The frequency of occult HBV infection in Eskisehir region of turkey between 2001-2015

Eskişehir bölgesinde okült HBV enfeksiyonu sıklığının 2001-2015 yılları arasında dağılımı

Occult HBV, frequency

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Öz

Amaç: Okült HBV enfeksiyonu (OBİ), HBs Ag negatif kişilerde, serumda, immün sistem hücrelerinde ve/veya karaciğerde düşük titrede HBV-DNA pozitifliği ile karakterize bir enfeksiyondur. Görülme sıklığı; test edilen hasta popülasyonuna, laboratuvar tanıda kullanılan yöntemlerin duyarlılığına bağlı olarak değişir. Ayrıca OBİ organ ve kan donörlerında virüs kontaminasyonu için kaynak olabilir. Bu çalışmada, viral hepatit B ön tanılı hastalarda, okült HBV enfeksiyon sıklığı değerlendırılmıştır. Gereç ve Yöntem: Bölgemizde 2001- 2015 yılları arasında gönderilen HBV enfeksiyonu ön tanılı hastalara ait serum örneklerınde HBV-DNA, gerçek zamanlı PZR (Qiagen); HBV, HCV ve HDV serolojik işaretleri ise, EIA (Axsym ve Architect i2000SR) yöntemi ile çalışılmıştır. Ayrıca HBsAg negatif, HBV-DNA pozitif serumların alanin aminotransferaz (ALT) ve aspartat aminotransferaz (AST) düzeyleri araştırılmıştır. Bulgular: HBV DNA'sı pozitif olan 4036 hastanın 105'inde (%2.6) HBsAg negatifliği saptanmıştır. Minimum ve maksimum DNA düzeyleri ise $1x10^1$-$1.7x10^8$ kopya/mL olarak belirlenmiştir. Okült HBV enfeksiyonu ön tanılı bu 105 hastanın tamamında Anti HBc IgM negatif ,31'inde (%29.5) tek başına anti-HBc (+), 3'ünde (%2.8) tek başına anti-HBs (+), 16'sında (%15.2) anti-HBs ve anti-HBc birlikte (+), 13'ünde (%12.3) ise tüm HBV serolojik işaretleri negatif olarak bulunmuştur. Beş hastada ise anti-HCV pozitif olup, tüm hastalarda anti HDV negatiftir. Ondokuz (%18) hasta immün düşkün konak özelliği taşımaktadır. Tartışma: OBİ, transplantasyon veya kan transfüzyonu yolu ile bulaşmasını takiben, tipik HBV enfeksiyonuna neden olabilmesi, özellikle immün süpresyon koşullarında reaktivasyonu ve karaciğer hastalığı varlığında progresyon veya hepatoselüler karsinomdaki rolü açısından önem taşımaktadır.

Anahtar Kelimeler

Hepatit B Enfeksiyonu; Görülme Sıklığı; PZR

Abstract

Aim: Occult HBV infection (OBI) is characterized by the detection of HBV DNA in low levels in serum and peripheral blood mononuclear cells and/or in the liver, in the absence of detectable hepatitis B surface antigen (HBsAg). The prevalence of OBI varies among patient populations tested and the sensitivity of the assay employed. Also OBI can be a source of virus contamination in both blood and organ donations. In this study, we evaluated the presence of occult HBV infection in patients diagnosed with viral hepatitis B infection. Material and Method: All samples were investigated for serological markers of HBV, HCV, and HDV by ELISA (AxSYM and Architect i2000SR, Abbott, USA) and also examined for the presence of HBV DNA by Real-time PCR in the clinical microbiology laboratory between 2001-2015. Also, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were evaluated. Results: We detected HBsAg negativity in the sera of 105 (2.6%) of 4036 patients having positive HBV DNA. The minimum and maximum DNA levels were $1x10^1$-$1.7x10^8$ copies/mL. Among the 105 patients, 31 (29.5%) were positive for only anti-HBc total, 3 (2.8%) were positive for anti-HBs, and 16 (15.2%) were positive for both anti-HBs and anti-HBc. Thirteen (12.3%) of the 105 patients were negative for serological markers of HBV infection. Nineteen (18%) patients were immunocompromised individuals. Discussion: Especially in immunocompromised individuals, occult HBV infection can reactivate and cause liver damage. Also OBI should be carefully assessed in particular clinical contexts: HBV infection transmission, liver disease progression, hepatocellular carcinoma onset, and HBV reactivation.

Keywords

Frequency; Hepatitis B Infection; PCR

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Introduction

The existence of occult HBV infection has been a matter of debate for years, but its existence and clinical relevance is now supported by many publications [1]. Occult HBV infection is characterized by the detection of HBV DNA in low levels in serum and peripheral blood mononuclear cells and/or in the liver in the absence of detectable hepatitis B surface antigen (HBsAg) with or without antibodies to hepatitis B core antigen (anti-HBc) or hepatitis B surface antigen outside the pre-seroconversion window period [2-5].

The gold standard for OBI diagnosis is liver tissue biopsy, although this is not usually feasible and standardization of this assay is not established. Therefore, most often OBI diagnosis is based on the results of a blood test. It is important to determine the optimal method to quantify HBsAg and HBV DNA. It is critical to use a very delicate and specific assay because OBI is usually associated with low levels of HBV DNA. An international consensus has suggested a cut-off value to detect HBV DNA by molecular assays (<200 IU/mL) [6].

The prevalence of occult HBV infection varies among patient populations tested, but also depends on the assay employed in routine serological or nucleic acid test screening [4,7]. Detection of HBV DNA in the absence of HBsAg has been increased with the improvements in the sensitivity of genomic amplification assays. Improvement in molecular diagnostic methods and in particular the development of increasingly sensitive PCRs has favored the recognition of occult HBV infections in an increasing number of clinical settings and geographical areas [1]. Recent technologies (nested-PCR, real-time PCR, and transcription based mediated amplification) have reduced the lower detection limit to >5 copies/mL and improved sensitivity. Prevalence of occult HBV has been found higher in seropositive persons, particularly those who are positive for anti-HBc only, than in seronegative individuals [8].

The prevalence of OBI in blood donors depends on the rate of HBV in the population. In developed countries this rates is under 1%, while in less developed and developing countries it is as high as 17%, reported rates in anti-HCV-positive blood donors range from 0-15%, and from 0-89% in HIV-infected individuals [1-6, 9,10]. OBI rates are reported as 45% and 51% in intravenous drug addicts and hemophilic patients, respectively [10].

OBI has been reported in 0.1-2.4% of HBsAg-negative, anti-HBc positive (anti-HBs positive or negative) blood donors in Western countries such as the United States, where only 5% of the population has prior exposure to HBV. In contrast, OBI has been reported in up to 6% of a similar cohort of donors (HBsAg-negative, anti-HBc positive) who reside in HBV endemic areas where 70-90% of the population has been exposed to HBV. When only anti-HBc data is evaluated (anti-HBe positive or negative), the rates range from 0% to 15% (median of 1.1%) [4]. Prevalence may also vary depending on the nature of biological material tested, with a higher proportion for the liver compared to serum specimen [1]. Occult HBV infection may follow recovery from disease, displaying antibody to hepatitis B surface antigen (anti-HBs) and persistent low-level viraemia, escape mutants undetected by the HBsAg assays, or healthy carriage with antibodies to anti-HBe and anti-HBc. All forms have been shown to be infectious in immunocompromised individuals, such as organ or bone marrow transplant recipients [6]. In several studies, occult HBV infection prevalence is high in immuncompromised situations. There are some studies which show that occult HBV infection was reactivated and caused liver damage and liver failure in 20% of immunocompromised cases such as anti-HIV positive patients, hematopoietic stem cell or solid organ transplant recipients, or patients treated with monoclonal antibody, anti-TNF antibody, or chemotherapy regimens because of rheumatological disorders [11-14].

In this study, we aimed to evaluate the presence of occult HBV infection in patients diagnosed with viral hepatitis B infection.

Material and Method

All samples were investigated for serological markers of HBV, HCV, and HDV by ELISA (AxSYM and Architect i2000SR, Abbott, USA) and also examined for the presence of HBV DNA by Real-time PCR in the clinical microbiology laboratory. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels of HBV DNA-positive sera were evaluated.

Serological markers. Tests for HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc total, anti-HBc IgM, anti-HCV, and anti-HDV were done with commercially available kits (AxSYM and Architect i2000SR, Abbott, USA).

Detection of HBV DNA. Serum samples were assayed for HBV DNA by using Artus HBV RG PCR kit (Qiagen, USA) between 2001-2010, Cobas TaqMan (Roche, Germany) between 2011-2013, and RTA HBV Real-time PCR kit (RTA, Turkey) after 2013. Total DNA extraction of HBV DNA and real-time PCR were performed according to the manufacturers' instructions. The limit of detection of Artus HBV RG PCR was 20 IU/mL, Cobas TaqMan HBV test was 20 IU/mL, and RTA HBV real-time PCR was 24 IU/mL.

Statistical analysis

Analysis was performed using the SPSS Version 20.0 (SPSS Inc., Chicago, IL, USA) software. The associations between baseline patient characteristics and the assay results were analysed using one-way ANOVA and Fisher's exact test. P value of <0.05 was considered statistically significant.

Results

In this study, 16,853 sera of 4036 patients who were considered as having HBV infection were tested in Eskisehir Osmangazi University Medical Faculty, Medical Microbiology Laboratory between 2001-2015. One hundred and five (2.6%) of the patients positive for HBV DNA were HBsAg negative. These 105 patients were from the following clinics: 36 (34.2%) Gastroenterology, 34 (32.3%) Infectious Disease, 15 (14.2%) Hematology, 8 Internal Medicine, 6 General Surgery, 5 Pediatrics, and 2 Gynecology and Obstetrics. The minimum and maximum DNA levels were $1x10^{1}$-$.7x10^{8}$ copies/mL.

Anti-HBc IgM was negative in all of the patients. Among 105 patients, 31 (29.5%) were only positive for anti-HBc total, 3 (2.8%) were positive for anti-HBs, and 16 (15.2%) were positive for both anti-HBs and anti-HBc total in their sera. Thirteen (12.3%) of all patients were negative for serological markers of HBV infection. The sera of 5 patients were anti-HCV positive. All of the patients positive for anti-HCV were also only anti-HBc

total positive. All of the patients were negative for anti-HDV.

The liver function test results were all in normal range, except that ALT and AST levels were abnormal in 40 (38%) and 38 (36.1%) patients, respectively. Both ALT and AST levels were abnormal in 21 patients (20.0%). The mean levels of ALT and AST were 79.58 and 72.78 IU/L, respectively. Nineteen patients (18%) were immunocompromised.

In the occult HBV group, the viral load was < 2x10 copies/ mL in 42 of 105 patients, and in non occult HBsAg positive group, in 16 patients the level was < 2x10 copies/ mL. We found statistically significant difference between the occult and nonoccult groups according to their viral loads ($p<0.05$). In terms of HBeAg positivity, Anti HBc positivity, Anti HBs positivity and Anti HBc-HBeAg positivity, the differences among the two groups were highly significant (Table 1).

Table 1. Characteristics and antibody status of occult and non occult hepatitis B (HBs Ag Positive) patients.

	Occult HBV (n:105)	Non occult HBsAg positive group (n: 114)	P value*
Mean age	48.31	43.85	0.019
Sex (male/female)	61/44	71/43	0.527
Mean viral load (copies/mL)	$2.1x10^4$(10-$1.7x10^8$)	$2.4x10^5$(10-$8.6x10^7$)	0.006
HBeAg positive	6 (5.7 %)	21 (18.4%)	0.004
Anti HBc positive	89 (84.7 %)	111 (97.3%)	0.001
Anti HBs positive	41 (39.0%)	9 (7.8%)	<0.0001
Anti HBc and Anti HBs positive	16 (15.2%)	7 (6.1 %)	0.088
Anti HBc and HBeAg positive	1 (0.9 %)	20 (17.5%)	<0.0001
Anti HBc, AntiHBs and HBeAg positive	1 (0.9 %)	1 (0.8%)	0.953

*P value <0.05 is statistically significant.

Discussion

Occult hepatitis B is defined by the presence of HBV DNA in the serum or liver of patients who are negative for HBsAg. The clinical significance of occult HBV infection remains largely unknown. This clinical entity has been reported in healthy blood donors, patients with chronic liver disease, and patients with hepatocellular carcinoma [4]. There is a clear association between occult HBV infection and liver diseases in the absence of other causes, such as HCV infection or excessive alcohol intake [15].

OBI in particular has a high prevalence, particularly in chronic HCV infection, cryptogenic, chronic and fulminant hepatitis patients [16]. Studies have suggested that, similar to HBV infection, OBI's obvious protooncogenic activity contributes to the development of cirrhosis or HCC [17-18]. In addition, non-Hodgkin's lymphoma and malignancies such as intrahepatic cholangiocarcinoma show a noteworthy relationship between HBV infection and OBI [19].

Occult HBV has also been detected in chronic HCV infection with or without antibody markers of HBV [20]. In our study, the rate of occult HBV was found as 2.6%. The rate of occult HBV infection in our study correlated with other studies in Turkey. Among 935 HBsAg-negative patients, occult HBV infection rate was found as 5.77% in a study of Akduman et al. in the Manisa region of Turkey [21]. In different studies from Turkey, among HBsAg negative patients 3.4%-19% OBI rates reported [22]. In Pakistan, Bhatti et al. selected 966 donors for testing of anti-HBc and HBV markers and detected HBV DNA in 5 blood donors who were negative for HBsAg [23]. O'Brien et al. tested 493,344 donors who made donations otherwise eligible for transfusion, and among them, 5,585 (1.13%) were detected as reactive for anti-HBc. Of these, 29 donors tested positive for the presence of HBV DNA but were negative for the presence of HBsAg (0.52% of all anti-HBc-reactive donors) [9]. Recently, Olotu et al. reported OBI prevalence of 5.4 % among anti-HBc positive blood donors in Ile-Ife, Nigeria. This means that after HBsAg screening, about 1 in 20 to 1 in 25 blood donations still have HBV DNA [24].

In our study, 3 (2.8%) patients were only anti-HBc total positive and 16 (15.2%) were positive for both anti-HBc total and anti-HBs. Recent data shows that about 20% of occult hepatitis B sera are negative for all serological markers of HBV infection except HBV DNA, 50% are positive for hepatitis B core antibody (± anti-HBs), and 35% are positive for hepatitis B surface antibody (± anti-HBc) [2,4]. Overall, the prevalence of occult HBV infection is higher in anti-HBc positive patients than in anti-HBc negative patients. The HBV DNA detection rate is greater in subjects who are anti-HBc positive but anti-HBs negative, and the infectivity of blood donations containing anti-HBc as the only marker of HBV infection has been known for several decades [4,5]. Blood products with these very low HBV viral loads are potentially infectious, as shown in recent reports [25].

Data on the rate of OBI in the general population is generally insufficient. In one study of seropositive and seronegative groups, the HBV DNA positivity rate was 18% and 8%, respectively. Other studies have reported an OBI rate of 15.3% for hematopoietic stem cell donors who were HBV/HCV negative, 16% in a group with normal serum transaminase values, and 8% in a group of individuals who were HBsAg negative and with a chronic family history of HBV infection [26-28].

When we looked at the rate (29.5%) of only anti-HBc total positive patients in our study, it was considered that anti-HBc screening of blood donors could identify most of the occult HBV infections.

In our study, 19 (18%) of patients were immunocompromised individuals and it is known that occult HBV infection can reactivate and cause liver damage in these patients. All forms of occult HBV infection have been shown to be infectious in immunocompromised individuals [6].

The limitation of our study is that we collected the data of the patients retrospectively and therefore we could not follow up the serological markers, viral loads, and clinical outcomes of the patients.

OBI has a variety of clinical impacts. The possible transmission of the infection (via blood transfusion, solid organ transplantation, hemodialysis, and also by close contact as has been reported recently), the contribution to liver disease progression, the development of hepatocellular carcinoma (HCC), and the risk of reactivation of HBV are the most relevant contexts [17,29]. Furthermore, detection of HBV DNA with highly sensitive PCR techniques should be routinely performed on blood

donations to minimize the occult HBV transmission risk.

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Competing interests

The authors declare that they have no competing interests.

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