

Detection of HBV-DNA and Its Correlation with the HBeAg/Anti-HBe Serological Status in HBsAg-positive Patients

Haris Widita*, Soewignjo Soemohardjo**, Zainul Muttaqin**
Putu Aditya Wiguna***, Shelly Olivia Rhamdiani***
Mahendra Wijaya***, Stephanus Gunawan**

* Department of Internal Medicine, West Nusa Tenggara Provincial General Hospital, Mataram

** Biomedical Research Unit West Nusa Tenggara Provincial Hospital, Mataram

*** Laboratory of Biomedika Hospital, Mataram

ABSTRACT

Background: In the past years, HBeAg and anti-HBe status in individuals with positive HBsAg were often correlated to viral replication. This study was aimed to find correlation between the HBV viremia and HBeAg/anti-HBe serological status in HBsAg-positive individuals.

Method: An observational-analytic design was performed in this study. The sera of all positive HBsAg patients at Biomedika Hospital Laboratory were collected and examined for HBeAg and anti-HBe using immunochromatography technique between January and April 2012. The sampling method was purposive sampling. Afterwards, the sera were examined for HBV-DNA by polymerase chain reaction (PCR).

Results: Sufficient amount of sera were collected from 44 patients consisting of 33 males and 11 females. The mean age was 15-68 years. Positive HBeAg and negative anti-HBe status was found in 11 (42%) patients. Negative HBeAg and positive anti-HBe was found in 26 (59.1%) patients. Both HBeAg and anti-HBe were negative in 7 (16.3%) patients. HBV-DNA was detected in all 11 (100%) patients with positive HBeAg and negative anti-HBe. HBV-DNA was also detected in 11 (42%) patients with negative HBeAg and positive anti-HBe. However, there was only one patient (14.3%) with both negative HBeAg and anti-HBe status, who had detectable HBV-DNA.

Conclusion: Positive HBeAg can be used as an indicator of viremia, but negative HBeAg cannot be used as an indicator of the absence of viremia without further HBV-DNA testing. Patients with negative HBeAg and positive HBV-DNA were suspected for having pre-core mutant.

Keywords: HBV-DNA, positive HBsAg, HBeAg, anti-HBe, pre-core mutant

ABSTRAK

Latar belakang: HBeAg dan anti-HBe pada individu HBsAg positif sering dihubungkan dengan replikasi virus. Penelitian ini bertujuan untuk mencari hubungan antara viremia virus hepatitis B (VHB) dengan status HBeAg/anti-HBe pada individu HBsAg positif.

Metode: Penelitian ini merupakan studi observasional-analitik. Sera dari pasien dengan HBsAg positif di Laboratorium Rumah Sakit Biomedika Mataram dikumpulkan dan diuji status HBeAg dan Anti-HBe dengan teknik imunokromatografi pada bulan Januari hingga April 2012. Pengambilan sampel dilakukan dengan metode purposif, kemudian DNA-VHB sera diuji dengan polymerase chain reaction (PCR).

Hasil: Sisa sera yang masih cukup diperoleh dari 44 pasien yang terdiri dari 33 laki-laki dan 11 perempuan, dengan rata-rata usia 15-68 tahun. HBeAg positif dan anti-HBe negatif didapatkan pada 11 (42%) pasien, HBeAg negatif dan anti-HBe positif didapatkan pada 26 (59,1%) pasien, HBeAg serta anti-HBe yang negatif didapatkan pada 7 (16,3%) pasien. DNA-VHB positif pada semua penderita, HBeAg positif dan anti-HBe negatif (100%). DNA-VHB positif pada 11 (42%) pasien dengan HBeAg negatif dan anti-HBe positif. Seorang pasien (14,3%) dengan HBeAg serta anti-HBe negatif menunjukkan DNA-VHB positif.

Simpulan: HBeAg positif bisa dipakai sebagai indikator adanya viremia. HBeAg negatif tidak dapat dipakai sebagai indikator tidak adanya viremia tanpa dilakukan pemeriksaan DNA-VHB. Pasien dengan HBeAg negatif dan DNA-VHB positif dicurigai menderita infeksi VHB dengan mutan pre-core.

Kata kunci: DNA VHB, HBsAg positif, HBeAg, anti-HBe, mutan pre-core

INTRODUCTION

The severity of hepatitis B virus (HBV) viremia is often correlated with the status of hepatitis B e antigen (HBeAg) and anti-HBe. Positive HBeAg and negative anti-HBe are correlated to the active replication of the virus, and negative HBeAg and positive anti-HBe are related to the non-replicative phase of the virus and the absence of viremia. Several decades ago, Carman et al, reported cases of pre-core mutant in which there was significant viremia despite the absence of HBeAg and the presence of anti HBe.¹ In the past years, it was thought that the condition was found only in the Mediterranean area. But many studies showed that it was also discovered in many parts of the world including Asia and it is caused by the mutation in the pre-core region resulted in the inability of the virus to produce HBeAg although the virus is in the replicative phase.² The condition is caused by point mutation in the nucleotide number 1,386 located in the pre-core region.

Due to limited biomolecular facilities in most laboratories in Indonesia, it is impossible to measure HBV-DNA routinely in all HBsAg positive patients for assessing the replication of hepatitis B virus. Thus, HBV-DNA test should be done selectively. This study was aimed to correlate the HBV viremia with HBeAg and anti-HBe status in HBsAg-positive individuals.

METHOD

The design of the study was an observational-analytic. The sera of all HBsAg positive patients (Entebe Mataram) in Biomedika Hospital Laboratory were collected and examined for HBeAg and anti-HBe using immunochromatography technique (Acon USA) from January to April 2012. The inclusion criteria were HBsAg positive patients with sufficient amount of remaining sera. The sampling method was purposive sampling. The sera were frozen in minus 20°C. Finally the sera were examined for HBV-DNA using qualitative polymerase chain reaction (PCR) method with primers derived from S gene. The primer used in PCR HBV-DNA were:³

S1F 5' CAT CAG GAT TCC TAG GAC CCC and
S3R 5' AGG ACA AAC GGG GCA ACA TAC

RESULTS

Sufficient amount of sera were collected from 44 patients consisting of 33 males and 11 females aged 15-68 years. The result of HBeAg and anti-HBe examination is summarized in Table 1. Table 2 shows the result of HBV-DNA testing. The presence of 300 bp band indicated HBV-positive (S2, S40); while the absence of the band showed negative HBV-DNA S39, S48 as shown in Figure 1.

Table 1. HBeAg/anti-HBe serological status in HBsAg-positive individuals

HBeAg and anti HBe status	n (%)
HBeAg positive, anti-HBe negative	11 (25.0)
HBeAg negative, anti-HBe positive	26 (59.1)
HBeAg negative, anti-HBe positive	7(16.3)
Total	44 (100)

Table 2. Detection of HBV-DNA and its correlation with HBsAg/Anti-HBe serological status in HBsAg-positive individuals

HBeAg and anti HBe status (n)	HBV-DNA positive n (%)
HBeAg positive, anti-HBe negative (11)	11 (100)
HBeAg negative, anti-HBe positive (26)	11 (42.3)
HBeAg negative, anti-HBe positive (7)	1(14.3)
Total	23 (52.3)

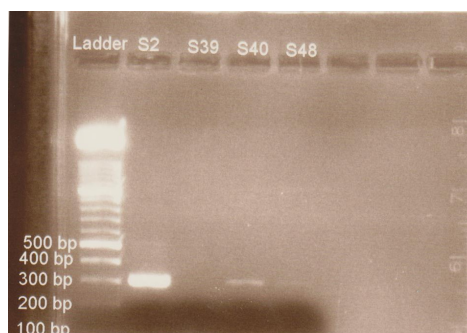


Figure 1. The results on electrophoresis of PCR amplification of HBV-DNA

DISCUSSION

This study showed that in limited facilities condition, viremia can be predicted by the positivity of HBeAg alone. However, in HBsAg-positive patients who have negative HBeAg status, the HBV DNA testing is mandatory to assess viremia. This study demonstrated that more than 40% of patients with negative HBeAg and positive anti-HBe had viremia. Therefore, it can be assumed that the occurrence of pre-core mutant is also high. The most frequent mutation in pre-core region is point mutation at nucleotide number 1896 in which the nucleotide G is replaced by A (G1896A) causing a stop codon resulting in the inability of the virus to produce HBeAg. In patients with pre-core mutant, we could find negative HBeAg and positive anti-HBe, but replication still occurs. It may occur due to the inability of the virus to produce HBeAg although it is still replicative. Anti-HBe is found positive because at the T cells level, HBeAg and HBcAg generate a similar humoral immune response.^{4,5} Individual with positive HBV-DNA and positive HBeAg and negative anti-HBe had active replication, while individual with positive HBV-DNA and negative HBeAg independent of anti-HBe status were suspected for having pre-core mutant.^{6,7}

HBV pre-core mutation is thought to be a mechanism of the virus to avoid immune response of the host considering that HBeAg is the target antigen of the host immune response. The absence of HBeAg causes failure of immune response to destroy the virus. On the other hand, many study reports show that there are relative resistance to anti-viral in patients with pre-core mutant.^{8,9} The presence of pre-core mutation is usually correlated to chronic and severe liver disease.¹⁰ A meta analysis from 85 case control studies indicated that pre-core HBV mutation possibly contributes to hepatocellular carcinoma risk.¹¹ In some countries, pre-core mutation were reported occurred in fulminant hepatitis cases; however, some reports in the other countries showed that the pre-core mutant was not related to fulminant hepatitis.^{12,13}

A study reported that pre-core mutant in HBV infection with genotype B was associated with the occurrence of acute or chronic liver failure.¹⁴ Many reports mentioned the presence of pre-core mutation in asymptomatic carrier.^{9,15} In our previous study on the HBeAg and anti-HBe status in various states of chronic HBV infections in Mataram, the most frequent condition that associated with negative HBeAg and positive anti-HBe was HBsAg asymptomatic carrier.¹⁶ The other study from Indonesia showed that

60.3% of patients with chronic liver disease was HBe negative and 79.6% of HBe negative patients had been seroconverted.¹⁷ The ignored diagnosis causing our inability to correlate the viremia and serologic patterns with clinical condition was one of this study limitation.

CONCLUSION

Positive HBeAg can be used as an indicator of viremia. In this group of patients, HBV-DNA test is not mandatory. However, negative HBeAg cannot be used as an indicator of the absence of viremia without HBV-DNA testing. Patients with negative HBeAg and positive HBV-DNA were suspected for having pre-core mutant. Therefore, all sera suspected for having pre-core mutant should be confirmed with DNA sequencing.

REFERENCES

1. Carman WF, Jacyna MR, Hadziyannis S, Karayiannis P, McGarvey MJ, Makris A, et al. Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 1989;2:588-91.
2. Alfaresi M, Elkoush A, Alshehhi H, Alzaabi A, Islam A. Hepatitis B virus genotypes and pre-core mutants in UAE patients. *Virology* 2010;7:160.
3. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J Hepatol* 2008;49:652-7.
4. Milich D, Liang TJ. Exploring the biological basis of hepatitis B e antigen in hepatitis B virus infection. *Hepatology* 2003;36:1075-86.
5. Pungpapong S, Kim WR, Petericha JJ. Natural history of hepatitis B virus infection: an update for clinicians. *Mayo Clin Proc* 2007;82:967-75.
6. Anonymous. B virus PCR qualitative & quantitative [cited 2012 April 6]. Available from: URL: <http://www.auroprobelabs.com/pcrhepatitisb.html>.
7. Shah SM, Singh SP. Hepatitis B virus serology: use and interpretation. *Hepatitis B Annu* 2007;4:39-54.
8. Ari BZ, Zemel R, Kazetsker A, Fraser G, Kasper TR. Efficacy of lamivudine in patients with hepatitis B virus precore mutant infection before and after liver transplantation. *Am J Gastroenterol* 1999;94:663-7.
9. Locarnini S. Molecular Virology of Hepatitis B Virus. *Semin Liver Dis* 2004;24:S3-10.
10. Rezende RE, Fonseca BA, Ramalho LN, Zucoloto S, Pinho JR, Bertolini DA, et al. The precore mutation is associated with severity of liver damage in Brazilian patients with chronic hepatitis B. *J Clin Virol* 2005;32:53-9.
11. Liao Y, Hu X, Chen J, Cai B, Tang J, Ying B, et al. Precore mutation of hepatitis B virus may contribute to hepatocellular carcinoma risk: evidence from an updated meta analysis. 2012; *PLoS ONE* [cited 2012 April 12]. Available from: URL: <http://www.plosone.org/article/info:doi/10.1371/journal.pone.0038394>.

12. Hasegawa K, Huang J, Wand JR, Obata H, Liang TJ. Association of hepatitis B viral precore mutations with fulminant hepatitis B in Japan. *Virology* 1991;185:460-3.
13. Liang TJ, Hasegawa K, Munoz SJ, Shapiro CN, Yoffe B, McMahon BJ, et al. Hepatitis B virus pre-core mutation and fulminant hepatitis in the United States: a polymerase chain reaction-based assay for the detection of specific mutation. *J Clin Invest* 1994;93:550-5.
14. Tillmann H, Trautwein C, Walker D, Michitaka K, Kubicka S, Boker K, et al. Clinical relevance of mutation in the pre-core genome of the hepatitis B virus. *Gut* 1995;37:568-73.
15. Ren X, Xu Z, Li X, Bai S, Ding N, Zhong Y, et al. Hepatitis B virus genotype and basal core promoter/pre-core mutation are associated with hepatitis B-related acute on chronic liver failure without pre existing liver cirrhosis. *J Viral Hepatol* 2010;17:887-95.
16. Widita H, Gunawan S, Laksono BT, Achwan WA, Wilusanta IG, Mahendra K, et al. HBeAg and anti-HBe status in patients with chronic hepatitis B infection. *Indones J Gastroenterol Hepatol Dig Endosc* 2010;11:125-7.
17. Utama A, Siburian MD, Purwantomo S, Intan MD, Kurniasih TS, Gani RA, et al. Association of core promoter mutation of hepatitis B virus load is different in HBeAg (+) and HBeAg (-) patient. *World J Gastroenterol* 2011;17:708-16.

Correspondence:

Haris Widita

Department of Internal Medicine

West Nusa Tenggara Provincial Hospital

Jl. Bung Karno 143 Mataram 83231 Indonesia

Phone: +62-370-645137 Facsimile: +62-370-645138

E-mail: klinikbiomedika@yahoo.co.id
