Profile Quantitative Hepatitis B Surface Antigen (qHBsAg) of Chronic Naïve Hepatitis B Patients in Dr. Soetomo Hospital, Surabaya, Indonesia

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Abstract

This study aimed to evaluate the profile of qHBs Ag profile, and also to investigate the correlation between qHBs Ag and HBV DNA. Seventy samples of chronic-naïve hepatitis B patients in Dr. Soetomo Hospital were analyzed in a cross-sectional study. Patients were categorized according to the HBe Ag positive (n=30) and HBe Ag negative (n=18), also based on qHBs Ag 1000 IU/mL and qHBs Ag >1000 IU/mL. qHBs Ag was correlated with HBV DNA. qHBs Ag by CLEIA method from Sysmex, KOBE HISCL, HBV DNA was measured by real-time Polymerase Chain method from Gene Xpert, Cepheid. 70 patients naïve CHB treatment showed a median of ALT level 60.21±70.76 U/L. 30 patients showed a positive-HBeAg, 18 patients showed negative-HBeAg, 22 patients were not evaluated (N/A). Positive-HBeAg patients had 70% qHBsAg >2500 mg/dL and median HBV DNA 7.49×10^7 IU/mL. Negative-HBeAg patients had 55.6% HBsAg ≤1000 mg/dL and median HBV DNA 9.66×10^2 IU/mL. qHBsAg correlated with HBV DNA ($p < 0.001$). This data demonstrated that quantitative HBsAg was associated with a phase of HBV-infection, quantitative HBsAg showed a moderate correlation with DNA HBV, quantitative HBsAg levels might be a predictor of initiation therapy for CHB patients.

Keywords: *Chronic-Naïve Hepatitis B Patients, Hepatitis B, Quantitative Hepatitis B Surface Antigen, Surabaya.*

Introduction

Hepatitis B virus (HBV), a small DNA virus, is Estimated globally as much as 400 million people are infected with chronic HBV infection worldwide. A High prevalence can be found in developing countries such as Indonesia. HBV patients are approximately 4.0-20.3% of the healthy population of Indonesia. However, The java island proportion is lower than the other islands[1,2]. The risk of developing chronic HBV infection based on the age of infection was as much as 90% if infection occurred from perinatal to 6 months and 20%-60% if infection occurred between the age 6 of the month-5 years[3].

HBs Ag is a seromarker used routinely to diagnose acute or chronic viral hepatitis B, screening blood donors or organ donors, surveillance persons at risk of acquiring or transmitting (HBV)[1]. Quantitative HBs Ag has been proposed to be used as monitoring the course of chronic hepatitis B infection including the immune tolerance, immune clearance, immune control/inactive carrier phase as well as reactivated negative HBe phase[4]. The decline of HBs Ag levels could be an early predictor to know viral efficacy for HBV therapy[5].

Appearance and disappearance of HBs Ag in infected HBV infection persons generally adhere to characteristic patterns. The highest level of HBs Ag is in

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the immune tolerance phase, declines during the immune clearance phase, and decreases slowly but progressively in seroconversion HBe antigen. The lowest HBs Ag level is in the inactive carrier phase, but increases in HBeAg-negative patients[6].

HBsAg, HBV quantification, and HBe Ag may be useful biomarkers to monitor therapy for HBV infection[5]. The novelty of this research so far was that there were no studies for clinical implication research of HBs Ag quantitative in Indonesia up to now.

The present study aimed to assess the clinical implication of quantitative HBs Ag in chronic HBV infection. We sought here to demonstrate the potential utility of quantitative HBs Ag in reactive HBe-Ag and non-reactive HBe-Ag patients. We also investigated the correlation between quantitative HBs Ag and the level of HBV DNA.

Methods

This was a case-controlled study. As many as 70 treatment-naïve chronic hepatitis B patients were enrolled in this study. Inclusion criteria were as follows age between 18-70 years old, positive HBs Ag at least 6 months. Exclusion criteria were no other chronic hepatitis causes including autoimmune hepatitis, or other viral hepatitis such as hepatitis C virus, hepatitis D virus, or human immunodeficiency virus and no history of anti-HBV treatment. In addition, qHbs Ag was quantified by Sysmex, KOBE HISCL-5000 with detectability range of 0.05-2,500 mg/dL, HBV DNA quantification by Gene Expert, with a detectability range 10-1,000,000,000 IU/mL.

The frequency and median values were determined for categorical and continuous variables. The differences between groups were analysed using the Mann-Whitney U test for ordinate data, while Fisher Exact test with continuity correction for continuous dichotomous variables, and The Spearman Rank test for correlation analysis. Statistical analyses were performed by SPSS 17 version.

Results and Discussion

A total of 70 patients, including 39 (55.7%) male and 31 (44.3%) females, with a median of age 36.86±12.73 years. The baseline median of Aspartate Transaminase (ALT) level was 60.21±70.76 U/L. Of these patients, 22.9% had ALT >2 ULN mean 137.6±118.7 U/L, 77.1% had ALT >2 ULN mean 37.4±14.82 (U/L). Among this prevalence, 30 patients were positive HBe Ag, 18 patients were negative HBe Ag, and 22 patients were not evaluated (N/A). Characteristics of 70 chronic hepatitis B patients are shown in Table 1.

Table 1. Characteristics of patients.

Characteristics	Value (percentage)	Mean
Total sample (n)	70	
Gender Male/Female n (%)	39 (55.7%)/31 (44.3%)	
Age (n)	70	36.86±12.732
Total HBe Ag Reactive	30 (62.5%)	
Total HBe Ag-negative	18 (37.5%)	
ALT (U/L)		60.21±70.765 (U/L)
ALT >2 ULN (U/L)	16 (22.9%)	137.6±118.7 (U/L)
ALT £2 ULN (U/L	54 (77.1%)	37.4±14.82 (U/L)

Based on this result, 30 patients were classified as positive HBe Ag and 18 patients were classified as negative HBe Ag. Patients with positive HBe Ag showed higher AST and ALT than negative HBe Ag patients (p <0.05). Of these positive HBe Ag showed a median of AST 40 U/L (range 14-236) and median ALT 65 U/L (range 20-559). Besides, positive HBe Ag patients had ALT 2 ULN (60%) higher than ALT >2 ULN 40%. Negative HBe Ag patients who had ALT £2 ULN 77.1% were higher than patients who had ALT >2 ULN 22.2% (p=0.343). positive HBe Ag patients had a median HBV DNA lower than negative HBe Ag patients (p <0.001).

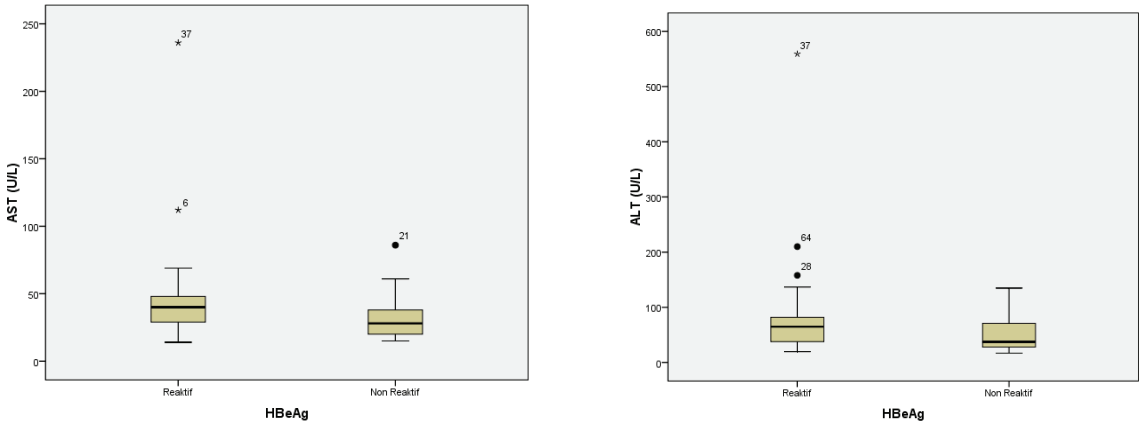

Figure 1. The differences AST and ALT levels between HBe Ag positive and HBe Ag negative.

Table 2. Baseline demographic data comparing HBe Ag positive and HBe Ag negative patients.

	HBe Ag Positive (30)	HBe Ag Negative (18)	p
AST	40 (14-236)	28 (15-86)	0.044[a]
ALT	65 (20-559)	37.5 (17-135)	0.041[a]
ALT >2 ULN	12 (40%)	4 (22.2%)	0.343[b]
ALT £2 ULN	18 (60%)	14 (77.8%)	
HBs Ag £1000 (mg/dL))	5 (16,7%)	10 (55.6%)	0.001[a]
HBs Ag >1000-2500 (mg/dL)	4 (13,3%)	4 (22.2%)	
HBs Ag >2500 (mg/dL)	21 (70%)	4 (22.2%)	
DNA HBV (IU/mL)	7.49×10^7 (26-7.22×10^8)	9.66×10^2 (10-2.71×10^8)	<0.001[a]

[a]Mann-Whitney test

[b]Chi square with continuity correction

However, between positive HBe Ag patients who had HBs Ag £1,000 mg/dL 16.7%, HBs Ag >1,000 mg/dL, 13.3%, HBs Ag >2,500 mg/dL 70%. negative HBe Ag patients who had HBs Ag £1,000 mg/dL 55% and HBs Ag >1,000 22.2%, HBs Ag >2,500 mg/dL 22.2% (p=0.001).

Table 3. HBV DNA levels in patient with HBs Ag >1,000 IU/mL and HBs Ag £ 1,000 IU/mL.

	DNA HBV (n)	Median	p
HBs Ag £1,000 IU/mL	27	7.70×10^3 (10-4.19×10^6)	< 0.001[a]
HBs Ag >1,000 IU/mL	41	5.00×10^6 (10-7.22×10^8)	

[a] Mann Whitney

Patients with HBs Ag £1,000 mg/dL had a median HBV DNA 7.70×10^3, patients HBs Ag >1,000 IU/mL had median HBV DNA 5.00×10^6 ($p < 0.001$).

Table 4. Correlation between HBs Ag and HBV DNA.

	HBs Ag vs HBV DNA	
Coefficient correlation HBs Ag vs HBV DNA	$r_s = 0.599$	p <0.001[a]

[a] Spearman correlation

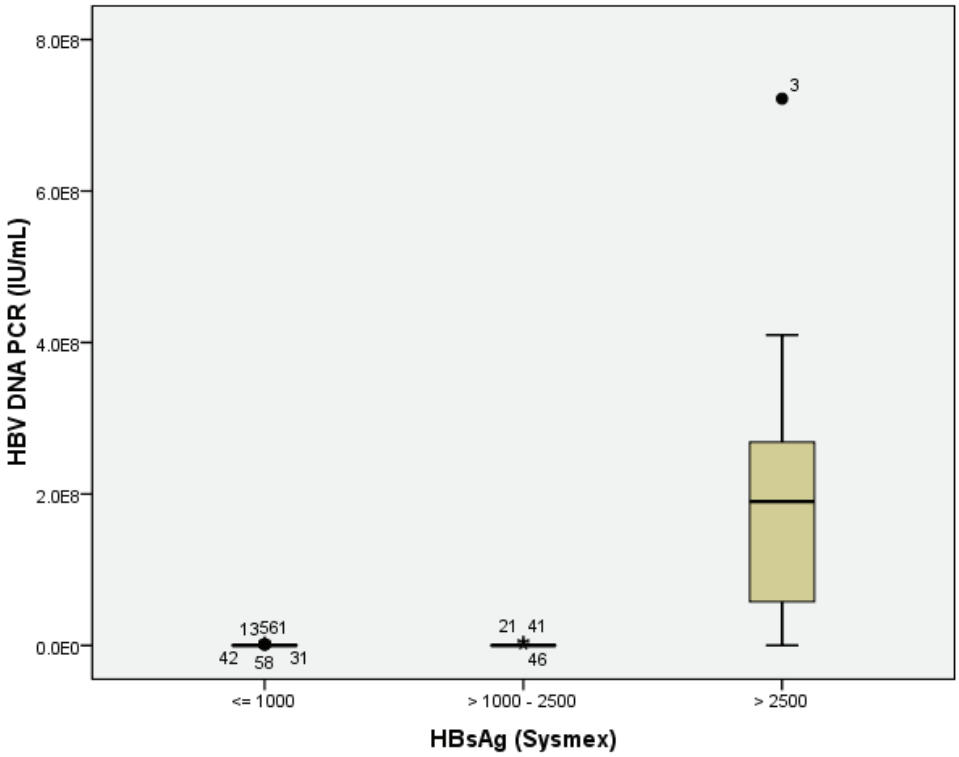

Figure 4. Correlation between quantitative HBs Ag and HBV DNA.

Indonesia is one of the major countries with a high prevalence of hepatitis B virus (HBV) infection. The endemicity of HBV in Indonesia is intermediate to high with a geographical difference. The most common HBV subgenotype in Indonesia is B3, followed by C1[7].

Chronic hepatitis B patients run through four phases, an initial HBe Ag positive-chronic infection Hepatitis B is characterized by high HBs Ag, positive HBe Ag, normal ALT, and null or minimal histologically damages. The second phase is HBe Ag positive chronic Hepatitis B, whereby the immune system recognizes HBV as a foreign-invader which can cause extensive liver cell inflammation. The third phase is HBe Ag-negative chronic infection or inactive phase showing persistently normal alanine aminotransferase (ALT) and lowers HBV DNA levels (<2,000 IU/mL). The fourth phase is characterized by late reactivation of infection with persistently or intermittently increased HBV DNA and ALT levels accompanied by progressive liver damage, then so-called HBe Ag- negative chronic hepatitis[8,9].

Based on the four phases of chronic hepatitis B, this study showed that among naïve Hepatitis B chronic patients, positive HBe Ag had higher AST and ALT than negative HBe Ag patients. Between positive HBe Ag patients, most of them had HBs Ag >2,500 IU/mL. Among HBe Ag negative patients, most of them had HBs Ag £1,000 IU/mL. Therefore 18 patients were immune tolerance, 12 patients were in immune clearance phase, 14 patients were in low replicative phase, and 8 patients were inactive carriers, and 10 patients were HBe-negative hepatitis B (data were not shown).

Positive HBe Ag patients had a higher HBV DNA than HBe Ag negative patients (median 7.49 x 10^7 IU/mL VS 9.6×10^2IU/mL) ($p<0.001$). Between quantitative HBsAg and HBV DNA showed a moderate correlation with a coefficient correlation $r_s= 0.599$ ($p<0.001$) especially in patients with quantitative HBs Ag >2,500 IU/mL. This finding was similar to a study by Gupta *et al* who stated that serum HBsAg correlates with HBV DNA in CHB patients, especially in high serum HBV DNA, HBe antigen-positive, and treatment-naïve group. Furthermore in a study from Pratiwi *et al.* who reported that quantitative HBs Ag had a significant correlation with HBV DNA in CHB patients[10]. These findings were similar to some previous studies, that HBs Ag titer and HBV DNA were higher in HBe Ag-positive patients than HBe Ag negative patients[4,11,12]. The kinetics of serum HBs Ag during the natural history of chronic HBV, infection have been studied, but the factors affecting them remain unclear.

HBs Ag titers varied significantly in different phases, with the highest in patients with HBe Ag-positive chronic HBV infection, while that among HBe-Ag positive patients, HBs Ag titers were correlated with HBV DNA [13]. These mechanisms are probably caused by the two different but cross-regulated synthesis pathways of HBs Ag and HBV DNA. HBs Ag is secreted in the serum as Dane particles and noninfectious filamentous or spherical subviral particles which are derived from different open reading frames of cccDNA[14], and can also be produced from HBV DNA integrated into the host genome[15,16]

In Another study from Thomas *et al*, HBe-positive patients showed a mean quantitative HBs Ag 5,410.17 IU/ml and mean HBV DNA 1.86×109 IU/ml with a correlation coefficient of -0.184 (p=0.256), while a mean quantitative HBs Ag 5,229.24 IU/mL and mean HBV DNA $1.8X10^7$ IU/mL with correlation coefficient -0.84 (p=0.256) while in HBe-negative patient. HBV DNA levels were significantly higher in positive HBe Ag patients, but there was no significant correlation between quantitative HBs Ag levels and HBV DNA[17]. HBe Ag-negative patients had higher levels of HBs Ag and HBV DNA, there was no significant correlation between HBs Ag and HBV DNA levels in CHB with predominant genotype D in Iran[18]. There are 2 forms HBs Ag, the first intact virion which includes small, medium, and large protein in the envelope, that is related to viral infectivity, the second are subviral particles in serum that are produced abundantly. These are predominantly S proteins and to a lesser extent are M and L proteins (these are not infectious but are strongly immunogenic, stimulating antibody production). This study suggested that examination should be used for two forms of HBs Ag and quantitate them, so the relationship between HBs Ag and HBV DNA could determined[18].

Monitoring quantitative HBs Ag has been suggested as a predictor of treatment response, especially for IFN- based therapy in chronic HBV-infection[4–6]. Since Chronic HBV infection had a highly dynamic based on

four chronological phases, this study aimed to determine HBs Ag levels in Reactive HBe Ag and non-reactive HBe Ag of HBV. HBs Ag levels were higher in reactive HBe Ag compared to nonreactive HBe Ag. We found a moderate correlation between HBs Ag and DNA HBV in chronic hepatitis B patients. The weak association between DNA HBV and HBs Ag in a narrow window of time for each HBV-phase may reflect a disconnection between HBV replication and HBs Ag production during persistent HBV infection. They are most likely due to various reasons such as the integration of HBV into the host genome that potentially provides a separate template for the production of HBs Ag or the cytokine dependant modification of viral replication pathways leading to disruption in the stability of cytoplasmic viral capsids[4]. Furthermore, the dissimilarity of quantitative HBs Ag as a predictor and discrepancies in studies about the association of HBs Ag and DNA HBV may be due to the highly dynamic nature of HBV infection as well as the influence of HBV genotype on HBs Ag-levels[4]. This study did not correlate the association between HBs Ag and HBV genotype.

Conclusion

In summary, these data demonstrated that (i) quantitative HBs Ag were associated with the phase of HBV-infection, (ii) while HBs Ag titers had higher in HBe Ag-positive CHB than HBs Ag-negative CHB, quantitative HBs Ag showed a moderate correlation with DNA HBV, and (iii) quantitative HBs Ag levels might be a predictor of initiation therapy for chronic hepatitis B patients. Further study with larger samples, examining genotype, monitoring therapy and liver biopsy as a need for future study.

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: This study funded by the Penelitian Unggulan Fakultas (PUF), Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

Acknowledgements: We thank Arif Nur Muhammad Ansori for editing the manuscript.

Ethical Approval: This study had been approved by the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

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