Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2019;44(3):753-757
DOI: 10.17826/cumj.419941

ARAŞTIRMA / RESEARCH

Prevalence of occult hepatitis B infection in patients with cryptogenic cirrhosis

Kriptojenik sirozlu hastalarda okült hepatit B sıklığı

Serkan Yalaki[1], Mehmet Suat Yalçın[2]

[1]Mersin Şehir Hastanesi, İç Hastalıkları, Gastroenteroloji Kliniği. Mersin, Turkey
[2]Aksaray Eğitim ve Araştırma Hastanesi. İç Hastalıkları Kliniği, Aksaray, Turkey

Cukurova Medical Journal 2019;44(3):753-757.

Abstract

Purpose: The frequency of occult hepatitis B is variable in patients with cryptogenic cirrhosis and is unknown in our region. The presence of HBV DNA in serum of cirrhotic patients can be determined by molecular techniques despite the lack HBV serological markers. We aimed to determine the frequency of occult hepatitis B virus in patients with cryptogenic cirrhosis in this study.

Materials and Methods: This cross-sectional study was designed to assess the prevalence of occult HBV infection in a group of patients with cryptogenic liver cirrhosis. Forty-four patients with cryptogenic cirrhosis who were examined in our hospital were enrolled in this study. Their serum were studied for HBV DNA levels using a real-time PCR method.

Results: There were 44 patients in the study group; Positive for anti-hepatitis B surface antibody was detected in 22 patients, and positive for hepatitis B core antibody was detected in 24 patients. In 2 patients, HBV DNA was found to be positive. In our study, occult HBV infection was found in 4.5% (2/44) of patients with cryptogenic cirrhosis.

Conclusion: The prevalence of occult HBV infection was relatively high in patients with cryptogenic cirrhosis; Occult hepatitis B infection may thus play a role in the development of decompensated cirrhosis and complications in cases of cryptogenic liver disease.

Keywords: Occult hepatitis B, cryptogenic cirrhosis, HBV DNA

Öz

Amaç: Kriptojenik sirozlu hastalarda okült hepatit B sıklığı değişkendir ve bölgemizde sıklığı bilinmemektedir. HBV serolojik belirteçleri olmamasına ragmen HBV DNA'sının bu sirotik hastalardaki varlığı moleküler tekniklerle belirlenebilir. Bu çalışmada kriptojenik sirozlu hastalarda okült hepatit B varlığının sıklığını belirlemeyi amaçladık.

Gereç ve Yöntem: Bu kesitsel çalışma, kriptojenik karaciğer sirozu olan bir grup hastada okült HBV enfeksiyonunun yaygınlığını değerlendirmek için tasarlanmıştır. Bu çalışmada kliniğimizce takip edilen kriptojenik sirozlu 44 hasta çalışmaya alındı. Bu hastaların serumları gerçek zamanlı bir PCR yöntemi kullanılarak, HBV DNA seviyeleri için incelenmiştir.

Bulgular: Çalışma grubunda 44 hasta vardı; 22 hastada anti-HBs antikoru pozitif saptandı, 24 hastada anti-HBc pozitif bulundu; 2 hastada HBV DNA pozitif bulundu. Bizim çalışmamızda kriptojenik sirozlu hastaların %4,5'inde (2/44) gizli HBV enfeksiyonu saptandı.

Sonuç: Kriptojenik sirozlu hastalarda okült HBV infeksiyonu prevalansı nispeten yüksek saptandı. Okült hepatit b enfeksiyonu kriptojenik karaciğer hastalığı vakalarında dekompanse siroz ve siroza bağlı komplikasyonların gelişmesinde rol oynuyor olabilir.

Anahtar kelimeler: Okült hepatit B, HBV DNA, kriptojenik siroz

INTRODUCTION

Hepatitis B virus (HBV) infection is one of the most common causes of acute and chronic liver disease (CLD) all over the world; it remains a serious global health problem[1]. The course of chronic HBV infection progresses from light to active liver disease[2,3]. It is estimated that approximately one third of the world's population (2 billion people) have been exposed to this virus. It is known that

Yazışma Adresi/Address for Correspondence: Dr. Serkan Yalaki, Mersin Şehir Hastanesi, İç Hastalıkları Gastroenteroloji Kliniği, Mersin, Turkey E-mail: serkanyalaki@hotmail.com
Geliş tarihi/Received: 30.04.2018 Kabul tarihi/Accepted: 29.12.2018 Çevrimiçi yayın/Published online: 08.09.2019

about 3–4 million people carry HBV in Turkey[4].

With the development of highly sensitive molecular techniques, HBV DNA has been detected more frequently in hepatitis B surface antigen-negative (HBsAg (−)) persons. The first occurrences were observed in 1980 in patients with hepatocellular carcinoma (HCC) or chronic hepatitis; these patients were hepatitis C virus negative [HCV (−)] and had no other underlying causes. In subsequent years, occult HBV infection (OHBI) has been observed in patients without liver disease and in persons with completely normal liver function[5–8].

OHBI is defined as the existence of HBV DNA in serum, lymphocytes or liver in individuals with negative serum HBsAg levels. The presence of hepatitis B core antigen (HBc Ag) or anti-HBs may be positive or negative in these individuals[5–7]. Today, PCR can be used as a diagnostic tool for the detection of HBV DNA in patient serum. HBV DNA levels exist at low titers in patients with OHBI. Today, the clinical and biological spectra of OHBI are not precisely known. HIV infection, nonalcoholic liver disease, and other liver diseases (e.g., in HIV-infected and hemodialysis patients) may be associated with OHBI[8–12]. OHBI can also serve as an additional risk factor for HCC, alcoholic cirrhosis, nonalcoholic fatty liver disease, and progression of HIV infection[13,14].

The gold standard method for the detection of OHBI is to demonstrate the presence of HBV DNA in the liver or serum. In most cases, it is not possible to detect HBV DNA in the liver. For this reason, the OHBI diagnosis is mostly based on the analysis of serum samples. However, general acceptance, OHBI is defined as the existence of HBV DNA in the livers of ones who test negative for HBsAg, regardless of the existence of HBV DNA in the sera[13,15,16].

The etiology is unknown in 10% of patients with liver cirrhosis, termed cryptogenic liver cirrhosis[16]. Cryptogenic cirrhosis, literally meaning cirrhosis of obscure or unknown origin, is a diagnosis of exclusion. OHBI may be an important etiological cause of cryptogenic cirrhosis.

OHBI frequency is not known in patients with cryptogenic cirrhosis in our region. We aimed to determine the frequency of OHBI in patients with cryptogenic cirrhosis who have been monitored in our hepatology clinic.

MATERIALS AND METHODS

This cross-sectional study was conducted at the gastroenterology and hepatology clinic of Adana Numune Training and Research Hospital. The records were retrospectively reviewed. The study was approved by the Ethics Committee of Cukurova University (No:32-2016).

Between January 2010 and January 2017, 57 patients with cryptogenic cirrhosis were diagnosed in our clinic. Forty-four patients who were still alive and whose records were complete were included in the study. A diagnosis of cryptogenic cirrhosis is typically given when all other causes of cirrhosis have been ruled out. Although biopsy is the gold standard for the diagnosis of cirrhosis, the diagnosis is usually made by the clinical, laboratory and radiological findings of liver failure and by the presence of portal hypertension. Forty-four patients were diagnosed with cryptogenic cirrhosis, according to the following criteria: Absence of viral etiologic agents such as HBs Ag, anti HCV; absence of serologic determinants of mitochondrial, nuclear, and smooth muscle antigens; normal levels of seruloplazmin, iron and alpha 1-antitrypsin; the absence of alcohol or other hepatic toxin use.

Blood samples were collected. Serological markers of liver function such as aminotransferase (AST), alanine aminotransferase (ALT), serum albumin, and bilirubin were assessed using an Abbott Architect 16200 autoanalyzer (Abbott Inc., Princeton, NJ, USA). Serological markers of HBV infection, such as HBs Ag, anti-HBc Ab, anti HBs Ab levels were assessed using the chemiluminescent microparticle immunoassay (Abbott Inc., Princeton, NJ, USA). All assay protocols, cutoffs and result interpretations were performed according to the manufacturers' instructions.

HBV DNA detection

A fully automated Cobas AmpliPrep instrument for nucleic acid extraction was used according to manufacturer's recommendations. This tool is an automated real-time PCR assay that targets the precore and core regions of the viral genome. HBV DNA levels were studied from 650 µL serum by this instrument. The data generated were analyzed by Amplilink software. HBV DNA quantities are uttered in international units per milliliter (IU/mL). The linear range of the system is 20–1.7 × 10^8 IU/mL.

Statistical analysis

Descriptive statistical data were presented using SPSS. Data are presented as mean ± standard deviation.

RESULTS

Forty-four patients were enrolled in this study. The clinical and biochemical characteristics of the patients are shown in the table 1. Among the patients with cryptogenic cirrhosis, 29 (65.1%) were males and 15 (34.9%) were females (male to female ratio = 1.93). The mean age at the time of the study was 62.93 ± 14.11 years.

Table 1. The clinical and biochemical characteristics of the patients

	All Patients (n = 44)
Age, y, mean ± SD	62.93 ± 14.11 (years)
Sex, No. (%)	
Male	29 (65.1)
Female	15 (34.9)
AST, IU/L, mean ± SD	45.28 IU/L ± 4.23
ALT, IU/L, mean ± SD	48.66 ± 5.36
Albumin, g/dL, mean ± SD	2.7 ± 0.6
Bilirubin, mg/dL, mean ± SD	1.9 ± 0.7

ALT, alanine transaminase; AST, aspartate transaminase; SD, standard deviation

The overall study group had a mild increase in serum transaminase levels, with median serum ALT and AST levels of 45 IU/L (range: 10–40 IU/L) and 48 IU/L (range: 0–40 IU/L), respectively. Furthermore, the bilirubin values of these patients were high and the albumin levels were low. Bilirubin and albumin values were 1.9mg/dL and (range: 0,3-1,2 mg/dL) and 2.7 g/dL (range: 3.4-5.4 g/dL), respectively.

Table 2. Prevalence of HBV serological markers as well as HBV DNA

	N	**%**
Anti-HBc Ab seropositivity	24	54.5
Anti-HBs seropositive y	22	50
HBV DNA positivity	2	4.5

Anti-HBc Ab: Anti hepatitis B core antibody; Anti-HBs Ab: Anti hepatitis B surface antibody; HBV DNA: Hepatitis B deoxyribonucleic acid

The frequency of HBV serological markers and HBV DNA in the study population is given in Table 2. Of the 44 study participants, 22 patients (50%) were positive for anti-HBs (anti-HBs antibody levels >10 mIU/mL) and 24 (54.5%) were positive for anti-HBc. HBV DNA was positive in 2 patients (2/44). In our study, the frequency of OHBI was 4.5% (2/44) in patients with cryptogenic cirrhosis.

DISCUSSION

Chronic HBV infection is characterized by the presence of HBV DNA in serum and persistent HBsAg. OHBI is the persistence of the viral genome in the livers of HbsAg (−) individuals with low titers of HBV DNA. It has been reported that there is a high prevalence of OHBI in those undergoing hemodialysis, in those with cryptogenic liver disease, in IV drug users, in those undergoing frequent blood transfusions (hemophiliacs, etc.), and in blood donors. It has also been reported that the prevalence of OHBI is high in HIV, chronic HCV or HCC patients[8–12]. OHBI can also be an additional risk factor for HCC, alcoholic cirrhosis, nonalcoholic fatty liver disease, and progressive HIV infection[13,14].

In our study, we investigated 44 patients with cryptogenic cirrhosis to evaluate the prevalence of OHBI. OHBI was found in 4.5% in patients with cryptogenic cirrhosis. The youngest OHBI patient was in the late fifties. We reviewed several studies on the prevalence of OHBI in CLD. Honarkar et al. reported the prevelance of OHBI to be 22% in 35 CLD patients using a tissue PCR method[17]. Kaviani et al. reported the prevalence of OHBI to be 1.9% in 104 cases of cryptogenic CLD using a real-time PCR method[18]. Finally, in a study conducted by Hashemi et al., OHBI frequency was identified to be 4% in patients with cryptogenic cirrhosis[19]. Unlike these studies, Heringlake et al. did not detect any hidden viral infections in 162 German patients with cryptogenic liver cirrhosis[20]. We also looked at studies that investigated the frequency of OHBI in the general populations of various countries. Fang et al. reported the prevalence of OHBI to be 10.6% in 359 Chinese patients[21]. Song et al. examined 1,091 HbsAg (−) adults in the general Korean population, finding an OHBI frequency of 0.7%[22].

Generally, the OHBI frequency is 0%–10% in people without liver disease, 11%–19% in patients with chronic hepatitis, and 12%–61% in HCC patients[23–25]. In our study, all patients were cirrhotic with an OHBI frequency of 4.5%. This finding is also consistent with previously published results[18,22].

In studies conducted, serum HBV DNA levels in patients with OHBI are generally less than 200 IU / mL, which is significantly lower than in HBsAg (+) individuals[26,27]. In our study, HBV DNA levels of two HBV DNA-positive cases were 22 and 2,345 IU/mL.

According to the determined antibodies, OHBI can be divided into two groups. OHBI may be seronegative [anti-HBc (−) and anti-HBs (−)] or seropositive [anti-HBc (+) and/or anti-HBs (+)]. Many of the people with OHBI (80%) are seropositive for these antigens. In this study, two patients who had detectable HBV DNA levels were also positive for anti-HBs and anti-HBc. When all cases were considered, 50% of patients were anti-HBs positive. The presence of positivity in Turkey was 4.0% for HBsAg, 32.0% for anti-HBs, and 30.6% for anti-HBc[28].

Although detection of HBV DNA is the gold standard for detection of occult infection, the presence of antibodies against HBV core antigen (anti-HBc) in peripheral blood is also very sensitive in the diagnosis of OHBI. The risk of occult hepatitis associated with anti-HBc seropositivity has been demonstrated extensively, and the presence of antibody response to HBc can be considered a sentinel marker of occult HBV infection. In some studies, OHBI is more common in patients who are positive for anti-HBc than in patients who are negative for anti-HBcAg[11,26,29]. In our study group, anti-HBc total positivity was 54%. Two patients with detectable HBV DNA were also positive for anti-HBc.

No other studies have investigated the frequency of OHBI in patients with cryptogenic cirrhosis in our country. The prevalence of OHBI has been reported to be 0%–36.4% in different patient groups and blood donors from Turkey. According to these studies, OHBI was 0%–36.4% in HCV(+) hemodialysis patients, 7% in HCV(−) hemodialysis patients, and 3.4%–19% in HBsAg(−) patients with detectable HBV DNA[30–33]].

Although OHBI may be a potential prognostic factor for CLD, information regarding effects of OHBI in patients with CLD is limited. The most important reason is that there are various limitations of the studies conducted to date. First, the detection of HBV DNA needs a liver biopsy; however, this is not routinely performed in clinical settings. In addition, these studies were conducted in small cohorts and heterogeneous populations and used cross-sectional methods or variable detection sensitivities for the analysis of liver or serum HBV DNA levels.

In all cases examined, liver biopsy specimens were unavailable; thus, we analyzed serum samples using real-time PCR, which may underestimate the prevalence of OHBI. The number of positive cases was thus insufficient to determine any correlation between OHBI and liver cirrhosis.

Our study revealed the incidence of OHBI to be 4.5% in patients with cryptogenic cirrhosis. OHBI can be a causative factor of cirrhosis and progressive liver decomposition in these patients. We recommend that patients with cryptogenic cirrhosis be examined for OHBI. It is also recommended that patients with OHBI be closely monitored and treated with highly potent antiviral drugs.

Yazar Katkıları: Çalışma konsepti/Tasarımı: SY, MSY; Veri toplama: SY, MSY; Veri analizi ve yorumlama: SY, MSY; Yazı taslağı: SY, MSY; İçeriğin eleştirel incelenmesi: SY, MSY; Son onay ve sorumluluk: SY, MSY; Teknik ve malzeme desteği: SY, MSY; Süpervizyon: SY, MSY; Fon sağlama (mevcut ise): yok.
Bilgilendirilmiş Onam: Katılımcılardan yazılı onam alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

Author Contributions: Concept/Design : SY, MSY; Data acquisition: SY, MSY; Data analysis and interpretation: SY, MSY; Drafting manuscript: SY, MSY; Critical revision of manuscript: SY, MSY; Final approval and accountability: SY, MSY; Technical or material support: SY, MSY; Supervision: SY, MSY; Securing funding (if available): n/a.
Informed Consent: Written consent was obtained from the participants.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support

REFERENCES

1. Lavanchy D. Chronic viral hepatitis as a public health issue in the world. Best Pract Res Clin Gastroenterol. 2008;22:991-1008.
2. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology. 2001;34:1225-41.
3. Lok AS. Chronic hepatitis B. N Engl J Med. 2002;346:1682
4. Akhan S, Aynıoğlu A, Çağatay A, Gönen İ, Günal Ö, Kaynar T et al. Management of chronic hepatitis B virus infection: a Consensus Report of the Study Group for Viral Hepatitis of the Turkish Society of Clinical Microbiology and Infectious Diseases. Klimik Derg. 2014;27:2-18.
5. Brechot C, Thiers V, Kremsdorf D et al. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely 'occult'? Hepatology. 2001;34:194-203.

6. Hu KQ. Occult hepatitis B virus infection and its clinical implications. J Viral Hep. 2002;9:243-257.
7. 7. Kwak MS, Kim YJ. Occult hepatitis B virus infection. World J Hepatol. 2014;6:860-9.
8. Raimondo G, Pollicino T, Cacciola I, Squadrito G. Occult hepatitis B virus infection. J Hepatol. 2007;46:160-170.
9. Rodriguez-Inigo E, Bartolome J, Ortiz-Movilla N, et al. Hepatitis C virus (HCV) and hepatitis B virus (HBV) can coinfect the same hepatocyte in the liver of patients with chronic HCV and occult HBV infection. J Virol. 2005;79:15578-81.
10. Chemin I, Zoulim F, Merle P, Arkhis A, Chevallier M, Kay A et al. High incidence of hepatitis B infections among chronic hepatitis cases of unknown aetiology. J Hepatology. 2001;34:447-54.
11. Minuk GY, Sun DF, Greenberg R, Zhang M, Hawkins K, Uhanova J et al. Occult hepatitis B virus infection in a North American adult hemodialysis patient population. Hepatology. 2004;40:1072-7.
12. Tramuto F, Maida CM, Colomba GM, Di Carlo P, Vitale F. Prevalence of occult hepatitis B virus infection in a cohort of HIV-positive patients resident in Sicily, Italy. Biomed Res Int. 2013:859583.
13. Nishikawa H, Osaki Y. Clinical significance of occult hepatitis B infection in progression of liver disease and carcinogenesis. J Cancer. 2013;4:473-80.
14. Kitab B, Ezzikouri S, Alaoui R, Nadir S, Badre W, Trepo C et al. Occult HBV infection in Morocco: from chronic hepatitis to hepatocellular carcinoma. Liver Int. 2014;34:e144-e150.
15. Lledo JL. Diagnostic strategy for occult hepatitis B virus infection. World J Gastroenterol 2011; 17: 1553-1557.
16. Berasain C, Betes M, Panizo A, Ruiz J, Herrero JI, Civeira MP et al. Pathological and virological findings in patients with persistent hypertransaminasaemia of unknown aetiology. Gut 2000;47:429-35.
17. Honarkar Z, Alavian SM, Samiee S, Saeedfar K, Zali MR. Occult hepatitis B among chronic liver disease patients. Saudi Med J. 2005;26:601-6.
18. Kaviani MJ, Behbahani B, Mosallaii MJ, Sari-Aslani F, Taghavi SA. Occult hepatitis B virus infection and cryptogenic chronic hepatitis in an area with intermediate prevalence of HBV infection. World J Gastroenterol. 2006;12:5048-50.
19. Hashemi SJ, Hajiani E, Masjedizadeh A, Makvandi M, Shayesteh AA, Alavinejad SP et al. Occult hepatitis b infection in patients with cryptogenic liver cirrhosis in southwest of Iran Jundishapur. J Microbiol. 2015;8:e16873.
20. Heringlake S, Schutte A, Flemming P, Schmiegel W, Manns MP, Tillmann HL. Presumed cryptogenic liver disease in Germany: High prevalence of autoantibody-negative autoimmune hepatitis, low prevalence of NASH, no evidence for occult viral etiology. Z Gastroenterol. 2009;47:417-23.
21. Fang Y, Shang QL, Liu JY, Li D, Xu WZ, Teng X et al. Prevalence of occult hepatitis B virus infection among hepatopathy patients and healthy people in China. J Infect. 2009;58:383-8.
22. Song EY, Yun YM, Park MH, Seo DH. Prevalence of occult hepatitis B virus infection in a general adult population in Korea. Intervirology. 2009;52:57-62.
23. Chemin I, Trepo C. Clinical impact of occult HBV infections. J Clin Virol. 2005;34:15-21.
24. Chaudhuri V, Nanu A, Panda SK, Chand P. Evaluation of serologic screening of blood donors in India reveals a lack of correlation between anti-HBc titer and PCR-amplified HBV DNA. Transfusion. 2003;43:1442-8.
25. Kasapoğlu B, Türkay C. Okült (OCCULT) hepatit B enfeksiyonu [Occult hepatitis B infection]. Güncel Gastroentrroloji 2007;11:51-56.
26. Alhababi F, Sallam TA, Tong CY. The significance of 'anti-HBc only' in the clinical virology laboratory. J Clin Virol 2003;27:162-9.
27. Noborg U, Gusdal A, Horal P, Lindh M. Levels of viraemia in subjects with serological marker sofpast or chronic hepatitis B virus infection. Scand J Infect Dis. 2000;32:249-52.
28. Tozun N, Özdoğan O, Çakaloğlu Y, Idilman R, Karasu Z, Akarca U et al. Seroprevalence of hepatitis B and C virus infections and risk factors in Turkey: a fieldwork TURHEP study. Clin Microbiol Infect. 2015;21:1020-6.
29. Urbani, S, Fagnoni, F, Missale G, Franchini M. The role of anti-core antibody response in the detection of occult hepatitis B virus infection. Clinical Chemistry and Laboratory Medicine. 2010;48:23-9.
30. Beşışık F, Karaca C, Akyüz F, Horosanlı S, Önel D, Badur S et al. Occult HBV infection and YMDD variants in hemodialysis patients with chronic HCV infection. J Hepatol. 2003;38:506-10.
31. Kanbay M, Gur G, Akcay A, Selcuk H, Yilmaz U, Arslan H. Is hepatitis C virus positivity a contributing factor to occult hepatitis B virus infection in hemodialysis patients? Dig Dis Sci. 2006;51:1962-26.
32. Altunay H, Kosan E, Birinci I, Kiralı K, Saribas S, Aslan M et al. Are isolated anti-HBc blood donors in high risk group? The detection of HBV DNA in isolated anti-HBc cases with nucleic acid amplification test (NAT) based on transcription-mediated amplification (TMA) and HBV discrimination. Transfusion and Apheresis Science. 2010;43:265-8.
33. Goral V, Ozkul H, Tekes S, Sit D, Kadiroglu AK. Prevalence of occult HBV infection in haemodialysis patients with chronic HCV. World J Gastroenterol. 2006;12:3420-4.