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Investigation of HBV DNA in HBsAg positive patients

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Abstract

Hepatitis B virus is an important health problem which about 400 million people are infected chronically and 500,000 to 1,200,000 people die every year due to HBV-related diseases. In the study we aimed to investigate the HBV DNA results in HBsAg positive patients. HBsAg positivity was detected by ELISA method in patients who were admitted to our hospital with suspicion of hepatitis. HBV DNA was performed by real time PCR. In this context, the presence of HBV DNA in serum sample results of 2437 HBsAg-positive patients which were sent to our Microbiology laboratory between August 2014 and August 2015 was retrospectively examined. HBV DNA was positive in 1037 (42.5%) and 1400 (57.5%) of the HBsAg positive cases. 42.2% of male patients' and 43% of female patients' HBV DNA was positive. HBV DNA positivity was detected in 42% of adult patients and 55% of children in child age group. As a result; we suggest that viral replication should be demonstrated in HBsAg-positive cases and HBV DNA level should be determined for follow-up the antiviral therapy.

Keywords: HBV, HBsAg, HBV DNA, Real-time PCR

Introduction

Hepatitis B virus (HBV) is an important health problem in all over the world. It is located in the genus Orthohepadnavirus and has enveloped and partially double-stranded DNA [1].

It has been reported that around 400 million people worldwide are infected with HBV and 500,000-1,200,000 people die from HBV-related diseases every year. And also, three and a half million people infected with HBV in our country [2,3]. HBV infections have a broad clinical spectrum; ranging from self-limiting acute disease, inactive carriers, chronic hepatitis, cirrhosis and hepatocellular carcinoma. On average, 5% of acute hepatitis cases that are caused by HBV become chronic, and some become cirrhosis. In cirrhotic cases, the likelihood of Hepatocellulare carcinoma development is high [2]. Although there is an effective and safe vaccine, 50 million new cases are diagnosed each year. Many factors, such as the health policy of the countries, measures taken, education, economic conditions, cause changes in the incidence of HBsAg [1].

HBV has infected persons by parenteral contact with infected blood or body secretions (percutaneous), sexual contact, infected mother to newborn (perinatal, vertical) and close contacts [2].

Our country is in the cmedium endemic regions in terms of hepatitis B. The main route of transmission is horizontal.

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The prevalence of HBsAg in Turkey has been determined as 2-21%, varying from region to region. In studies conducted in our country, the prevalence of hepatitis B increases from west to east [3].

HBsAg is used as a screening test in serological methods for HBV diagnosis. HBsAg screening test sensitivity is not always sufficient to detect HBV infection [4]. Also HBcAg, Anti-HBc IgM, Anti-HBc IgG, Anti-HBe and Anti-HBs are used for serological diagnosis with HBsAg. In recent years molecular diagnostic techniques have been developed. Molecular methods are being used to detect mutant strains and to elucidate the mechanisms of HCC formation, following antiviral therapy, especially in cases where serologic methods are inadequate and unusual hepatitis B serology [2,4].

One of the most important steps in molecular biology is in vitro propagation of the nucleic acid sequence. In this context, infection diagnosis, treatment follow-up and drug resistance could be determined. The use of the methods has increased depending to understanding the importance of identifying hepatitis B virus DNA (HBV DNA). Real-time PCR is a fast and simple test that allows the quantification of HBV DNA and is frequently used in the detection of HBV DNA.

The aim of this study was to compare the results of HBV DNA by retrospective analysis of real-time PCR method with HBsAg positive detected cases by ELISA in our study.

Material and Methods

In this study we aimed to investigate the comparison of HBsAg

and HBV DNA results in HBsAg positive patients. In this context, HBsAg positive 2437 patient's serum samples which sent between August 2014 and August 2015 to our laboratory were examined retrospectively.

Blood samples from patients were separated the sera and after then stored at -20 degrees freezing until analyzed.

For the detection of HBsAg in serum samples; HBsAg Qualitative II eliza kit (ARCHITECT 2000, Abbott Diagnostic, USA), for the detection of HBV DNA; Real-time PCR kit (artus HBV QS-RGQ Kit, QIAGEN, Germany) was used. The test sensitivity of the real-time PCR kit was <50 copies / ml (1 IU / ml = 5.68copies / ml). A value of over 400 copies / mL was assessed as HBV DNA positive.

Results

In our study, 2437 HBsAg positive patient sera were examined for HBV DNA. 1548 (63.5%) were male and 889 (36.5%) were female. 2368 (97.2%) were over the age of eighteen (adult) and 69 (2.8%) were under eighteen years of age (childhood). The mean age of the patients was 47 (range 2-92 years).

In our study, among the 2437 HBsAg positive patients, 1037 (42.5%) of them were HBV DNA positive and 1400 (57.5%) were HBV DNA negative. It was observed that the amount of viral load in HBV DNA positive cases was between 435 copies / mL-9 billion 782 million copies / mL. According to gender, 42.2% male and 43% female patients were HBV DNA positive (Figure). According to age; 42% of 2368 adult and 55% of 69 children patients were HBV DNA positive (Figure 1).

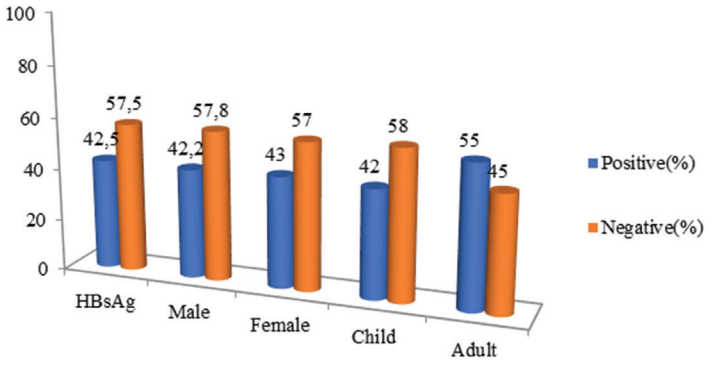

Figure 1. HBV DNA positivity rates according to HbsAg, gender and age.

Discussion

HBV is widespread in whole the world and in our country. It is in the important hepatotrophic viruses because of liver tropism and leading to hepatocellular carcinoma. It has become an issue of public health concern. World Health Organization reported that; nearly 2 billion people have been exposed to HBV, and more than 400 million of them are chronic HBV carriers. Every year 50 million new cases are added to these patients. Chronic and acute complications, such as cirrhosis, hepatocellular carcinoma caused by HBV leads to death [2,3].

Turkey is in the medium endemic regions in terms of HBV frequency. According to the European Center for Disease Control and Prevention's report about hepatitis B in September 2010; the prevalence of hepatitis B in Turkey ranges change between 2-8% depending to the region. This shows that approximately 3.5 million people in our country are HBV carriers [3]. Studies in our country show that the prevalence of hepatitis B is lower in the western regions than eastern regions. In a study done in Malatya, among patients who were suspected of hepatitis; HBsAg positivity were found 13% [5] and in a study including Diyarbakır, Batman, Mardin and Şanlıurfa were found %7 [6]. In the studies made in western cities HBsAg positivity were reported 2%-3% [7,8]. In the thirty different studies investigated the seroprevalence of HBsAg shown that infecting age by HBV has reduce to childhood in East and Southeast Anatolia [9].

HBV carriers are the most important factor in the spread of HBV infection. HBV is mainly transmitted with vertical (mother to child), parenteral (blood and blood products, intravenous drug use), horizontal (hygiene habits, common toothbrush use), sexual intercourse and medical procedures. There is a close relationship between the grade of endemia and transmission factors of HBV infection [1,2]. Horizontal transmission is the most common factor in the Middle Eastern and Mediterranean countries. In our country, horizontal transition is the main transmission pathway. It has been reported that in vertical transmission cases, HBV tends to become more chronic. Vertical transmission is likely to be less common due to the low prevalence of HBsAg in pregnancies. HBV transmission occurs primarily in childhood and adolescence in the family. This shows that vaccination of newborns and children and informing families about transmission pathways are very important [10].

Although the results of studies in different age groups in our country show some differences, it is observed that the encounter with HBV tends to increase from the beginning of the adolescence. According to these results, people in adolescence who do not have vaccination should also be vaccinated. The Ministry of Health gradually spread vaccination to risk groups, pre-school and school-age children [3]. The risk of chronic HBV infection occurrence after an acute encounter with HBV is; in babies who born from HBeAg positive mothers is 90%, in children under five years is 25-30% and in adults is less 5%. Furthermore, the risk of chronic HBV infection is higher in immunosuppressed patients [4].

HBsAg is the first detectable antigen during acute HBV infection and it is used as a screening test. In the sera, it can be detected 2-8 weeks before the onset of symptoms and it disappears within 2-6 months [1,4]. HBeAg was evaluated as the most important marker of viraemia before HBV DNA was detected by molecular diagnostic tests. However, after the identification of HBeAg negative mutants, it has been understood that evaluating HBeAg and anti-HBe as viraemia markers is not a reliable method [11]. HBV DNA is used to detection of HBV infection in cases whose serologic markers are inadequate and also detection of viral replication and infectivity, and the evaluation of treatment and prognosis. Recently, the rapid development of nucleic acid amplification tests has enabled to determine the amount of HBV DNA, especially for identification of viral replication and the follow up of patient response to treatment [11,12].

In our country; Külah et al [12] 53%, Sağlık et al [13] 92%, Otlu et al [14] 71% and Koçoğlu et al [15] 45% reported HBV DNA positivity in HBsAg positive patients, respectively. Eroğlu et al [16] was compared HBV DNA detection with PCR and hybridization methods; HBV DNA positivity was detected 36% by PCR, and

32% by hybrid capture method. In other studies; Peignoux et al [17] %86, Odaibo et al [18] 82% and Hasan et al [19] 45% reported HBV DNA positivity in HBsAg positive patients. Poljak et al [20] was used Hybrid Capture and Cobas-HBV methods for HBV DNA detection in HBsAg positive specimens and found 69% HBV DNA positivity in both methods. In our study, we found HBV DNA positive in HBsAg positive cases 42.5% (1037/2437).

In our country; HBsAg positivity rate varies between 0.1% and 12% in studies conducted in children [21]. Sharing the same towel, chewing gum or toothbrush, facilitates transmission of HBV infection under 6 years of age children. Therefore, routine Hepatitis B vaccination in children is very important for prevention of HBV infection and for decrease of carriers. Vaccination has become more important because of increasing in Chronic liver disease by taking the infection in the early period of life. Since 1998, hepatitis B vaccine has been in routine vaccination schedule under the "National Hepatitis B Vaccine Program" (UHBAP), and 3 doses have been started to done since the newborn period [22]. In studies before the start of national vaccination program; Üner et al [23] 10% and Arabacı et al [24] 9.5% reported HbsAg positivity. In studies after the beginning of the national vaccination program; Araz [25] was found HBsAg positivity rate as 1.1%, Şahin et al [26] as 1.3%, Çopur Çiçek et al [22] as 2.4%. According to the studies there is a decrease in HBsAg positivity rates after the starting of the vaccination program. In our study, 2.8% of HBsAg positive cases were under 18 years of age.

When the rate of HBsAg positivity was assessed according to the gender of the patients; İnci et al. found 4.7% in males and 3.3 % in females in their study which made in Artvin [27], Iraz et al. found 6.7% in males, 4.4% in females, and Tunç et al [28] found 61% in males and 39% in females in Siirt. Similar to other studies, we also found that HBsAg seropositivity in males were higher than in women. In our study, 63.5% of HBsAg positive cases were male and 36.5% were female. Studies show that HBsAg seropositivity in men is higher than in women because of men are being in public places more than women (Dormitory, cafeteria, military service, barber) and using common materials (shaving, etc.) [28].

Various studies evaluating the HBV DNA-HBsAg associations have yielded different results. A great deal of studies has found a positive relationship between HBsAg and HBV DNA levels. However, there are also studies in which no relationship has been established or a negative relationship has been established [13]. Although HBsAg is positive; (In the nonreplicative period after the infection's immunoreaction phase), HBV DNA can be detected negative, which is an indicator of active viral replication. In this period, HBV DNA may not be shown, despite HBsAg can detect in serum and can be shown by immunohistochemical methods in hepatocyte cytoplasm. Also, HBV DNA can be detected negative in treated cases which in asymptomatic or healthy hepatitis B carriers (29). In studies which made in HBsAg positive patients reported HBV DNA negativity; Külah et al [12] as 47%, Sağlık et al [13] as 8%, Otlu et al [14] as 29%, Koçoğlu et al [15] as 55%, Peignoux et al [17]as 14%, Odaibo et al [18] as 18%, Hasan et al [19] as 55.2% and Poljak et al [20] as 31%, respectively. Similarly in other studies, in our study we found HBV DNA negativity 57.5% in HBsAg positive cases.

HBsAg is still important as a serologic marker for the detection of hepatitis B virus infection. Furthermore, HBV DNA detection is the most important molecular test in determining viral replication and infectivity, and also in the evaluation of antiviral response and the prognosis

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