). Antibody to anti-HBs appears

following the disappearance of HBsAg. Anti-HBs shows recovery and immunity. Anti-hepatitis B core (HBc) immunoglobulin G (IgG) positivity, which is detected together with anti-HBs, is defined as natural immunity (2,4,5). Presence of HBV-DNA is the most sensitive indicator of viral replication.

Detection of serological markers is important in the identification of infection but has little clinical importance. Polymerase chain reaction (PCR) detects low levels of viral DNA in serum or liver in some patients whose HBsAg levels are undetectable or in patients who have HBsAg disappearance with HBV treatment. Occult hepatitis B (OHB) infection (OBI) is defined as the presence of HBV-DNA in serum or liver in the absence of HBsAg. OBI has also been defined as a serological condition characterized by the presence of isolated hepatitis B core antigen anti-HBc in the absence of HBsAg and anti-HBs. OBI can be classified into 2 groups on the basis of the HBV antibody profile: seropositive OBI (anti-HBc and/or anti-HBs-positive) and seronegative OBI (anti-HBc- and anti-HBs-negative) (6). HBV-DNA level in OBI is generally measured lower than 200 IU/mL (7).

OBI has been reported to be more prevalent in patients with HCC, chronic hepatitis C virus (HCV) infection, cryptogenic cirrhosis, hemodialysis patients, substance users, intravenous drug abusers, patients with human immunodeficiency virus infection, and patients who receive frequent blood transfusions (8). If patients with OBI diagnosis undergo dialysis in the same dialysis machines with HBsAg-negative patients, HBV transmission may occur.

There are not sufficient studies performed on the frequency of OBI in our country and our province. In this study, we aimed to detect OHB in hemodialysis patients who are at a higher risk for OBI.

## Materials and Methods

This randomized prospective study included 567 patients with chronic renal failure (CRF) aged 18 and older who underwent hemodialysis in 10 dialysis centers in Izmir province between May 2013 and July 2013. Patients younger than 18 years of age, having a previous diagnosis of HBV infection and undergoing peritoneal dialysis were excluded. Ethic committee approval was taken from the Ethics Committee Board of University of Health Sciences, Izmir Tepecik Training and Research Hospital (approval number: 47/1, date: 24.04.2013).

A form questioning risk factors for HBV contamination including socio-demographic characteristics, medical history such as co-morbidities, IV drug administration, surgery, blood transfusion and time of hepatitis B vaccination was completed by the patients. Written consent form was taken from each patient. Blood samples were taken before dialysis session; serum samples were centrifuged in 3000 rpm for 5 minutes and stored at -80 °C. HBsAg, anti HBc IgG, and anti-Hbs were investigated by the ELISA method (Liaison, Diasorin, Italy) in accordance with the instructions of the manufacturing company. HBV-DNA levels were evaluated by PCR (Roche, Taqman, Switzerland). HBV-DNA levels lower than 20 IU/mL were considered as negative. HBV-DNA presence in serum without HBsAg positivity was defined as OBI.

## Results

Forty-nine percent of 567 patients were male and the mean age was 62.2 (range: 24-78) years. The patients were receiving hemodialysis treatment three times a week for four hours. The mean duration of hemodialysis treatment was 60.7 months (range: 4-180 months). The indications for dialysis were diabetes mellitus (42.7%), hypertension (31.3%), glomerulonephritis (12.5%), polycystic kidney disease (4.2%), and others (9.3%) (Table 1). Mean AST: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were 14 (5-42 u/L) and 17 (8-36 u/L), respectively.

Fifty-seven percent of the patients had a history of vaccination with 40 mcg recombinant DNA vaccine in months 0, 1, 2 and 6 according to the standard vaccine schedule. In 1.5% (n=5), insufficient antibody response (anti-HBs <10 IU/mL) was detected despite administration of vaccine twice. Fifteen percent of the patients were considered to have natural immunity due to past infection.

As shown in Table 2, HBsAg was negative in all cases. Isolated anti-HBc IgG positivity was detected in 8 patients while HBV-DNA was negative. Serum HBV-DNA level was 270 IU/mL in only one patient aged 53 years (0.2%) with anti-HBc IgG and anti-Hbs negativity. AST and ALT levels of the patient who was considered as seronegative for OBI, were within the normal range (0-35 IU/L). Hemodialysis duration of the patient was lower than the average (12 months). The patient had a history of diabetes mellitus and hypertension. In terms of chronic hepatitis B, no other risk factors (history of past HBV infection or inactive chronic hepatitis, previous surgery, blood transfusion, suspicious coitus, history of dentist appointment, and family history of hepatitis), beyond hemodialysis and catheterization, were detected.

<b>Table 1. The questionnaire including demographics, dialysis reasons and risk factors for hepatitis B virus infection</b>	
<b>Socio-demographic characteristics</b>	
Mean age	62.2
Gender	49%
History of hepatitis B virus vaccination	57%
<b>Dialysis reasons</b>	
	(%)
Diabetes mellitus	42.7
Hypertension	31.3
Glomerulonephritis	12.5
Polycystic kidney disease	4.2
Others	9.3
<b>Risk factors</b>	
	(%)
IV drug administration	32.6
Operation	13.8
Blood transfusion	26.7
Family history	3.4
Suspicious coitus	2.9

**Table 2.** The serological parameters of hepatitis B virus in hemodialysis patients

n (%)	HBsAg	Anti-HBc	Anti-HBs	HBV-DNA
8 (1.4%)	(-)	(+)	(-)	(-)
1 (0.1%)	(-)	(-)	(-)	(+)
318 (56%)	(-)	(-)	(+)	(-)
240 (42.3%)	(-)	(-)	(-)	(-)

HBsAg: Hepatitis B surface antigen, HBc: anti-hepatitis B core, HBs: Hepatitis B surface, HBV: Hepatitis B virus

## Discussion

HBV infection continues to be a significant issue in hemodialysis units despite vaccine schedules and precautions. Risk of the HBV transmission between hemodialysis patients is explained by the presence of OBI (HBsAg negative but HBV-DNA positive) in addition to the presence of immunosuppression, shared use of dialysis machines, insufficient response to vaccine, blood transfusions and interventions which are performed more frequently for hemodialysis patients than for normal population (9).

The prevalence of OBI in healthy subjects has been reported to vary between 0% and 90% based on the endemicity (10,11,12). In hemodialysis patients, the prevalence of OBI reported in the literature varies greatly, ranging from 0% to 50% (9,13). In a study investigating the prevalence of anti-HBc in hemodialysis patients, HBV-DNA was detected in 1 of 3 anti-HBc positive patients. HBV-DNA was undetectable in 123 anti-HBc negative patients (14). In a study by Ramezani et al. (15), HBV-DNA was detected in 1% of HBsAg negative patients. Similar to that study, Mucho et al. (16) found nil prevalence of OBI in chronic hemodialysis patients and a very low prevalence (<1%) in renal transplant patients suggesting that routine screening for HBV-DNA was not required in chronic hemodialysis population in their region.

The frequency of rate of OBI in hemodialysis patients reported in studies performed in our country varies between 0% and 12.4% (17,18,19). The variability in the reported prevalence is related with the regions where the studies were performed, PCR method used, and patient population included. In our study, it was found to be 0.2%. Possible reasons for low frequency of OBI in our study may be regular vaccination and anti-HBs screening of patients, good physical conditions of the hemodialysis units and complete compliance of the study team to the standard precautions. On the other hand, vaccine runaway replication mutants and antiHBs, immune escape mutants in patients who received hepatitis B immune globulin (HBIG), diagnostic escape mutants which could not be detected by some tests used in detecting HBsAg due to change in HBs structure have been reported to be the result of the mutations of the "a" determinant region of HBV S gene in subjects who were immunized with recombinant vaccines (20).

In patients with CRF, the most common reasons for dialysis are diabetes mellitus (22.8%), hypertension (18.1%), and glomerulonephritis (14.2%) (21). CRF etiologies in patients included in this study are consistent with the literature.

Since cellular and humoral immune responses are insufficient in CRF patients, elevations of liver function tests are mild to moderate and, most of the time, the patients have asymptomatic HBV infection. As a result, chronic disease may occur more frequently in patients who undergo hemodialysis compared to normal population (22). Thus, establishing the diagnosis of OBI in

hemodialysis patients is difficult. In a study by Yoo et al. (13), there was no significant difference in the liver function tests between HBV-DNA positive and negative hemodialysis patients. Laboratory tests in patient who was considered to have OBI in this study were also normal.

HBV-DNA levels were observed to be low (<200 IU/mL) in cases with OBI in all studies reported in the literature (23,24). Consistent with the literature, the patient with OBI in this study had a HBV-DNA level of 270 IU/mL. This finding suggests that OBI may occur due to low concentrations of undetectable HBsAg rather than presence of mutant HBV. Furthermore, HBV-DNA levels are also very low in these patients; the sensitivity of the test preferred in the diagnosis of OBI is of crucial importance (25).

The possibility of transmission of HBV infection from dialysis patients with OBI to others using same dialysis machine is not clearly known. No detection of OBI in other 26 cases who undergo hemodialysis in the same center with the OBI case suggests that possibility of transmission is low.

Presence of fulminant liver disease, chronic hepatitis, cirrhosis, and HCC has been reported in patients with OBI (26). This suggests a role of OBI in development of cirrhosis and HCC. Carcinogenesis may start with integration of the viral genome into liver cells together with the cytotoxic liver injury due to long-term HBV positivity. Therefore, OBI-seropositive patients without HBV-DNA in serum may also require liver biopsy for further OBI detection. We also detected isolated anti-HBc IgG positivity in 8 patients and suggested liver biopsy.

Initiation of antiviral treatment should be considered in patients with OBI diagnosis. The patients should be screened for HCC in regular intervals. A favorable response to treatment may be expected in patients with a low viral load (27).

## Conclusion

HBsAg alone is not an adequate serological test to detect HBV infection. HBV-DNA should be tested with molecular diagnostic methods in patients with OBI suspicion. For diagnosis of OBI, DNA nucleic acid tests should be performed especially in high-risk patients, those living in endemic regions, individuals with cryptogenic chronic hepatitis, with potential prior exposure before blood or organ donation, transplantation and chemotherapy, and those receiving hemodialysis, even if these patients have anti-HBc and anti-HBs negativity (28). Further studies investigating cost-effectiveness and the role of PCR in diagnosis are warranted.

## Ethics

**Ethics Committee Approval:** Ethic committee approval was taken from the Ethics Committee Board of University of Health

Sciences, Izmir Tepecik Training and Research Hospital (approval number: 47/1, date: 24.04.2013).

**Informed Consent:** Written consent form was taken from each patient.

**Peer-review:** Externally peer-reviewed.

#### Authorship Contributions

Surgical and Medical Practices: P.A., Concept: Ş.K., Design: B.T., Data Collection or Processing: P.A., Analysis or Interpretation: B.T., Literature Search: P.A., Writing: P.A.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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