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Prevalence of Occult Hepatitis B Virus Infection in Hemodialysis Patients

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Background/Aims: The prevalence of occult HBV infection depends on the prevalence of HBV infection in the general population. Hemodialysis patients are at increased risk for HBV infection. The aim of this study was to determine the prevalence of occult HBV infection in hemodialysis patients.
Methods: Total of 98 patients undergoing hemodialysis in CHA Bundang Medical Center (Seongnam, Korea) were included. Liver function tests and analysis of HBsAg, anti-HBs, anti-HBc and anti-HCV were performed. HBV DNA testing was conducted by using two specific quantitative methods.
Results: HBsAg was detected in 4 of 98 patients (4.1%), and they were excluded. Among 94 patients with HBsAg negative and anti-HCV negative, one (1.1%) patient with the TaqMan PCR test and 3 (3.2%) patients with the COBAS Amplicor HBV test were positive for HBV DNA. One patient was positive in both methods. Two patients were positive for both anti-HBs and anti-HBc and one patient was negative for both anti-HBs and anti-HBc.
Conclusions: The present study showed the prevalence of occult HBV infection in HBsAg negative and anti-HCV negative patients on hemodialysis at our center was 3.2%. Because there is possibility of HBV transmission in HBsAg negative patients on hemodialysis, more attention should be given to prevent HBV transmission. **(Korean J Gastroenterol 2013;61:209-214)**

Key Words: Hepatitis B virus; DNA; Hepatitis B surface antigens; Hemodilaysis

INTRODUCTION

Chronic HBV infection is characterized by persistent detection of HBsAg and HBV DNA for 6 or more months after acute infection.[1-3] It is known that the clearance of HBsAg in patients with HBV infection is associated with the disappearance of HBV DNA and active viral replication. However, several studies revealed that low levels of HBV DNA still remain detectable in the serum or liver tissue of some patients whose HBsAg disappeared spontaneously or by successful anti-viral treatment. The presence of HBV DNA in the liver tissue (with detectable or undetectable HBV DNA in the serum) of HBsAg negative individuals is defined as occult HBV infection.[4,5]

Although the mechanism and clinical implications of occult HBV infection have not identified clearly, occult HBV infection has the risk of disease transmission through transfusion, hemodialysis, and organ transplantation. Occult HBV infection may contribute to the development and acute exacerbation of HBV associated diseases such as cryptogenic

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liver disease, fulminant hepatitis, liver cirrhosis (LC), and hepatocellular carcinoma (HCC). It may also affect disease progression and treatment response of chronic HCV infection.[3-5]

The prevalence of occult HBV infection is related to the overall prevalence of HBV infection in the general population.[6] South Korea is still an endemic area for HBV infection and chronic renal failure (CRF) patients on hemodialysis are at risk for HBV infection. The prevalence of occult HBV infection in HBsAg negative patients on hemodialysis ranges between 0% and 58% in published reports.[7-12] There has been limited data about the prevalence of occult HBV infection among CRF patients in South Korea. The aim of this study was to investigate the prevalence of occult HBV infection in patients receiving hemodialysis at a single center in South Korea.

MATERIALS AND METHODS

1. Patients

Among CRF patients undergoing hemodialysis in CHA Bundang Medical Center (Seongnam, Korea) between May and June 2007, a total of 98 patients were included in the study. Four patients were HBsAg positive and they were excluded. Patients with history of other liver disease including LC, HCC, autoimmune hepatitis, or alcoholic hepatitis, history of alcohol drinking over 20 g/day for recent 6 months, and other risk factors such as human immunodeficiency virus (HIV) and intravenous drug abuse were not included. All patients have no family history of HBV infection and they were anti-HCV negative. The study was approved by the Institutional Review Board at the CHA Bundang Medical Center of CHA University and informed consent was obtained from the subjects.

2. Blood samples

Blood samples were obtained from patients when they visited the hospital for hemodialysis and centrifuged at 2,500 rpm for 5 min. Separated serum samples were used serological tests and the rest of serum samples were stored at −70°C for PCR assay. Blood was tested for complete blood count, AST, ALT, total bilirubin, and hepatitis B and C viral markers (HBsAg, anti-hepatitis B surface antigen [anti-HBs], anti-hepatitis B core antigen [anti-HBc] and anti-HCV antibody).

3. Detection of HBV DNA

HBV DNA testing was performed using two different PCR methods. COBAS Amplicor HBV monitor test (Roche Molecular Diagnostics, Basel, Switzerland) is widely used automated PCR assay for the quantitation of HBV DNA in serum. This test is based on the four major processes: specimen preparation, PCR amplification of target DNA with biotinylated primers, hybridization of the amplicon to oligonucleotide probes specific for the target, detection of the amplicon-probe complex by colorimetric determination.[13] The biotinylated HBV-104UB primer and the nonbiotinylated HBV-104D primer were used to define a sequence of 104 nucleotides within the highly conserved precore-core region of the HBV genome. TaqMan real-time PCR test was performed with an Applied Biosystems 7300 real-time PCR system (Applied Biosystems, Foster City, CA, USA). This is based on the 5'-3' exonuclease activity of the Taq DNA polymerase, which results in cleavage of fluorescent dye-labeled probes during PCR. Two PCR primers (forward primer 5'-CTCCCC-GTCTGTKCCTTCTCATC-3' [HBVRT1F; K=G or T]; reverse primer 5'-GGCGTTCACGGTGGTCTCCATGC-3' [HBVRT1R]) and TaqMan probe 5' FAM-CCGTGTGCACTTCGCTTCACCTCTGC-TAMRA 3' (HBV1TAQ) were created against a region of the HBV genome overlapping the genes encoding the X-protein and DNA polymerase. Quantitation of HBV DNA by the COBAS Amplicor HBV monitor test and Taqman PCT test was performed according to the protocol previously described.[13,14] Linear dynamic range of detection in the COBAS Amplicor HBV monitor test and TaqMan PCR test was found to be 300-2×10^5 copies/mL and 120-1.2×10^{10} copies/mL, respectively.

4. Statistical analysis

IBM SPSS Statistics 19.0 (IBM Co., Armonk, NY, USA) was used for the statistical analysis. All data are expressed as means±standard deviation or median and range in continuous variables and percentage in categorized variables. Mann-Whitney test and Fisher's exact test were used for comparing variables between HBV DNA positive patients and negative patients. All p-values less than 0.05 were considered statistically significant.

RESULTS

Four of 98 patients (4.1%) were HBsAg positive, and they were excluded. The baseline characteristics of remaining 94 patients are provided in Table 1. Among 94 patients with HBsAg negative, 45 were males and 49 were females. The median age was 58 (25-86) years and the median dialysis period was 38.5 (3-216) months (Table 1). Patients with positive IgG anti-HBc and positive anti-HBs were 60 (63.8%) and 68 (72.3%), respectively, and 14 patients were negative for both IgG anti-HBc and anti-HBs (Table 2). HBV DNA was detected in 3 of 94 patients (3.2%) by COBAS Amplicor HBV monitor test and 1 of 94 patients (1.1%) by Taqman PCR test. One patient was positive in both methods. There was no significant difference of age, sex, dialysis period, total bilirubin, AST, ALT, anti-HBc, and anti-HBs between HBV DNA positive patients and negative patients in the COBAS Amplicor HBV monitor test (Table 3). Clinical characteristics of HBV DNA positive patients are detailed in Table 4. The highest serum HBV DNA level in Taqman PCR test and COBAS Amplicor HBV monitor test were 3,940 copies/mL and 11,309 copies/mL, respectively. Two patients were positive for both IgG anti-HBc and anti-HBs and one patient was negative for both IgG anti-HBc and anti-HBs.

Table 1. Baseline Characteristics of HBsAg Negative Patients

Characteristic	Data
Age (yr)	58 (25-86)
Sex (male : female)	45 : 49
Dialysis period (mo)	38.5 (3-216)
Total bilirubin (mg/dL)	0.28±0.15
AST (IU/L)	18.2±10.4
ALT (IU/L)	16.1±8.7

Values are presented as median (range), n (%), or mean±SD.

DISCUSSION

The prevalence of occult HBV infection varies widely depending on the sensitivity of the assay used, the general prevalence of the HBV in different parts of the world, and the different populations studied.[3] Since HBV DNA level is usually less than 10^3 copies/mL in occult HBV infection, the detection of occult infection is strongly dependent on both the sensitivity and specificity of the assay.[15] Occult HBV infection is more frequently reported in the countries with high HBV infection, such as China, Japan and Taiwan, compared with Western countries.[6,11] South Korea is still an endemic area for HBV infection, and Kim et al.[16] reported the prevalence of occult HBV infection was high (16%) in HBsAg negative subjects with normal serum ALT levels in Korea. Occult HBV infection is also more frequent in some study populations, such

Table 2. Frequency of Various Combinations of Hepatitis B Virus Markers

Anti-HBc	Anti-HBs	Patient
−	−	14 (14.9)
+	−	12 (12.8)
−	+	20 (21.2)
+	+	48 (51.1)
Total		94 (100.0)

Values are presented as n (%).
Anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to hepatitis B surface antigen.

Table 3. Comparison of Baseline Characteristics between Positive HBV DNA and Negative HBV DNA Group

Characteristic	Total	HBV DNA			p-value[a]
		Both method (+)	Amplicor method (+)	Both methods (−)	
Patient	94 (100.0)	1 (1.1)	3 (3.2)	91 (96.8)	
Age (yr)	58 (25-86)	85	62 (58-85)	58 (25-86)	0.268
Gender (male)	45 (47.9)	1 (100.0)	1 (33.3)	44 (48.4)	0.532
Dialysis period (mo)	38.5 (3-216)	10	82 (10-118)	38 (3-216)	0.471
Total bilirubin (mg/dL)	0.28±0.15	0.25	0.28±0.15	0.26±0.06	0.761
AST (IU/L)	18.2±10.4	9	19.7±3.1	18.1±10.6	0.307
ALT (IU/L)	16.1±8.7	18	15.7±3.2	16.2±8.9	0.699
Anti-HBc	60 (63.8)	1 (100.0)	2 (66.7)	58 (63.7)	0.919
Anti-HBs	68 (72.3)	1 (100.0)	2 (66.7)	66 (72.5)	0.871

Values are presented as n (%), median (range), or mean±SD.
[a]This p-value is the result of comparison between HBV DNA positive group and negative group in the COBAS Amplicor HBV monitor test.

Table 4. Clinical Characteristics of Patients with Positive HBV DNA

Number	Age (yr)	Gender	Anti-HBs	Anti-HBc	HBV DNA	
					Taqman method (copies/mL)	Amplicor method (copies/mL)
1	85	Male	+	+	3,940	11,309
2	62	Female	−	−	ND	1,557
3	58	Female	+	+	ND	1,126

Anti-HBs, antibody to hepatitis B surface antigen; anti-HBc, antibody to hepatitis B core antigen; ND, not detected.

as HCV infected patients, patients on hemodialysis or intravenous drug abuser.[17-19]

According to the recent definition of occult HBV infection, gold standard for the detection of occult HBV infection is the analysis of HBV DNA in liver tissue. However, standardized and effective assays for the detection of HBV DNA in the liver tissue are not available currently. Although, liver biopsy is safe and the best way to get liver tissue specimens, it is an invasive procedure and cannot be performed in the majority of the patients. The analysis of serum HBV DNA is still the most common assay to identify occult HBV infection.[4,5]

In the present study, HBV DNA test was performed using two different methods and the prevalence of occult HBV infection was 3.2% with COBAS Amplicor HBV monitor test and 1.1% with Taqman PCR test. The prevalence of occult HBV infection in patients undergoing hemodialysis at this center was not high compared to that of low endemic regions. However, previous study from Korea reported no occult HBV infection in chronic hemodialysis patients.[10] The difference of HBV DNA testing might caused an difference in the prevalence of occult infection. In this study, the prevalence of occult infection was different according to the assays used. Also, the difference between communities studied might affect the result.

Large numbers of HBV infection cases of both patients undergoing hemodialysis and medical personnel who worked with the hemodialysis patients were reported in 1977.[20] For this reason, a guideline was introduced for the prevention of HBV infection.[21] Consequently, the prevalence of HBV infection was reduced in the developed counties, such as Europe and North America.[22] The following methods were also introduced into Korean hospitals for hemodialysis treatment: the examination of viral markers for HBV and HCV in hemodialysis patients, vaccinations for the patients with negative HBsAg or negative anti-HBs, and the segregation of tools and equipments that are used for hemodialysis treatment. These guidelines for the control of infection were also performed in our dialysis center.

Pre-exposure HBV vaccination of three injections at 0, 1, and 6 months was performed for all susceptible hemodialysis patients at our center. Actually, all patients had a history of HBV vaccination. If patients had anti-HBs $<$ 10 mIU/mL after last vaccine injection, they were revaccinated with three additional injections. If they had anti-HBs $\geq$ 10 mIU/mL, test of HBsAg and anti-HBs was performed every 6 months. They received a booster dose of vaccine if anti-HBs $<$ 10 mIU/mL.

In the current study, the concentrations of HBV DNA detected in patients with occult HBV infection were 11,309 copies/mL, 1,557 copies/mL, and 1,126 copies/mL. Of the currently used PCR techniques, these two techniques were sensitive enough to diagnose occult HBV infection. Although both techniques were sensitive enough to detect HBV DNA less than 10^3 copies/mL, it was possible to increase the chance of a false positive because of the contamination or amplification of other products.[1] We reduced false positive rate by repeating PCR test from patients who recorded positive responses using the COBAS Amplicor HBV monitor test.

Because humoral and cell-mediated immune responses were compromised in CRF patients, the elevation of liver enzyme is mild and clinical symptom such as jaundice is usually absent.[23] Moreover, it is highly likely for HBV patients undergoing hemodialysis to become chronic carriers compared with the general population.[20] It is difficult to detect occult HBV infection in hemodialysis patients just with clinical symptoms and liver function tests, including viral markers. This study showed similar finding that there was no significant difference of laboratory characteristics between HBV DNA positive patients and negative patients. However, this result had a limitation because of small sample size of occult HBV infection.

Among three patients with occult HBV infection, two patients were both positive for anti-HBc and anti-HBs, whereas

the third patient was both negative. Occult HBV infection is related to markers of HBV. HBV can be transmitted if anti-HBc is positive. When the tests for HBV DNA are not carried out, anti-HBc is used for the surrogate marker of occult HBV infection.[1] Although, occult HBV infection is most commonly reported in patients with anti-HBc positive, it can be detected in those patients with negative for both IgG anti-HBc and anti-HBs.[24] There is no clear association between IgG anti-HBc and occult infection. Thus, negative IgG anti-HBc may not exclude occult HBV infection. Of 94 patients, 61.2% of patients were anti-HBc positive, whereas 66.7% of patients were anti-HBc positive in patients with occult HBV infection. These results are not significantly different when compared with the prevalence of anti-HBc (61.6%) in the Korean adult population.[25] If the presence of HBV DNA in the liver tissue is proved, occult HBV infection can be diagnosed without detectable HBV DNA in the serum. Although, undetectable HBV DNA in the serum also may not exclude occult HBV infection and there is a problem of false positive, detection of HBV DNA in the serum is still reliable marker of occult HBV infection.

It is necessary to study the relationship between the amount of HBV DNA and the infectivity in the future. Because this study was carried out at a single dialysis center, there was a possibility that the result was biased for the differences in the communities. Further study should be performed in collaboration with other hospitals.

In conclusion, the prevalence of occult HBV infection in HBsAg negative and anti-HCV negative patients undergoing hemodialysis at our hospital was 3.2%. Because, there is a possibility of HBV transmission by contaminated blood or organ, more attention should be given to prevent the transmission of HBV infection.

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