Occult Hepatitis B Prevalence in Hepatitis B Vaccinated Dialysis Patients

Hepatit B Aşılı Diyaliz Hastalarında Occult Hepatit B Prevalansı

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ABSTRACT

OBJECTIVE: Occult hepatitis B (OHB) virus infection is defined as the presence of hepatitis B virus (HBV) DNA in the liver tissue or serum of subjects seronegative for hepatitis B surface antigen. OHB leads to the potential risk of transmission in dialysis service. Routine HBV vaccine in dialysis patients is recommended. However, HBV vaccine response rates are lower than the community. The aim of this study was to determine the prevalence of OHB in hepatitis B vaccinated dialysis patients.

MATERIAL and METHODS: This study was performed at the Nephrology Department, Faculty of Medicine, Cumhuriyet University, between 1st January - 31st December 2014. Sera from 200 dialysis patients with negative HbsAg were investigated for HBV DNA using the polymerase chain reaction (PCR).

RESULTS: The mean age of the patients was 59.57±14.89 (18-91) years; 179 of them were on hemodialysis and 21 were on peritoneal dialysis. Of the patients included in the study, anti-HBs positivity was present in 135 (67.5%) and anti-HBs negativity in 65 (32.5%). The OHB prevalence was 1.5% (n=3).

CONCLUSION: In our study, the OHB prevalence was 1.5%. We assume that HBV infection would be reduced further by routinely applying HBV PCR tests for all patients who start dialysis and by taking precautions against transmission.

KEY WORDS: Occult hepatitis B, Dialysis, Hepatitis B vaccine

ÖZ

AMAÇ: Occult hepatit B (OHB) virüs enfeksiyonu, hepatit B yüzey antijeni seronegatif olan hastalarda, karaciğer dokusunda ve / veya serumda hepatit B virüsü (HBV) DNA'sının tespit edilmesi olarak tanımlanır. OHB diyaliz servislerinde, potansiyel bulaşma riskine yol açar. Diyaliz hastalarına rutin HBV aşısı önerilmektedir. Fakat aşılamaya rağmen HBV aşısına yanıt oranları topluma göre düşük bulunmaktadır. Çalışmanın amacı, hepatit B aşısı yapılmış diyaliz hastalarında OHB prevelansını saptamak.

GEREÇ ve YÖNTEMLER: Çalışma, Cumhuriyet Üniversitesi, Tıp Fakültesi, Nefroloji Bölümünde 1 Ocak-31 Aralık 2014 yılında yapıldı. HBsAg negatif 200 diyaliz hastasının serumunda polimeraz zincir reaksiyonu (PCR) kullanılarak HBV DNA araştırıldı.

BULGULAR: Hastaların yaş ortalaması 59,57±14,89 (18-91), 179 hemodiyaliz ve 21 periton diyaliz hastasıydı. Çalışmaya alınan hastaların, anti-HBs pozitifliği 135 (%67,5), anti-HBs negatifliği 65 (%32,5) idi. OBH prevelansı %1,5 (n=3) tespit edildi.

SONUÇ: Çalışmamızda OHB prevelansı %1,5 olarak tespit ettik. Diyalize başlanacak hastaların hepsinde HBV DNA PCR testinin rutin olarak gerçekleştirilmesi ve önlemlerin alınması ile HBV enfeksiyonunun daha da azaltılacağını düşünmekteyiz.

ANAHTAR SÖZCÜKLER: Occult hepatit B, Diyaliz, Hepatit B aşısı

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INTRODUCTION

Hepatitis B virus (HBV) is a double-stranded DNA virus from the hepadnavirus family. There are more than 2 billion people worldwide in whom HBV infection can be shown serologically and 400 million of these are chronic hepatitis B carriers. Every year, 1.2 million people die of chronic liver diseases such as cirrhosis and hepatocellular carcinoma (1,2).

Occult hepatitis B (OHB) infection is the detection of HBV deoxyribonucleic acid (DNA) in the blood or tissues in the presence or lack of anti-HBs against HBsAg antigen while HBsAg is negative, or antibody with an immunoglobulin G-structure (anti-HBcIgG) against Hepatitis B core antigen serologically, outside the window period. Hoofnagle et al. first reported in 1978 that HBV infection developed following negative HBsAg and anti-HBs level with a positive anti-HbcIgG blood transfusion (3). The OHB incidence is closely associated with the HBV infection prevalence of the country. Moreover, it is reported more frequently in groups with hepatocellular carcinoma, chronic hepatitis C infection, those who have received a liver transplant from an anti-HBcIgG positive donor, those who are anti-HBcIgG positive, who have chronic HCV infection, who have cryptogenic cirrhosis, hemodialysis (HD) patients, and intravenous drug users (4-9). It is known that HD patients are at high risk regarding parenteral infection transmission. The reasons for the increased risk are the numerous blood transfusions in HD patients, invasive attempts, and immunosuppression of the patients. HBV infection is higher in HD patients than the general population. In a study performed in Mexico, HBV infection was found in 7% of HD patients, and this rate was 35 times higher than the general population (10).

As the immune system is suppressed in HD patients, hepatitis B vaccine should be applied in a double dose, though a sufficient response may not be achieved even with a double dose. The vaccination dose scheme in HD patients is an intramuscular double dose (40 mcg) at the 0^{th} /1^{st} /2^{nd} and 6^{th} months. While the response to the vaccine is approximately 70% of hemodialysis patients with this vaccination, it is more than 90% in the general population (11). In addition, the rate of response to hepatitis B vaccine was higher in pre-dialysis patients. Seroconversion rates between 1.5-3 mg/dl of serum creatinine level were 87.5% after 3 doses and 100% after 4 doses. Seroconversion rates between 3-6 mg/dl of serum creatinine level were 66.6% after 3 doses and 77% after 4 doses. If the serum creatinine level is > 6mg/dl, seroconversion rates were 35.7% after 3 doses and 36.4% after 4 doses. In another study, seroconversion rates in chronic renal disease were 80% in stage 3, 76% in stage 4, 67% in stage 5 and 79% in HD patients (12-14).

In this study, we aimed to define the OHB prevalence by PCR in HD and peritoneal dialysis patients who were hepatitis B vaccinated.

MATERIAL and METHODS

This study was performed with 200 patients who presented to the Cumhuriyet University HD, peritoneal dialysis and nephrology clinics between 1^{st} January -31^{st} December 2014. Patients in the dialysis program for whom four doses of hepatitis B vaccine were completed and who agreed to participate were included in the study. The study was carried out with Cumhuriyet University Scientific Research Projects support. Patients under 18 years, cancer patients and immunosuppressive drug users were excluded from the study. Routine evaluation results of the patients were taken from the monthly evaluations; their HD duration and diagnosis were noted. Patient sera were studied fully automatically with Abbott-brand test kits (USA) with macro ELISA for HBsAg, anti-HBs, anti-HBc total and anti-HCV (USA) in an Abbott Architect i2000 SR model device with the chemiluminescent assay method.

DNA isolation was done to determine OHB presence in the serum samples of patients in which hepatitis B surface antigen was negative. The HBV DNA test was performed with Roche-brand (Germany) test kits, and the isolation process was done in Roche-brand Cobas Ampliprep Model devices. Amplification-Detection-Quantification tests were carried out in a Roche Cobas Taqman 48 model device with the Real Time PCR method.

Statistical Analysis

The results of our study were uploaded into the SPSS (data 20.0) program, and when a parametric test hypothesis was executed in the evaluation of the data, the results were expressed as the mean ± standard deviation. The chi-square test was used in the evaluation of results obtained from the patients and the Mann-Whitney U test was used for comparison of the mean values of the patients. The P value was accepted as statistically meaningful if less than 0.05.

RESULTS

In our sample of 200 patients, 114 were female and 86 were male. The mean age of the patients was 59.57±14.89 (18-91) years, and their duration of dialysis was between 8-288 (average 65.09±52.89) months. 179 of the patients were in an HD program, and 21 were in a peritoneal dialysis program. For the chronic viral hepatitis serologic indicators, anti-HBs positivity was 67.5% (135), anti-HBc total positivity was 29% (58), anti-HBe positivity was 9.5% (19), anti-HCV positivity was 3.5% (7) and HBV DNA positivity was 1.5% (3). The liver enzymes of the 200 patients showed an ALT level of 2-83 (average 18.9±12.5) and AST level of 2-144 (average 20±14.9) IU/ml. Clinical and laboratory data of the patients are given in Table I.

The diagnosis and post-vaccine anti-HBs response rates of the patients are given in Table II. Other causes of chronic renal failure included existing diagnoses such as glomerulonephritis, nephrolithiasis, and vasculitis. In our study, the most frequent cause of end-stage renal failure was diabetes mellitus, and the

Table I: Clinic and laboratory data of the patients.

Gender	Female	114
	Male	86
Mean age		59.57±14.89(18-91)
Dialysis duration (month)		65.09± 52.89 (8-288)
Dialysis type	Hemodialysis	179
	Peritoneal dialysis	21
Anti-HBs positivity		135 (67.5%)
Anti-HBs titers (IU/ml)		282,91±324,57(11-1000)
Anti-HBs negativity		65 (32.5%)
Anti-HBc positivity		58 (2%9)
Anti-HBc negativity		142 (71%)
Anti-HBeAg positivity		19 (9.5%)
Anti-HBe negativity		181 (90.5%)
Anti-HCV positivity		7 (3.5%)
HBV DNA positivity		3 (1.5%)
Alanine aminotransferase (IU/L)		13.31±10.30 (3-71)
Aspartate aminotransferase (IU/L)		16.44±9.94 (3-78)
Serum creatinine (mg/dl)		7.4±2.65(1.9-17)
Blood urea nitrogen (mg/dl)		62.36±17.98(22-137)
Potassium (mmol/L)		4.98±0.85(2.9-6.6)
Calcium (mg/dl)		8.64±0.81(5.2-10.6)
Phosphorus (mg/dl)		4.48±1.39(0.70-10.0)
25 (OH) D vitamin (ng/ml)		11.13±5.63(0.7-45)
Albumin (gr/dl)		3.80±0.51(2.2-5.2)
C-reactive protein (mg/L)		24.4±37.1 (0.1-263)
Hemoglobin (gr/dl)		11.06±1.64 (7-15)
Hematocrit (%)		34.42±5.27 (21.6-46)
URR		68.39±8.53 (26-89)
Kt/v		1.54±0.54(0.4-5.6)

URR: Urea reduction ratio, **Kt/v:** K - dialyzer clearance of urea, t - dialysis time, V - volume of distribution of urea.

second ranked cause was idiopathic. The non-responder rate of diabetes mellitus patients among the non-responder patients was 50.7%.

Anti-HBs positivity was determined in 121 of the HD patients and 14 of the peritoneal dialysis patients. Anti-HBs positivity was present in 135 (67.5%) patients. Anti-HBc positivity was found in 58 patients; there was anti-HBs positivity in 46 of the patients and for 12 there was only anti-HBc positivity. HBV DNA positivity was found in 3.44% (2/58) of the anti-HBc positive patients. HBV DNA positivity was found in 2.17% (1/46) of both anti-HBs and anti-HBc positive patients. The OHB prevalence was 1.5%. There was anti-HBc positivity in two of these patients. Anti-HBs positivity (27 IU/ml) was found in one of the anti-HBc positive patients. Liver enzymes were within normal limits in HBV DNA positive cases. When patients were classified according to dialysis type, HBV DNA positivity was 1.67% (3/179) in HD patients and 0% (0/21) in peritoneal dialysis patients.

DISCUSSION

HBV infection is still a serious problem in HD patients, despite effective vaccinations and infection controls. HBV infection is accompanied by increased morbidity and mortality in end-stage renal failure.

According to the national data from the Turkish Society of Nephrology's, HBsAg seropositivity in HD patients was 4.3% in 2011 in Turkey (15). Interestingly, with the introduction of lower HBV DNA level detection with more sensitive PCR techniques, the observed frequency has increased. As a result of the improvements in PCR methods, OHB diagnosis has begun to be made in HBsAg-negative patients (16). Interpretation of HBV serologic markers is given in Table III.

OHB infection is thought to be a risk for transmission. Hoofnagle et al. reported that HBV infection developed following HBsAg and anti-HBs negative, anti-HBcIgG positive blood transfusions (3). In another study, when the serum of patients who were HBsAg negative and HBV DNA positive was injected into chimpanzees, acute hepatitis developed; and when DNA samples were taken from humans and the chimpanzees after acute hepatitis development were compared, they were the same (17).

Table II: Anti-HBs positivity and causes of end-stage renal failure.

	DM	**HT**	**ADPKD**	**Idiopathic**	**Others**	**Total**
Anti-HBs positive	45	33	8	36	13	135
Anti-HBs negative	33	8	3	14	7	65
Total	78	41	11	50	20	200

DM: Diabetes mellitus, **HT:** Hypertension, **ADPKD:** Autosomal dominant polycystic kidney disease.

Table III: Hepatitis B serology.

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Comment
-	-	-	-	Not infected
+	-	-	-	Acute infection (early stage)
+	+	+	-	Acute infection
-	+	-	+	Acute infection recovery
-	+	-	+	Vaccinated or recovered from the infection
+	+	-	-	Chronic infection
-	+	-	-	False positive or low viremia chronic infection
-	-	-	+	Vaccinated

To prevent HBV transmission in HD patients, patients are normally involved in a vaccine program. However, there are both humoral and cellular immune defects in chronic kidney disease. As a result, response to the HBV vaccine in HD patients is lower than the general population. 45-66% of chronic renal disease patients develop sufficient anti-HBs positivity, but their anti-HBs levels show a faster decline when compared with immune competent individuals (18-20).

The clinical and biological spectrum of OHB is not entirely understood. Though OHB is detected in patients with chronic hepatitis and hepatocellular cancer for unknown reasons, it is evident that it causes liver disease. However, a significant majority of OHB patients are asymptomatic and are detected only during scanning. There are studies showing that all anti-HBs positive OHB patients have normal serum ALT and AST levels (21). The viral load is very low (generally below 200 IU/ml) or at undetectable levels in OHB patients (22). The viral load of the three patients in whom we detected HBV DNA positivity with PCR was <200 IU/ml.

OHB can appear in different clinical conditions. One of these is defined as a convalescence period of the acute infection determined by anti-HBs positivity. In some individuals,HBV DNA replication still continues at low levels despite anti-HBs production to neutralize HBsAg antigen. This HBV DNA can persist in the blood or tissues for years (23-25). The second clinical condition is chronic hepatitis developing due to fugitive mutants. These mutant viruses cannot be recognized by an antibody. Mutations in different zones of HBV have been known for years. The host immune response to these mutants is different. These mutations occur by selection of natural variants due to immune response pressure (26).

In the study performed by Sowole et al., HBV DNA was determined by PCR in 138 anti-HBc positive, HBsAg negative patients and OHB was detected in 3 patients (2.2%) (HBV DNA 3, 5 and 9 IU/ml). The liver function tests of these patients were within normal limits (27). In the study of Fontenele et al. with 301 HD patients, anti-HBc positivity was detected in 114 (38%) patients, both anti-HBs and anti-HBc positivity were found in 104 (35%) patients, and anti-HBs positivity in 132 (44%) patients. Anti-HCV positivity was detected in 15 (5%) patients. HBV DNA positivity was found in 7 (2.3%) patients. Three of these events with HBV DNA positivity were found to be anti-HCV positive (28). In our study, we detected OHB prevalence in 1.5% of 200 dialysis patients. When this rate was classified into dialysis types, the frequency was 1.67% (3/17) in HD patients and 0% (0/21) in peritoneal dialysis patients. In two of the three HBV DNA positive patients, anti-HBc positivity was detected and in one of them, anti-HBs positivity was detected.

In a study analyzing 2919 patients between ages 19-21 who had been vaccinated against hepatitis B in the neonatal period, HBsAg positivity was 2.1%, HBsAg negative, anti-HBs positive and anti-HBc positive or negative was 43.9%, only anti-HBc positive was 10.4 %, and both anti-HBs and anti-HBc positive was 4.3%. HBV DNA was positive in 81 of 106 patients (76.4%) who were HBsAg antigen negative, anti-HBs positive and anti-HBc positive (29). In another study involving 46 HBsAg negative vaccinated children, the anti-HBs positivity was 50%, and occult hepatitis B frequency was 10.9% (30). In our study, when anti-HBs and anti-HBc positive patients were considered, the OHB prevalence was found to below, 2.17% (1/46).

In conclusion, the OHB prevalence in our study was 1.5% in HBsAg negative dialysis patients. There are still contradictions about the etiopathogenesis and clinical importance of OHB. It is necessary to consider the route of transmission in HD patients and we recommend performing HBV DNA tests of all HD patients. The rate of response to hepatitis B vaccination in dialysis patients is lower than in pre-dialysis kidney disease, so it is important to emphasize the vaccination of patients in the pre-dialysis period. It is important to check the HBV DNA of patients before hemodialysis, but it seems to be costly. Moreover, although there is no exact information on how OHB

positive patients should be handled in HD, the latest consensus is to perform chemical disinfection on the device after the patient exits dialysis. In this way, increased morbidity and mortality due to HBV infection in end-stage renal failure patients could be prevented by eliminating transmission.

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