

RAPID COMMUNICATION

Prevalence of occult HBV infection in haemodialysis patients with chronic HCV

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Abstract

AIM: To study the prevalence and clinical effects of occult HBV infection in haemodialysis patients with chronic HCV.

METHODS: Fifty chronic hemodialysis patients with negative HbsAg, and positive anti-HCV were included in the study. These patients were divided into two groups: HCV-RNA positive and HCV-RNA negative, based on the results of HCV-RNA PCR. HBV-DNA was studied using the PCR method in both groups.

RESULTS: None of the 22 HCV-RNA positive patients and 28 HCV-RNA negative patients revealed HBV-DNA in serum by PCR method. The average age was 47.2 ± 17.0 in the HCV-RNA positive group and 39.6 ± 15.6 in the HCV-RNA negative group.

CONCLUSION: The prevalence of occult HBV infection is not high in haemodialysis patients with chronic HCV in our region. This result of our study has to be evaluated in consideration of the interaction between HBsAg positivity (8%-10%) and frequency of HBV mutants in our region.

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Key words: Chronic HCV; Haemodialysis; Occult HBV infection

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INTRODUCTION

In most cases, occult HBV infection is defined with low existence of HBV infection in HbsAg deficiency. Serum HBV-DNA level in these patients is generally lower than 10^4 copies/mL. It is reported that there is a high prevalence of occult HBV infection in patients with chronic hepatitis C, HCC and haemodialysis patients, criptogenic liver disease, drug injection users and HIV patients, and in those undergoing frequent blood transfusion (those with hemophilia disease, etc.) and blood donors^[1-8]. The existing data is limited on occult HBV among patients on long-term dialysis. It has been recently suggested that occult HBV infection might be associated with a lower response of hepatitis C virus (HCV) to standard interferon therapy in patients with normal renal function. The prevalence of occult HBV infection in renal dialysis patients ranges between 0% and 58% in published reports. The aim of this survey is to address epidemiological and clinical significance of occult HBV infection in patients receiving regular dialysis in Turkey.

MATERIALS AND METHODS

A total of 50 patients with chronic renal failure receiving haemodialysis have been chosen with negative HbsAg and positive anti-HCV for at least 6 months and accepted for this study from among the patients of dialysis unit, and from among patients who have applied to Internal Diseases, Faculty of Medicine, Dicle University. These patients were divided into two groups, HCV-RNA positive (22 patients) and HCV-RNA negative (28 patients). Average haemodialysis periods were 4.9 ± 2.3 years in the HCV-RNA positive group^[1-9] and 4.6 ± 2.1 years in the HCV-RNA negative group^[1-8]. There were 13 male and 9 female subjects in the HCV-RNA positive group, with an average age of 47.2 ± 17.0 years^[18-24]. There were 17 male and 11 female subjects in the HCV-RNA negative group, with an average age of 39.6 ± 15.6 years^[13-16]. Cirrhosis existence in patients was externalized both clinically and through laboratory tests.

Fifteen ml blood was taken from the patients, centrifuged at 2500 r/min for 5 min, and stored at -25 °C with separated serum samples. Hepatitis panel (HBsAg, anti-HBs, anti-HBc IgG) was studied by "Macro Enzyme Immune Assay (Macro EIA)" method in Elecsy 2010 (Roche) device and with similar brand kits; and anti-HCV was studied with the Cobas Core II Immunochemical system (Roche). The "Real-time PCR method" with Robo Gene

(Roboscreen, Leipzig, Germany) kits in ABI PRISM 5700 (Applied Biosystems) device HCV-RNA was used. HBV-DNAs of cases included in the study were quantitatively evaluated by PCR method. Techne Cylogene Thermal Cycler (Techne Cambridge Ltd) Duxford-Cambridge U.K. device was used for HBV-DNA PCR amplification. For PCR amplification, viral DNA was insulated from 100 µL serum in the incubation tampon containing 1 mg/mL Proteinase K at 37°C through phenol-chloroform extraction and ethanol precipitation following a 3-h incubation.

RESULTS

Patients included in the study were divided into two groups, HCV-RNA positive and HCV-RNA negative. Demographic and clinical characteristics of these patients are shown in Table 1. Thirty (60%) of 50 patients were male, while 20 (40%) patients were female, with an age range of 13-84 years, and averaging 42.9 ± 16.5 years.

HCV-RNA PCR examination was positive in 22 (44%) patients, and negative in 28 (56%) patients. No HBV-DNA (0%) was detected through the high-sensitivity PCR study in both patient groups with positive and negative HCV-RNA. Accordingly, occult HBV infection was detected in none of the 50 patients in our study.

Results in anti-HCV positive and HCV-RNA positive group (22 patients) are as shown in Table 1. Considering both groups together, the results in 50 patients are as follows.

Serum ALT levels are above normal in 12 (24%) of the 50 patients, 8 showed persistent and meaningful ALT level in their six-month monitoring. HCV-RNA was positive in 7 of the 8 patients, and negative in 1 patient. With regard to the duration of haemodialysis, only 6 of the 50 patients were receiving haemodialysis for less than 3 years, 44 patients were receiving haemodialysis treatment for 3 years and over. The minimum haemodialysis duration was 1 year. Twelve (57.1%) out of a total of 21 patients in the two groups with insulated anti-HBs seropositivity received HBV vaccination in their history.

DISCUSSION

In most cases, occult HBV infection is defined with low existence of HBV infection in HbsAg deficiency. Serum HBV-DNA level in these patients is generally lower than 10^4 copies/mL. It is reported that there is a high prevalence of occult HBV infection in patients with chronic hepatitis C, HCC and haemodialysis patients, in those with cryptogenic liver disease, drug injection users and HIV patients, and in those who underwent frequent blood transfusion (those with hemophilia disease, etc) and blood donors^[1-8].

As they are similar transmission models, HBV and HCV co-infection are prevalent clinical presentations. Anti-HBc prevalence reported in patients with chronic HCV infection is 50%-55%^[1,2,9]. This ratio is a close value, 59% (13/22) in our study. Most studies show that HBV-DNA genome existence is 22%-87%^[10,11] in patients with negative HBsAg and positive HCV-RNA. HBV-DNA is seen in 46% of anti-HBc positives, and in 20% of anti-HBc nega-

Table 1 Demographic and clinical characteristics of patients

	HCV-RNA Positive (n = 22)	HCV-RNA Negative (n = 28)	Total (n = 50)
Average age (yr) (age range)	47.2 ± 17 (18 - 24)	39.6 ± 15.6 (13-76)	42.9 ± 16.5 (13 - 84)
Sex			
Male	13 (59.1%)	17 (60.7%)	30 (60%)
Female	9 (40.9%)	11 (39.3%)	20 (40%)
Average Haemodialysis period (yrs) (haemodialysis range)	4.9 ± 2.3 (1 - 9)	4.6 ± 2.1 (1-8)	4.8 ± 2.2 (1-9)
ALT (IU/mL)	52.9 ± 52.8 (4 - 186)	29.3 ± 20.9 (2-88)	39.7 ± 39.7 (2 - 186)
Insulated anti-HBs Seropositivity	7 (31.8)	14 (50%)	21 (42%)
Insulated anti-HBc IgG seropositivity	3 (13.6%)	1 (3.5%)	4 (8%)
Anti-HBs and anti-HBc IgG Seropositivity	10 (45.4%)	6 (21.4%)	16 (32%)
Anti-HBs and anti-HBc IgG Seronegativity	2 (9.1%)	7 (25%)	9 (18%)
HBV-DNA (PCR) Positivity	0 (0%)	0 (0%)	0 (0%)

tives^[11]. In chronic HCV related liver diseases, frequency of detectable HBV-DNA is apparently higher than HCV non-related liver diseases^[11]. However, variable prevalence of occult HBV infection is seen in the permanence of HBV-DNA PCR^[12]. And this makes us think that HBV viremia shows fluctuations during occult HBV infection.

Clinical interaction of HCV and occult HBV infection is still controversial. Some studies report that cirrhosis is seen more frequently in those patients with HCV and occult HBV co-infection than in those with HCV infection alone^[11]. However, there are also studies that are not in support of this^[13]. Parallel to this, HCV and occult HBV co-infection had a lower incidence in non-cirrhotic hepatitis^[14]. Occult HBV co-infection is related to higher ALT levels and histological activity. Despite this, other studies reveal that the incidence of liver related complication in these patients is at a level comparable to those with HCV infection alone^[9-13]. Patients with active HBV and HCV co-infection tend not to respond to interferon treatment. Some studies show that such low interferon response exists in patients with HCV and occult HBV co-infection^[14-16]. However, other studies reveal that occult HBV co-infection has no effect on the interferon response of HCV infection. Interferon treatment may accelerate HCV clearance in patients with HCV and occult HBV co-infection, but HBV-DNA remains at detectable level. HCV-RNA level is apparently higher in patients with occult HBV co-infection than patients with HCV infection alone. Despite all these, three recent studies show a lower prevalence of chronic HCV and occult HBV co-infection and that the effects of occult HBV infection on chronic HCV infection remained low in these patients^[17-19]. While one of these studies report occult HBV co-infection prevalence at 6.7% in patients

with chronic HCV infection, no difference was found in the frequency of occult HBV infection between those with and without a marker for HBV infection in their history^[18]. Another study found that occult HBV infection had no effect on the early phase response of chronic HCV infection combined treatment^[17]. And the last study states that occult HBV infection has no significant effect on treatment response upon HCV viral titre, liver enzymes, histological parameters and HCV and interferon- α and ribavirin in patients with chronic hepatitis C^[19].

In a study on chronic haemodialysis patients, serum in PCR and HBV-DNA in PBMC was studied in 67 patients. While serum HBsAg and HBV-DNA were found negative in all of these patients, HBV-DNA was positive in 5 patients (7.5%) in PBMC^[6].

Another study focused on the disease etiology in 107 patients with negative HCC and cirrhosis HbsAg and 192 chronic hepatitis patients as control group with negative HbsAg. In the group with HCC, HCV was blamed as etiological factor in 73 (68%) patients, cryptogenic liver disease in 29 (27%) patients and alcohol in 5 (5%) patients. HCV was blamed in 153 (80%) patients of the group with chronic hepatitis HCV, cryptogenic liver disease was blamed in 32 (17%) patients and alcohol was blamed in 7 (4%) patients^[2]. HBV-DNA PCR was checked in these patients, being positive in 68 (64%) patients with HCC, 63 (33%) patients with chronic hepatitis. In patients with HBV-DNA positive HCC, anti-HBc positivity was found higher than anti-HBc negativity percentage (64% and 56%, respectively). Frequency of occult HBV infection varies in patients with HCC. The prevalence of anti-HBc/Anti-HBs is 43% in such patients, and the HBV-DNA prevalence varies between 5%-80%^[2-20].

Although there are strong evidences aimed at the relationship between occult HBV infection and HCC, the role and place of HBV is not clear in this series of incidents. Is it a silent bystander, a cofactor, or criminal alone? There are strong evidences about the etiological relationship between chronic HBV infection and HCC. It is unlikely that occult HBV infection is only a silent bystander in patients with HCC. The high prevalence of occult HBV infection in patients with HCC supports the role of occult HBV infection in the development of HCC compared with the results in patients with cirrhosis or chronic hepatitis. It is quite possible that occult HBV infection plays a role as cofactor in HCC development. Two viruses may mutually interact, causing more painful inflammation and quicker cirrhosis progress. Alternatively, direct oncogenic effect of two viruses may be additive or synergistic^[2]. This has been an issue of interest in patients with occult HBV co-infection. Evidences relating to occult HBV infection in patients with HCC related to HCV are being gradually seen more frequently^[1,2,4]. In a study made in Spain, in 109 anti-HCV negative and HBsAg negative patients with increased liver enzymes with unknown etiology, 19% HBV-DNA was detected in serum^[21]. Again in another study, in 50 patients with cryptogenic chronic hepatitis, there was detectable HBV-DNA in 15/50 (30%) patients. Of these 15 patients, 66% had increased liver enzymes and 53% suffered from painful fibrosis and cirrhosis. In their repeated liver biopsies during follow-up, in 2/11 (18.2%) patients,

the disease progressed from chronic hepatitis to cirrhosis. Generally considering, clinical results of occult HBV infection after delayed HBsAg clearance vary. These results depend upon the underlying liver disease, duration of active HBV infection and degree of liver damage before HBsAg clearance^[1,2].

An increased frequency of occult HBV infection was reported in immune-dominant patients^[1]. Lymphoma, aplastic anemia or cytotoxic treatment for leukemia, allogeneic or autolog bone marrow transplantation practice in patients with occult HBV infection chronic might cause reactivation of Hepatitis B or development of fulminant hepatic insufficiency^[22,23]. However, chronic hepatitis B reactivation with induced treatment is less frequent and painful in those with occult HBV infection before the treatment than those with chronic hepatitis B with positive HBsAg before the treatment^[1]. Viral infection progresses with the application of immune-suppressive agent, destruction starts with immune intermediary in hepatocytes infected with HBV after the regaining of immunity with the interruption of this agent.

In patients with fulminant hepatic insufficiency, the pathogenetic role of occult HBV infection has been very well defined. In these patients, positivity of serum HBV-DNA ranges between 0%-47%^[24-26]. Recurrence of HBV infection following liver transplantation for fulminant hepatic insufficiency shows possible HBV replication in these patients^[24,25]. However, the pathogenetic role of occult HBV infection in patients with fulminant hepatic insufficiency is doubtful and weak. A multi-centered study in the USA shows that occult HBV infection is not related with acute liver insufficiency^[26].

Occult HBV infection was found in 81/180 patients (45%) in drug injection users in Baltimore^[7]. In a study, 10% occult HBV infection was detected in patients with HIV^[8]. In Japan, occult HBV infection was detected in 22 (51.2%) of 43 hemophilia patients^[5]. It has been reported that in numerous studies covering wide populations conducted in many different countries, occult HBV infection incidence varied between 0%-15% in blood donors^[3].

Most of the relevant authors are mostly unable to provide clear replies to the question what is the responsibility and effect of occult HBV infection in the pathogenesis of diseases where there is a high prevalence of occult HBV infection and where the pathogenetic role of apparent HBV infection is well known. And they are still suspicious on most things. Although there are questions in minds about the subject, pathogenetic role of occult HBV infection is accepted best in HCC.

It is reported that the prevalence of occult HBV infection is parallel with the prevalence of apparent HBV infection in that region^[1,2]. For example, occult HBV infection prevalence varies between 7%-19% among blood donors in endemic regions where 70%-90% of the population are exposed to HBV^[2]. In Western countries (e.g., there is pre-reported HBV exposure in 5% of the American population), frequency of occult HBV infection ranged between 0%-9%^[2]. Prevalence of occult HBV infection can also be affected by incorrect negative and incorrect positive results based on the sensitivity and specificity of the HBV-DNA PCR method. Therefore, the

specificity of the methods has to be verified and cross-contamination has to be prevented. Moreover, in order to positively consider the result in HBV-DNA PCR method, one needs to show at least two different primers from different areas of the HBV genome clone. Most of the studies so far mentioned the sample collection method at very few occasions^[1,2,4]. Cross contamination may occur particularly in retrospective studies where the blood samples used in the past are collected.

In a study made by Beşişik *et al.* in 33 patients with chronic hepatitis C and at the same time chronic haemodialysis with negative HBsAg and positive HCV-RNA, serum HBV-DNA PCR study yielded positive results in 12 (36.4%) patients. YMDD mutation was detected in 6 (50%) of these 12 patients^[27]. According to this study, there is increased occult HBV infection incidence in patients with chronic hepatitis C treated with chronic haemodialysis and YMDD mutation is the responsible agent in an important part of them. In our study, according to the HCV-RNA condition of the 50 cases chronic haemodialysis with negative HbsAg and positive anti-HCV, the study group was divided into two groups, positive HCV-RNA (22 patients) and patients with negative HCV-RNA (28 patients). None (0%) of the patient groups revealed HBV-DNA in serum by PCR method despite the HCV-RNA positive group has been studied twice in different times. Contrary to the above-mentioned similar study, there is no increased incidence of occult HBV infection in patients with chronic haemodialysis with chronic hepatitis C in our region. In our study, 3 patients showed insulated anti-HBc IgG seropositivity and positive HCV-RNA (13.6%), and 1 patient showed negative HCV-RNA (3.5%). And the percentage of having at least one of the markers of past HBV infection was 89.9% (20 patients) in HCV-RNA positive group and 75% (21 patients) in HCV-RNA negative group. However, no increase was detected in occult HBV infection incidence in our study.

The result of our study has to be evaluated in consideration of the interaction between the HBsAg positivity (8%-10%) for Diyarbakır region and the frequency of HBV mutants in this region. Besides, it has become necessary to review the sensitivity and specificities of still non-standardized HBV-DNA PCR methods and define a common method of diagnosis accordingly.

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