Prevalence of occult hepatitis B virus infection in haemodialysis patients from central Greece

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Abstract

AIM: To assess the hepatitis B virus (HBV)-DNA and the prevalence of occult HBV infection in end-stage renal failure (ESRF) patients from Central Greece.

RESULTS: HBV-DNA was detected in 15/366 patients (4.1%) and HBsAg in 20/366 (5.5%). The prevalence of occult HBV infection was 0.9% (3/346 HBsAg-negative patients). Occult HBV was not associated with a specific marker of HBV infection or anti-HCV or anti-HEV reactivity. There was no significant difference in HBV-DNA titres, demographic and biochemical features, between patients with occult HBV infection and those with HBsAg-positive chronic HBV infection.

CONCLUSION: In central Greece, 4% of ESRF patients had detectable HBV-DNA, though in this setting, the prevalence of occult HBV seems to be very low (0.9%).

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Key words: Hepatitis B virus-DNA; Occult hepatitis B virus infection; Haemodialysis; Hepatitis B; Real-time polymerase chain reaction

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INTRODUCTION

Infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are well-known and important causes of liver disease in end-stage renal failure (ESRF) patients on haemodialysis (HD) [1-3]. The adoption of preventive
measures and extensive infection control guidelines, along with a decreased need for transfusions after the introduction of erythropoietin, and the development of an effective HBV vaccine, have significantly contributed to the progressive reduction of HCV and HBV prevalence in HD patients. However, the relatively low response rates to HBV vaccination in this group of patients might contribute, under some specific circumstances, to the ongoing HBV transmission in this setting.

On the other hand, three studies from different parts of Greece have reported a high prevalence of antibodies against the hepatitis E virus (HEV) - an icosahedral non-enveloped single stranded RNA virus- in ESRF patients on HD, compared to the healthy population, suggesting that the oral-faecal route may not be the only mode of transmission in HD. However, a similar prevalence of anti-HEV antibodies in Greek patients who have undergone open-heart surgery might indicate that past infections, and not viral exposure during HD or other interventional procedures, are responsible for the acquisition of HEV.

In chronic renal failure patients undergoing maintenance HD, the diagnosis of liver disease based on biochemical tests is difficult; taking into account that aminotransferase levels in HD patients are usually suppressed. It has been hypothesized that reduced immune competence of chronic uremic patients is a possible cause of attenuated inflammatory reactions in the liver and consequently reduced hepatocyte destruction. Therefore, the quantitative detection of HBV-DNA has been shown to be the most efficient method to evaluate viral replication in HD patients infected with HBV. Besides, several studies using sensitive HBV-DNA polymerase chain reaction (PCR) assays have revealed the presence of HBV-viremia in patients who cleared HBsAg from either acute self-limited or chronic HBV infection, or even after a successful anti-HBV treatment. Demonstration of this clinical entity has resulted in the introduction of the concept of “occult” HBV infection, which is characterised by undetectable serum HBsAg but detectable HBV-DNA in serum or the liver. Occult HBV infection has been reported in patients with chronic HCV infection, hepatocellular carcinoma, and cryptogenic or autoimmune liver diseases. Data on occult HBV infection among patients on long term HD is scarce, as most of the studies have investigated the presence of occult HBV infection in the context of chronic HCV infection. Greece belongs to the intermediate endemicity countries with a wide variance of HBsAg seropositivity among different regions and various populations, ranging from 3%-5% and markers of previous HBV infection between 17% and 25%. Unpublished data from our group (Gatselis, Stefos, Koukoulis and Dalekos, data in preparation), have estimated the prevalence of HBV infection in 2006, in a representative sample of the general population of the region of Thessaly to be 4.26% (range 3.6%-5.2%, age range 18-80 years, n = 1174).

For these reasons, we conducted a large study in Thessaly, Central Greece to: (1) determine the presence of HBV-DNA in all 366 consecutive ESRF patients with or without chronic HBV infection attending the five out of six Nephrology and Dialysis units in Thessaly region; and (2) assess the prevalence of occult HBV infection in this cohort. Additionally, the possible clinical impact of HBV-DNA positivity in these patients (either in the context of chronic or occult HBV infection) was assessed by comparing epidemiological, clinical, laboratory and HBV, HCV and HEV serological markers between HBV-DNA-positive and -negative patients.

**MATERIALS AND METHODS**

Thessaly is one of the thirteen regions of Greece and covers the largest part of central Greece. The prefectures of Larissa (capital city Larissa), Magnesia (capital city Volos), Trikala (capital city Trikala) and Karditsa (capital city Karditsa) constitute the Thessaly region.

The population is approximately 800,000 people (census 2001; 7.5% of the national population and 0.22% of the EU population). All 366 ESRF patients (243 men, 123 women; mean ± age 60.5 ± 14 years and mean duration of HD 49.2 ± 48.2 mo) attending five out of the six Nephrology and Dialysis units of Thessaly region in Central Greece were studied. Sixty patients were from the Nephrology and Dialysis unit of the General Hospital in the city of Larissa (unit 1), 70 from the only private dialysis centre in the city of Larissa (unit 2), 119 from the dialysis unit of the General Hospital in the city of Volos (unit 3), 56 from the dialysis unit of the General Hospital in the city of Trikala (unit 4) and 61 from the dialysis unit of the General Hospital in the city of Karditsa (unit 5). The aetiology of renal failure was as follows: diabetic nephropathy (16.7%), idiopathic glomerulonephritis (14.5%), vascular renal disease (9.3%), urinary tract obstruction (9.3%), polycystic kidney disease (7.7%), systemic diseases (3.3%), tubulointerstitial diseases (1.6%), other causes (3.3%), and unknown (34.3%). The patients’ characteristics are shown in Table 1.

Serum samples were collected between May and August 2001 (before HD) and then stored at -80°C in aliquots until tested. Serological markers of HBV infection (HBsAg, anti-HBs, anti-HBe, HBeAg, and anti-HBc) were determined using standard third generation commercially available enzyme immunoassays (Abbott GmbH, Wiesbaden-Delkenheim, Germany). The samples were investigated for the presence of HBV-DNA using a sensitive commercially available real time PCR kit (COBAS Taqman HBV Test; cutoff of detection: 6 IU/mL). The COBAS TaqMan HBV Test is an in vitro nucleic acid amplification test for quantitation of HBV in human serum or plasma, using the High Pure Viral Nucleic Acid kit for manual specimen preparation and the COBAS TaqMan 48 Analyzer for automated amplification and detection. The highly conserved HBV precore/core region was...
amplified. Every sample with detectable HBV-DNA by real time PCR was tested again in another experiment. Only serum samples with repeatedly detectable HBV-DNA were considered positive for HBV-DNA.

Anti-HCV antibody was tested by a third generation enzyme linked immunosorbent assay (ELISA) (HCV 3.0 ELISA Ortho, Raritan, NJ) according to the manufacturers’ instructions. For the detection of anti-HEV IgG antibodies two commercially available ELISAs (Abbott Diagnostika, Wiesbaden-Delkenheim, Germany and Genelabs Diagnostics, Singapore Science Park, Singapore) were used. These assays included two recombinant antigens, which correspond to the structural regions of the HEV genome[7]. Both ELISAs were performed according to the manufacturers’ instructions. Only repeatedly reactive samples in both assays were considered positive for the presence of anti-HEV IgG. According to the above criteria, initially reactive specimens that according to the manufacturers’ instructions. Only repeatedly reactive samples in both assays were considered positive for the presence of anti-HEV IgG. According to the above criteria, initially reactive specimens that were non-reactive on retesting were considered negative.

Levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using standard techniques. Informed consent was obtained from all patients involved in the study. The Local Ethical Committee of the Medical School, University of Thessaly, approved the study protocol.

**Statistical analysis**

Results were expressed as mean ± SD or median and range as appropriate. Data were analyzed by unpaired t-test, \( \chi^2 \) (two by two with Yates’ correction), and Fisher’s exact test, where applicable. A two-sided \( P \) value < 0.05 was considered as statistically significant. Confidence intervals (95% CI) were determined using the formula

\[ P = p \pm 1.96 \left( \frac{pq/n} \right)^{1/2} \]

where \( p \) is the frequency, \( q \) is 1 - \( p \) and \( n \) is the number of individuals tested from each group.

**RESULTS**

In total, HBV-DNA was detected in 15 of the 366 patients with ESRF (4.1%; 95% CI: 2%-6.1%). Twenty patients (5.5%) were HBsAg positive (overt HBV infection) and 245 were anti-HBs positive (66.9%) (Table 2). Eighty-eight (24%) and 15 (4.1%) were anti-HCV and anti-HEV positive, respectively (Table 2). The prevalence of HBV-DNA seropositivity ranged from 1.4%-10% among the five Dialysis units (\( P = 0.163 \)) (Table 2). The median HBV-DNA titre was 25 IU/mL (range: 7-10 584 IU/mL). HBV-DNA seropositivity was not associated with age, sex, history of hepatitis, history of blood transfusions, the number of blood units transfused, the dialysis type, or with the aetiology of renal failure, aminotransferase activity or anti-HEV reactivity. As expected, there was a significant association between the presence of HBV-DNA and HBsAg, anti-Hbc, anti-HBe positivity (\( P < 0.05 \) for all, Table 3). The absence of HBV-DNA was associated with the presence of anti-HBs and anti-HCV (\( P < 0.05 \) for both, Table 3).

Amongst the 15 HBV-DNA positive patients, 12 had overt HBV infection (80%), including one HBeAg positive, 10 anti-HBe positive and one anti-HBe positive patient who was also anti-HBs positive. Of the three remaining HBV-DNA positive patients, one had markers of past HBV infection (anti-HBc and anti-HBs positive) and two had no serological markers of present or past HBV infection (Figure 1). Thus, the overall prevalence of occult HBV infection amongst the 346 HBsAg negative ESRF patients in the five Nephrology and Dialysis units in the Thessaly region was 0.9% (3/346; 95% CI: 0%-1.9%). Actually, the prevalence of occult HBV infection in Volos Dialysis Unit was 2.6% (3/116) compared to zero prevalence in all other Dialysis Units in the Thessaly region (\( P = 0.199 \)). HBV-DNA titres (median titre: 19 IU/mL, range: 7-37 IU/mL) in patients with occult HBV infection were not significantly different compared to those with overt HBV infection (median: 27 IU/mL, range: 9-10 584 IU/mL, \( P = 0.391 \)). Occult HBV infection was associated neither with age, sex, history of hepatitis, history of blood transfusions, the number of blood units transfused, biochemical parameters (AST, ALT) nor with the aetiology of renal failure (data not shown). Moreover, occult HBV-DNA detection was not associated with a specific marker of past HBV infection and anti-HCV or anti-HEV reactivity. No statistical significance was found comparing the age, HD duration, biochemical parameters, and serological markers of HCV and HEV infections or the duration of erythropoietin treatment.

**Table 1 Characteristics of 366 ESRF patients (mean ± SD)**

| Parameter                                | Mean ± SD   |
|-------------------------------------------|-------------|
| Sex (M/F)                                 | 243/123     |
| Age (mean/range, yr)                      | 60.5/17-85  |
| Haemodialysis duration (mo)               | 49.2 ± 48.2 |
| Dialysis type (haemodialysis/peritoneal dialysis) | 352/14     |
| Known history of viral hepatitis (yes/no) | 51/315      |
| History of transfusions (yes/no)          | 129/247     |
| Number of blood units                     | 6.8 ± 4.7   |
| Duration of erythropoietin treatment (mo) | 46.2 ± 35.8 |

1Haemodialysis duration was calculated from the date of the first haemodialysis procedure to the date of the collection of the sera; 2Proven history of viral hepatitis, either icteric or anicteric; M: Male; F: Female; ESRF: End-stage renal failure.

**Figure 1 Flowchart of the profile of 15 hepatitis B virus (HBV)-DNA positive haemodialysis patients.**
between patients with occult and overt HBV infection (data not shown). Serological markers of HBV, HCV and HEV infections in the 15 HBV-DNA positive patients are presented in Table 4.

### DISCUSSION

The present study demonstrated that 4.1% of Greek patients with ESRF from Central Greece had detectable HBV-DNA by real-time PCR, including 0.9% with occult HBV infection. Interestingly, 5.5% of HD patients had chronic HBV infection (overt HBV infection) but only 60% of them (thus 3.3% of total ESRF patients) were HBV-DNA positive. HBsAg prevalence in patients on maintenance HD varies among studies and geographical areas, from 0.8% to 17%, remaining very high in less developed countries. Further supported by a recent study from our group, the prevalence of HBsAg might suggest contact with HBV during adulthood, though this cannot be safely ascertained if it has taken place in an HD setting. Nevertheless, a similar prevalence of markers of previous HBV infection has already been reported in our country for several high-risk groups of HBV infection, such as HD patients, alcoholics, and heroin addicts, while in refugees this prevalence can be as high as 70% [22,26-33,35]. Furthermore, with the development of sensitive PCR-based testing for HBV-DNA, studies in dialysis units have demonstrated that the prevalence of occult HBV infection ranges from 0% to 58% in published reports [18-23]. These variations could be attributed to differences in the sensitivity of the methods used for the detection of HBV-DNA, in the patients’ sample investigated in each study, and in geographic variations of HBV prevalence. Though our aim was to strictly assess the prevalence of occult HBV infection in a very large cohort of HD patients, a recent publication on Greek blood donors with occult HBV infection was able to successfully perform the molecular characterization of HBV strains in less than half of occult cases, revealing several amino acids substitutions related to diagnostic escape and antiviral resistance [34].

It should be stated herein, however, that the detection of HBV-DNA in serum samples rather underestimates the true prevalence of occult HBV infection [31]. Indeed, the most correct and precise methodological approach for the determination of the prevalence of occult HBV infection is the analysis of liver DNA extracts. This is further supported by a recent study from our group,
which assessed the prevalence of occult HBV infection in patients with autoimmune liver diseases[17]. However, the availability of liver tissues is often limited by restrictions on the performance of liver biopsies, which in the setting of HD is often very difficult and usually contraindicated.

In agreement with previous reports, we found that both groups of ESRF patients with chronic HBV infection and occult HBV infection had low levels of HBV-DNA[2,18,23]. HBV-DNA titres have been reported to remain low and stable over time, which can probably explain the low mortality rates due to liver disease in ESRF patients in developed countries[24]. The mechanisms responsible for the inhibition of HBV activity are as yet undefined in this specific group of patients. The passage of HBV-DNA from serum into the dialysate compartment during HD session, the destruction of HBV genome during HD procedure, and the interplay between HBV and the immune system could all play a role[25]. The low viral load observed in these subjects could also be attributed to multiple amino acid substitutions in the polymerase region, which might give rise to less fit viral strains, as was recently demonstrated in Greek blood donors with occult HBV infection[26]. The longer half-life of interferon in dialysis patients is another important factor, though none of the patients in our study was receiving antiviral treatment. Moreover, viral interactions can maintain HBV infection in a latent state, as in ESRF patients with chronic HCV infection and occult HBV viremia[22,23]. In these cases, a negative interference has been considered between the two viruses, leading to low HBV-DNA levels[26,27]. In our study, HBV-DNA positivity was negatively associated with HCV infection, further supporting the possibility of reciprocal replicative suppression of the two viruses. In this context, all three HD patients with occult HBV infection in our study were negative for anti-HCV and HCV-RNA by a transcription mediated amplification assay[28]. On the contrary, HBV-DNA positivity was not associated with markers of HEV infection.

The emerging evidence of the potential clinical significance of occult HBV infection is the main reason for the growing interest in this topic. Accumulating data shows that occult HBV infection might be transmitted in cases of blood transfusions, in the event of organ transplantation, and in particular, in cases of orthotopic liver transplantation as an obvious consequence of the fact that hepatocytes are the reservoir of the viral strains[21]. However, the risk of occult HBV transmission in cases of kidney transplantation seems to be low[26,41]. Nevertheless, virological and clinical reactivations of occult HBV infection have been repeatedly observed in several clinical conditions, where an immunosuppressive status evolves, including haematological or other malignancies, HIV infection, haematopoietic stem cell transplantation, and organ transplantation[11]. Therefore, the theoretical basis of HBV reactivation in HD cases with occult hepatitis B, who had undergone kidney transplantation and are under immunosuppression, is still unresolved.

In conclusion, approximately 4% of ESRF patients on HD from Central Greece have detectable HBV-DNA levels, amongst which 20% have occult HBV infection, which might indicate that the absence of HBsAg in the blood of HD patients is not sufficient to ensure lack of circulating HBV. However, in the total group of HBsAg negative HD patients, the prevalence of occult HBV infection in our region seems to be one of the lowest worldwide (0.9%). The demographic and biochemical features of HBV-DNA positive subjects, including those with occult HBV infection, do not help to distinguish these individuals from those who are HBV-DNA negative. We believe that longitudinal studies including even larger numbers of patients are needed to further clarify the clinical significance and outcome of occult HBV infection in this setting.

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COMMENTS

Background

The prevalence and clinical significance of occult hepatitis B virus (HBV) infection in the haemodialysis (HD) setting in large series of patients with end-stage renal failure (ESRF) on HD has not been adequately addressed.

Research frontiers

This situation might have considerable implications, either from the public health point of view (due to the risk transmission of the virus among individuals in the hemodialysis units) or from the clinical point of view, as attested by well-known cases of acute and severe exacerbations of HBV infection under some circumstances, such as transplantation and the subsequent immunosuppressive treatment.

Innovations and breakthroughs

Occult HBV infection in HD patients ranges from 0% to 58% in published reports. Though their large study in 366 ESRF patients on HD from Central Greece revealed the presence of HBsAg in 5.5% and HBV- viremia in approximately 4%, the actual presence of occult HBV infection was identified in only 0.9% of HBsAg negative patients (one of the lowest frequencies of occult HBV infection in HD patients worldwide). These discrepancies in the frequencies of occult HBV infection in a HD setting could be ascribed to differences in the number of patients tested, the sensitivity of the methods used for the detection of HBV-DNA, the patients’ specimen investigated in each study (serum or liver tissue), and geographical variations regarding in HBV prevalence.

Applications

Based on the potential clinical significance of occult HBV infection in the HD setting, the authors believe that further multicentre longitudinal studies, including larger numbers of patients, are needed to further clarify whether occult HBV infection is a major clinical problem in this setting or not.

Terminology

Occult HBV infection, is a clinical entity characterised by undetectable HBsAg in the serum but detectable HBV-DNA in serum or the liver. The use of sensitive HBV-DNA polymerase chain reaction assays in serum samples or, preferably, in liver tissues when available using the highly conserved HBV precore/core regions for amplification, appears to be the assay of choice for the diagnosis of this condition.

Peer review

This study described the occult HBV infection in 366 ESRF patients on HD in Nephrology and Dialysis units of the Thessaly region in Central Greece. The authors concluded that the prevalence of occult HBV infection was 0.9%, although the HBsAg prevalence was 5.5% in this cohort. This study provides
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