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Evaluation of viral load distribution of HBV DNA positive patients at Suleyman Demirel University Hospital

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ABSTRACT

Hepatitis B virus (HBV) may cause a wide spectrum of liver diseases ranging from an asymptomatic carrier state to severe chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC). In patients with Chronic hepatitis B (CHB) infection, serum HBV viral load has been shown to be significantly associated with disease activity and disease progression. The aim of this study was to determine the viral load distribution of HBV DNA in patients with HBV infection in Isparta region. Hepatitis B surface antigen (HBs Ag) and HBV DNA levels in serum samples of 1054 patients were analysed retrospectively between January 2013 and December 2013. HBs Ag tests in the serum of patients were measured with chemiluminescence method (Vıtros, Johnson&Johnson, USA). The levels of sera HBV DNA were quantified with real-time PCR method by Cobas/AmpliPrep/Cobas Taqman HBV test v 2.0 (Roche Diagnostics GmbH, Mannheim, Germany) and HBV Quantification Kit v1, Magnesia 16, Montania 483 (Anatolia Geneworks, Turkey). HBV-DNA levels were determined as 1-10^2 IU/ml (28.6%), 10^{2-3} IU/ml (32.6 %), 10^{3-4} IU/ml (22.7%), 10^{4-5} IU/ml (7.2%), 10^{5-6} IU/ml (2.4%), 10^{6-7} IU/ml (2.4 %), 10^{7-8} IU/ml (1.5%), 10^{8-9} IU/ml (2.4%), ≥10^9 IU/ml (0.2%). Amounts of the viral load of HBV DNA positive samples were observed to concentrate around the lower levels (1-10^5 IU/ml) in Isparta region (91%) which predicted lower risk of progression to HCC.

1. Introduction

Hepatitis B virus (HBV) is a member of the Hepadnaviridae family and has a relaxed circular, partially double stranded deoxyribonucleic acid (DNA) genome of about 3200 nucleotides (Awan et al., 2010). HBV is present in blood, saliva, semen, vaginal secretions, and menstrual blood of infected patients. Because HBV is resistant to breakdown outside the body, it is easily transmitted by contact with infected body fluids (Lavanchy, 2004). Transmission of HBV occurs through sexual contact, sharp equipment, and under skin contacts, blood transfusions, organ transplants, and blood products, mother-to-child (vertical transmission), diagnostic and therapeutic services, and body fluids. (Mast et al., 2005).

HBV infection is a globally important health problem. Because HBV may cause a wide spectrum of liver diseases ranging from an asymptomatic carrier state to severe chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC). Chronic hepatitis B (CHB) infection is found in more than 400 million people across the world (van Zonneveld et al., 2004). HBV accounts for about 50-80% HCC patients worldwide (Nguyen et al., 2009).

Diagnosis of HBV infection is made through some

serological and virological markers. Hepatitis B surface antigen (HBs Ag) is fundamental for diagnosis of HBV infection. On the other hand, other markers are important in differentiating between active and inactive infections. Hepatitis B e antigen (HBeAg) is indicative of active viral replication (Liaw and Chu, 2009). Serum alanine aminotransferase (ALT) and HBV-DNA levels are an important components in evaluating and managing patients with CHB (Kennedy et al., 2008).

Since serum HBV viral load have been shown to be significantly associated with disease activity and disease progression in patients with CHB infection, serum HBV DNA level is routinely measured to monitor the disease status and treatment response (Hu and Vierling, 1994; Dufour et al., 2000; Iloeje et al., 2006) More recent studies have demonstrated HBV DNA levels to be more sensitive in reflecting viral replication and predicting risk of HCC (Yang et al., 2002; Tang et al., 2004; Yu et al., 2005; Chen et al., 2006). Real-time PCR is a highly sensitive molecular tool with a wide dynamic range, which allows rapid HBV DNA measurement. Real-time PCR gradually replaced other methods for HBV DNA quantification (Loeb et al., 2000; Pawlotsky, 2002; Stelzl et al., 2004; Sum et al., 2004; Gordillo et al., 2005). Using sensitive real-time polymerase chain reaction (PCR), a level of HBV DNA more than 105 copies/ml has been associated with a 7 fold to 9 fold increase in risk of HCC (Yu et al., 2005).

The aim of this study was to determine the viral load distribution of HBV DNA in respect of predicting the risk of progression to HCC in patients with HBV infection in Isparta region.

2. Materials and methods

HBs Ag and HBV DNA levels of 1054 (442 women and 612 men) patients who admitted to various clinical departments of Suleyman Demirel University Research and Education Hospital between January 2013 and December 2013, were analysed retrospectively. The patients' ages ranged from 5 to 88 years.

HBs Ag tests in the serum of patients were measured with chemiluminescence method (Vitros, Johnson&Johnson, USA). According to the product reference, positivity was defined by signal-to-cut-off (s/co) ratio ≥1.00, negativity was defined by s/co ratio <0.90, and gray zone was defined by s/co ratio between ≥ 0.90-<1.00. The levels of sera HBV DNA were quantified with real-time PCR method by Cobas/AmpliPrep/ Cobas Taqman HBV test v 2.0 (Roche Diagnostics GmbH, Mannheim, Germany) and HBV Quantification Kit v1, Magnesia 16, Montania 483 (Anatolia Geneworks, Turkey). The linear range of quantitation was $1x10^1$-$1x10^9$ IU/ml and the analytic sensitivity was 10 IU/ml for Anatolia geneworks. The linear range of quantitation was 20-$1.7x10^8$ IU/ml and the analytic sensitivity was 20 IU/ml for Cobas/AmpliPrep/ Cobas Taqman. After the expiration of the duration of the contract of Anatolia geneworks devices, Roche Diagnostics devices have begun to be used, in our laboratory, between January and December 2013.

3. Results

In this study, the mean age of the patients was 45.84±14.28 years and 58.07% of the patients were men while 41.93% of them was women. HBV DNA was detected in 821 of the 1054 HBs Ag positive samples (77.9%), while it was negative in 233 of the 1054 HBsAg positive samples (22.1%). HBV-DNA levels were determined as 1-10^2 IU/ml (28.6%), 10^{2-3} IU/ml (32.6%), 10^{3-4} IU/ml (22.7%), 10^{4-5} IU/ml (7.2%), 10^{5-6} IU/ml (2.4%), 10^{6-7} IU/ml (2.4%), 10^{7-8} IU/ml (1.5%), 10^{8-9} IU/ml (2.4%), ≥10^9 IU/ml (0.2%). The groups have been created to determine the distribution of patients according to viral load levels. The distribution of patients according to viral loads are summarized in Table 1.

4. Discussion

Individuals with an intact immune system who become infected with HBV generally recover without antiviral treatment, and the risk of developing a CHB infection after acute hepatitis B is less than 5% (Hyams, 1995; Jindal et al., 2013). Viral load is probably an important factor in the natural history of HBV infection, with patients with active replication being at greater risk of disease progression than those without detectable HBV DNA in serum sample. On the other hand, patients in the immune tolerant phase of disease may have a high level of replication without significant liver damage. Risk of HCC is related in part to a direct effect of viral replication and genomic integration, and in part to the host immune response including necroinflammation and hepatic regeneration (Chin and Locarnini, 2003).

A high HBV DNA level seems very important for the development of chronic liver disease. Some prospective studies with the use of sensitive molecular methods for the detection of HBV DNA have demonstrated a dose response increase in HCC risk with circulating HBV DNA levels. Yu et al. (2005) have reported that HBV carriers with plasma HBV DNA levels greater than 4.22 log 10 copies/ml have at least a two fold excess risk of HCC compared with HBV carriers with lower HBV DNA levels (Yu et al., 2005). Chen et al. (2006) have reported that, elevated serum HBV DNA level (≥10 000 copies/ml) is a strong risk predictor of hepatocellular carcinoma independent of HBeAg, serum ALT, and liver cirrhosis. In another study, it has been reported that the viral load of the HBeAg-positive patients was higher than that of the HBeAg-negative patients and that viral load is closely related to the serum ALT level and the grade of histological inflammation. (Ohata et al., 2004) In our study, although the HBeAg test results, serum ALT levels, and the degrees of histological inflammation could not be evaluated, amounts of the viral load of HBV DNA positive samples were observed to concentrate around the lower levels (1-10^5 IU/ml) in Isparta region (91%). Thus, this finding of our study is considered to be important to predict the lower risk of progression to HCC according to the low viral load of HBV DNA in patients with HBV infection in Isparta region. However, the fact that some samples might be samples of the patients who received treatment should be taken into

Table 1. The distribution of patients according to HBV viral load

HBV DNA	1-10^2 IU/ml	10^{2-3} IU/ml	10^{3-4} IU/ml	10^{4-5} IU/ml	10^{5-6} IU/ml	10^{6-7} IU/ml	10^{7-8} IU/ml	10^{8-9} IU/ml	10^9 IU/ml	Total
Number of Positive Samples	235	267	186	59	20	20	12	20	2	821

consideration since treatment duration or whether the patients are under any treatment regimen are not taken into account and this might be the reason of this low viral load detected in this study.

Various reports have shown that viral load of HBV is associated with recurrence after resection of HBV related HCC and high viral load of HBV is a risk factor for recurrence after curative resection of HCC. Sohn et al. (2014) analyzed the predictive factors including HBV DNA and HBsAg levels for early recurrence (within 2 years) and late recurrence (after 2 years) of HCC after curative resection for 248 patiens. Recurrence of HCC after surgical resection was detected in 86 patients (35%). Early recurrence and late recurrence were observed in 61 and 25 patients, respectively. The cumulative 6-month, 1-year, and 2-year recurrence-free survival rates after resection were 96.9, 88.7, and 82.3%, respectively, in patients with a preoperative HBV DNA level <20,000 IU/ml; and 90.5, 76.0, and 60.1%, respectively, in patients with a preoperative HBV DNA level ≥20,000 IU/ml. Early recurrence after resection was significantly different according to the preoperative HBV DNA level. On the other hand, HBsAg levels were associated with late recurrence after curative resection in HBV-related HCC (Sohn et al., 2014). In our study, the relationship between the viral load of HBV and recurrence after resection of HBV related HCC could not be assessed.

In conclusion, quantitative detection of HBV DNA viral replication is one of the best indicators for liver injury and disease progression. In this study, compared to titers reported to be for higher risk of HCC, amounts of the viral load of HBV DNA positive samples were observed to concentrate in the lower levels in Isparta region. However, the role of the level of viremia observed in the patients should be validated by further investigation of clinical findings such as serum HBeAg, ALT levels, and the degrees of histological inflammation and more intensive monitoring of HCC should be considered in chronic HBV patients.

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