

Correlation between Quantitative HBsAg and HBV-DNA in Chronic Hepatitis B Infection

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ABSTRAK

Background: Methods used to diagnose and monitor chronic hepatitis B (CHB) by quantitation of hepatitis B virus-deoxyribonucleic acid (HBV-DNA) levels is expensive. Cheaper laboratory test as an additional marker is needed, thus we studied serum quantitative HBsAg to be used as surrogate marker in CHB patients. This study was aimed to investigate correlation between serum quantitative HBsAg and HBV-DNA in CHB patients.

Method: In this cross-sectional study, we enrolled 62 CHB patients between January 2010 and December 2012 who had quantitative HBsAg and HBV-DNA assays in a private laboratory at Denpasar. HBV-DNA was measured by real-time polymerase chain reaction and quantitative serum HBsAg was measured by chemiluminescent microparticle immunoassay (CMIA). Statistical analysis was performed by Mann-Whitney and Spearman's correlation.

Results: Of 62 patients, most subjects were males (82.26%). Mean HBsAg titer of CHB in HBeAg positive and negative patients were 281,000 and 4,900 IU/mL, respectively; while mean HBV-DNA in HBeAg positive and negative patients were 59,000,000 and 7,530,000 IU/mL, respectively. We found that quantitative HBsAg and HBV-DNA in HBeAg positive and HBeAg negative patients were statistically significant ($p = 0.0001$, $p = 0.0001$, respectively). Significant correlation was found between serum quantitative HBsAg and HBV-DNA ($r = 0.737$; $p = 0.000$). Quantitative HBsAg was significantly correlated with HBV-DNA in HBeAg-positive subgroup ($r = 0.717$; $p = 0.0001$); and significant correlation was also found in HBeAg-negative subgroup ($r = 0.443$; $p = 0.006$) although the correlation was weak.

Conclusion: Quantitative HBsAg has significant correlation with HBV-DNA in CHB patients.

Keywords: quantitative HBsAg Assay, HBV-DNA, HBeAg, chronic hepatitis B infection

ABSTRAK

Latar belakang: Metode yang digunakan untuk diagnosis dan memantau hepatitis B kronis menggunakan kadar deoxyribonucleic acid-virus hepatitis B (DNA-VHB) secara kuantitatif masih cukup mahal. Saat ini masih dibutuhkan pemeriksaan laboratorium yang lebih murah sebagai petanda tambahan. Hal tersebut yang mendorong kami untuk melakukan penelitian mengenai HBsAg serum kuantitatif yang dapat digunakan sebagai petanda pengganti pada pasien hepatitis B kronis. Penelitian ini bertujuan untuk mengetahui korelasi antara serum HBsAg kuantitatif dan DNA-VHB pada pasien hepatitis B kronis.

Metode: Desain penelitian potong lintang dilakukan pada 62 pasien hepatitis B kronis dari Januari 2010 sampai Desember 2012 yang menjalani pemeriksaan HBsAg kuantitatif dan DNA-VHB di laboratorium swasta, Denpasar. DNA-VHB diukur menggunakan real-time polymerase chain reaction, dan serum HBsAg kuantitatif diukur dengan chemiluminescent microparticle immunoassay (CMIA). Analisis statistik dilakukan dengan uji Mann-Whitney dan korelasi Spearman.

Hasil: Dari 62 pasien, sebagian besar adalah pria (82,26%). Rerata titer HBsAg pada pasien hepatitis B kronis dengan HBeAg positif dan negatif masing-masing adalah 281.000 dan 4.900 IU/mL; sedangkan rerata DNA-VHB pada pasien dengan HBeAg positif dan negatif masing-masing adalah 59.000.000 dan 7.530.000 IU/mL. HBsAg kuantitatif dan DNA-VHB pada pasien dengan HBeAg positif dan HBeAg negatif ditemukan berbeda bermakna ($p = 0.0001$). Korelasi bermakna juga ditemukan antara serum HBsAg kuantitatif dengan DNA-VHB ($r = 0,737$; $p = 0,000$). HBsAg kuantitatif berkorelasi bermakna dengan DNA-VHB pada kelompok HBeAg positif ($r = 0,717$; $p = 0,0001$); korelasi bermakna juga ditemukan pada kelompok HBeAg negatif ($r = 0,443$; $p = 0,006$) meskipun korelasinya lemah.

Simpulan: HBsAg kuantitatif memiliki korelasi bermakna dengan DNA-VHB pada pasien hepatitis B kronis.

Kata kunci: pemeriksaan HBsAg kuantitatif, DNA-VHB, HBeAg, infeksi hepatitis B kronis

INTRODUCTION

Hepatitis B virus (HBV) causes a wide range of clinical consequences, from acute and chronic infection till cirrhosis and hepatocellular carcinoma, and represents a global public health problem.^{1,2} Level of HBV-DNA, alanine aminotransferase (ALT), and histological findings are important to determine HBV treatment. Active viral infection can be detected by HBV-DNA level but this assay is expensive. Therefore, cheaper laboratory test that can be used as surrogate marker is needed.³

Quantitative HBsAg is believed might be helpful in the management of HBV. This assay can be used as a biomarker to evaluate infection treatment response in chronic hepatitis B (CHB). HBsAg level reflects the transcriptional activity of covalently closed circular DNA (cccDNA) rather than the absolute amount of cccDNA copies.⁴

Various studies have shown the clinical utility of HBsAg quantitation, but studies where these two markers have been compared are scarce, with conflicting results.⁵⁻¹⁰ Therefore, the present study was undertaken to find correlation between HBV DNA and quantitative HBsAg in CHB patients.

METHOD

A cross-sectional study was performed in 62 CHB patients consecutively between 2010 and 2012 who had quantitative HBsAg and HBV-DNA assays in a private laboratory in Denpasar, Bali. All chronic HBV patients, with HBeAg positive and negative who underwent quantitative HBsAg and HBV-DNA assays were included.

HBV-DNA was measured by real-time polymerase chain reaction with limit of detection 20 IU/ml (conversion: 1 IU is equivalent to 5.82 copies/mL), measuring range of 20-170,000,000 IU/mL, specificity

100%. Quantification of HBsAg was performed using the ArchitectTM HBsAg assay, a two-step immunoassay based on the use of chemiluminescent microparticles immunoassay (CMIA). To determine human serum and plasma HBsAg concentrations quantitatively, CMIA with flexible assay protocols, referred to as Chemiflex, was used. In the first step, the sample and hepatitis B surface antigen antibody (anti-HBs) coated with paramagnetic microparticles are combined. HBsAg present in the sample binds to the anti-HBs coated microparticles. After washing, acridinium-labeled anti-HBs conjugate is added. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by the Architect Immunoassay System optics. This is a fully automated system and can detect as low as 0.2 ng/mL of HBsAg with a dynamic range of 0.05-250 IU/mL.

Continuous variables were compared between groups using Mann-Whitney test. Spearman's correlation was used because variables were not normally distributed. We used 95% confidence interval with significance level of 0.01 (2-tailed). All statistical analyses were performed using SPSS.

RESULTS

Of 62 patients, 51 (82.26%) were male and 11 (17.7%) were female. Mean age of subjects was 42.34 ± 13.07 years. Mean HBsAg titer in CHB with HBeAg positive and negative patients were 281,000 and 4,900 IU/mL, respectively; while mean HBV-DNA in HBeAg-positive patients and HBeAg-negative patients were 59,000,000 and 7,530,000 copies/mL, respectively (Table 1).

We found that quantitative HBsAg and HBV-DNA in HBeAg-positive and HBeAg-negative patients

were statistically different ($p = 0.0001$, $p = 0.0001$, respectively) (Table 1). Significant correlation was found between serum quantitative HBsAg and HBV-DNA ($r = 0.737$; $p = 0.0001$). Quantitative HBsAg was significantly correlated with HBV-DNA in HBeAg-positive subgroup ($r = 0.717$; $p = 0.000$). Similarly, significant correlation was also found in HBeAg-negative subgroup ($r = 0.443$; $p = 0.006$), although the correlation was weak (Table 2).

DISCUSSION

This study results showed significant and strong correlation between quantitative HBsAg and HBV-DNA. But in subgroups, HBsAg had strong correlation with HBV-DNA in HBeAg-positive CHB group, while the correlation was found weak in HBeAg-negative group. Thompson et al, showed similar results in HBeAg-positive CHB group, in which HBsAg had strong correlation with HBV-DNA ($r = 0.69$; $p < 0.01$), while in HBeAg-negative CHB group, HBsAg had weak correlation with HBV-DNA ($r = 0.28$; $p = 0.01$).¹¹ Gupta et al, found weak but significant correlation between quantitative HBsAg and HBV-DNA in all the groups ($r = 0.443$; $p < 0.01$); this correlation was stronger in HBe antigen-positive ($r = 0.402$; $p < 0.01$).¹² Similarly, Lee et al, demonstrated that the overall correlation between quantitative HBsAg and HBV DNA was significant but very weak ($r = 0.121$; $p = 0.004$).¹³ Chan et al, showed that quantitative HBsAg correlated well with log [cccDNA] ($r = 0.54$; $p = 0.004$) and log [total intrahepatic HBV DNA]

($r = 0.43$, $p = 0.028$).⁷ In contrast, Ganji et al, demonstrated no significant correlation between HBsAg and HBV-DNA ($r = 0.53$, $p = 0.606$).³

Hepatitis B virus, a small DNA virus, is a prototype virus of the *Hepadnaviridae* family. HBV infection leads to a wide spectrum of liver diseases ranging from acute to chronic hepatitis, cirrhosis, and hepatocellular carcinoma.¹⁴ Upon entry into the hepatocyte nucleus, the host and viral polymerase repair the partially double-stranded HBV genome to a fully double-stranded (cccDNA), which as a non-integrated minichromosome, acts as a template for transcription of viral genes.¹⁵ Assay of cccDNA in liver tissue is the most accurate index of infected hepatocytes but the complexity of the testing renders it unavailable for routine use. Serum HBsAg has been shown to correlate with transcriptionally active cccDNA.¹⁶

Measuring serum HBV-DNA is the gold standard for monitoring viral load, but it is relatively expensive and not yet readily available in some areas. Hence, there is a definite need of a tool which is economical, reliable, and easy to perform. HBsAg quantitation is a recent serological marker being evaluated.¹⁷ The technique for detecting quantitative HBsAg is fairly easy and inexpensive.¹⁸ The first assay for quantitative HBsAg using enhanced chemiluminescence was reported nearly 20 years ago but was limited by lack of standardization.¹⁵ Serum HBsAg quantification has been suggested to reflect the concentration of cccDNA in the liver which serves as the template for viral replication inside hepatocytes.^{19,20}

Table 1. Baseline subject characteristics

Parameters	Patients (%)	Mean \pm SD	p*
Age (years)		42.34 \pm 13.07	
Sex			
Male	51 (82.3)		
Female	11 (17.7)		
HBeAg-positive CHB	25 (40.32)		
HBsAg titer (IU/mL)		2.81 $\times 10^5 \pm 1.3 \times 10^6$	
HBV-DNA (copies/mL)		5.9 $\times 10^7 \pm 5.45 \times 10^7$	0.0001*
HBeAg-negative CHB	37 (59.68)		
HBsAg titer (IU/mL)		4.9 $\times 10^3 \pm 2.05 \times 10^4$	
HBV-DNA (copies/mL)		7.53 $\times 10^6 \pm 2.55 \times 10^7$	0.0001*

*Mann-Whitney; CHB: chronic hepatitis B; HBV-DNA: hepatitis B virus-deoxyribonucleic acid

Table 2. Statistical analysis for correlation between quantitative HBsAg and HBV-DNA

	Mean (95% CI)		r	p*
	HBsAg (IU/mL)	HBV-DNA (copies/mL)		
All Subject	1.16 $\times 10^5$	2.83 $\times 10^7$	0.737	0.000
HBeAg positive (n = 25)	2.81 $\times 10^5$	5.9 $\times 10^7$	0.717	0.000
HbeAg negative (n = 37)	4.9 $\times 10^3$	7.53 $\times 10^6$	0.443	0.006

*Spearman's correlation test; HBV: hepatitis B virus; significant at the 0.01 level (2-tailed); HBV-DNA: hepatitis B virus-deoxyribonucleic acid

HBsAg is encoded by the envelope gene, which contains three open-reading frames: the pre-S1, pre-S2 and S domains. There is subsequent conversion to small, medium and large forms of HBsAg proteins. Newly synthesized HBsAg proteins are secreted from the hepatocyte. HBsAg synthesis is separated from the viral replication pathway. Existing quantitative HBsAg serology can detect all three forms of HBsAg in the circulation.¹²

Based on the results of this study and the discussion above, we can consider quantitative HBsAg as a surrogate marker to HBV-DNA. Limitation of this study was the relatively small number of patients.

CONCLUSION

Quantitative HBsAg has significant correlation with HBV-DNA in CHB patients. Further studies are required to investigate the possibility of using quantitative HBsAg as an aid, if not an alternative, for HBV-DNA.

REFERENCES

1. Lok AS. Chronic hepatitis B. *N Engl J Med* 2002;346:1682-3.
2. Ahn SH, Han KH, Park JY, Lee CK, Kang SW, Chon CY, et al. Association between hepatitis B virus infection and HLA-DR type in Korea. *Hepatology* 2000;31:1371-3.
3. Ganji A, Esmaeilzadeh A, Ghafarzadegan K, Helalat H, Rafatpanah H, Mokhtarifar A. Correlation between HBsAg quantitative assay results and HBV-DNA levels in chronic HBV. *Hep Monthly* 2011;11:342-5.
4. Chan HL, Thompson A, Martinot-Peignoux M, Piratvisuth T, Cornberg M, Brunetto MR, et al. Hepatitis B surface antigen quantification: why and how to use it in 2011—a core group report. *J Hepatol* 2011;55:1121-31.
5. Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. *Hepatology* 2009;49:1151-7.
6. Wursthorn K, Jung M, Riva A, Goodman ZD, Lopez P, Bao W, et al. Kinetics of hepatitis B surface antigen decline during 3 years of telbivudine treatment in hepatitis B e antigen-positive patients. *Hepatology* 2010;52:1611-20.
7. Chan HL, Wong VW, Tse AM, Tse C, Chim AM, Chan H, et al. Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. *Clin Gastroenterol Hepatol* 2007;5:1462-8.
8. Kuhns MC, Kleinman SH, McNamara AL, Rawal B, Glynn S, Busch MP. Lack of correlation between HBsAg and HBV DNA levels in blood donors who test positive for HBsAg and anti-HBc: implications for future HBV screening policy. *Transfusion* 2004;44:1332-9.
9. Kohmoto M, Enomoto M, Tamori A, Habu D, Takeda T, Kawada N, et al. Quantitative detection of hepatitis B surface antigen by chemiluminiscent microparticle immunoassay during lamivudine treatment. *J Med Virol* 2005;75:235-9.
10. Ozaras R, Tabak F, Tahan V, Ozturk R, Akin H, Mert A, et al. Correlation of quantitative assay of HBsAg and HBV DNA levels during chronic HBV treatment. *Dig Dis Sci* 2008;53:2995-8.
11. Thompson AJ, Nguyen T, Iser D, Jackson K, Littlejohn M, Slavin J, et al. Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers. *Hepatology* 2010;51:1933-44.
12. Gupta E, Kumar A, Choudhary A, Kumar M, Sarin SK. Serum hepatitis B surface antigen levels correlate with high serum HBV DNA levels in patients with chronic hepatitis B: a cross-sectional study. *Indian J Med Microbiol* 2012;30:150-4.
13. Lee JH, Kim SJ, Ahn SH, Lee JH, Park YJ, Kim HS. Correlation between quantitative serum HBsAg and HBV DNA test in Korean patients who showed high level of HBsAg. *J Clin Pathol* 2010;63:1027-31.
14. Kao JH, Chen DS. Global control of hepatitis B virus infection. *Lancet Infect Dis* 2002;2:395-3.
15. Seth AK. HBsAg quantification in clinical practice. *J Clin Exp Hepatol* 2012;2:75-80.
16. Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, et al. Persistence of cccDNA during natural history of chronic hepatitis B and decline during adefovirdipivoxil therapy. *Gastroenterology* 2004;126:1750-8.
17. Deguchi M, Yamashita N, Kagita M, Asari S, Iwatani Y, Tsuchida T, et al. Quantitation of hepatitis B surface antigen by an automated chemiluminescent microparticle immunoassay. *J Virol Methods* 2004;115:217-22.
18. Lee JM, Ahn SH. Quantification of HBsAg: basic virology for clinical practice. *World J Gastroenterol* 2011;17:283-9.
19. Chan HL, Wong VW, Wong GL, Tse CH, Chan HY, Sung JJ. A longitudinal study on the natural history of serum hepatitis B surface antigen changes in chronic hepatitis B. *Hepatology* 2010;52:1232-41.
20. Zoulim F. New insight on hepatitis B virus persistence from the study of intrahepatic viral cccDNA. *J Hepatol* 2005;42:302-8.

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