Measuring HBsAg and HBV DNA Levels in Cilegon

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ABSTRACT

Background: *Implication of measuring HBsAg level is still not recognized well. The aim of this study to recognize the correlation of serum HBsAg level and serum HBV DNA level between in HBeAg positive patients and HBeAg negative patients.*

Method: *Quantitative serum HBV DNA were collected retrospectively between January 2006 and May 2009. We stratified the patients into four groups, that were; HBeAg positive and (a) ALT > 2 x upper limited normal (UNL) (group A),(b) ALT < 2 x UNL (group B), HBeAg negative and:(a) ALT > 2 x UNL (group C) (b) ALT < 2 x UNL (group D). We studied the correlation of serum HBsAg and HBV DNA level in each group. In addition, we also studied the accuracy of HBsAg titers to predict serum HBV DNA levels in each group by using receiver operating characteristic (ROC) curve analysis.*

Results: *Eighty nine patients were recruited in this study. Most of them 63 (70%) were male; the mean age of the patients was 38.49 ± 11.21 years. The number of patients with HBeAg positive and negative were 28 and 61 respectively. Based on the group stratification, the A, B, C and D groups we found 16, 12, 11, 50 respectively. There was a positive correlation between HBsAg titers and HBV DNA level in HBeAg positive patients but it was statistically not significant. Similar result was also found in HBeAg negative patients. There were positive correlation in group A, C, and D but they were not statistically significant. In group B the correlation was negative (r = -0.40). We found 100% sensitivity and 100% specificity of predicting serum HBV DNA levels in group A with HBsAg cut-off level of 7.91 IU/mL and baseline serum HBV DNA cut-off level > 20,000 IU/mL. In group B, C and D the accuracy to predict serum HBV DNA level were not so good.*

Conclusion: *There were positive correlation between HBsAg titers and HBV DNA levels in HBeAg positive and HBeAg negative patients as demonstrated in the three group stratification; however, it was not significant. There were weak positive correlations between HBsAg level and HBV DNA serum level, for group A, C and D patients, but again, they were not significant. While in other groups, the correlation were not so good.*

Keywords: *serum HBsAg level, serum HBV DNA level, hepatitis B, correlation*

INTRODUCTION

Several studies in quantify the serum HBsAg levels have been done. Chen's et al found a good correlation between HBsAg and HBV DNA levels in asymptomatic hepatitis B virus carriers.[1] Chan et al showed that serum HBsAg levels were well correlated to the cccDNA and intrahepatic hepatitis B virus (HBV) DNA.[2] Low pret-reatment HBsAg level is better than HBV DNA for predicting good response to peg-interferon and lamivudine treatment in HBeAg positive patients.[2] However, they did not find correlation between serum HBsAg level and serum HBV DNA level. Jinlin showed three dynamic patterns of serum HBsAg levels following treatment with adevovir and peg-interferon-alfa-2a in HbeAg positive chronic hepatitis B patient.[3] Ozaras et al concluded that HBsAg quantitative measurement could be a surrogate marker for viral loading during

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the management of patient with HBeAg positive and chronic HBV infection.[4]

In 2004 Krakatau Medika hospital provided HBsAg serum examination and the result are expressed as countable HBsAg titer. When receiving the laboratory result, the patients frequently asked about the progression of their disease. We regularly suggested them to repeat the examination, and if the result of HBsAg titer increased, the patients worried as it indicated that they had worse condition. At that time, we could only provide qualitative result of serum HBV DNA. Since 2006, we have performed quantitative measurement of serum HBV DNA, and therefore, the quantitative serum levels are available for HBsAg and HBV DNA.

The cost of HBV DNA examination is relatively expensive; while the HBsAg examination relatively cheap and affordable. If HBsAg titer can predict serum HBV DNA levels, then it would bring a great value for management of patients. In order to recognize the correlation of serum HBsAg level and serum HBV DNA level in HBe positive and HBe negative patients, retrospectively we search data of patients with HBsAg positive.

METHOD

Patients who had been examined for their serum HBV DNA levels quantitatively were recruited recto spectively between January 2006 and May 2009. Patients with non countable HBV DNA levels were excluded. HBsAg titers were noted, as well as the serum HBeAg, anti HBeAg, and ALT, AST level.

Patients who had laboratory data between HBsAg level and HBV DNA level, did not exceed one month were recruited. AST and ALT serum that time examined closed to time HBSAg examination were noted. All examinations were performed at Krakatau Medika hospital. Patients who were receiving treatment with interferon or nucleosid analog were excluded from this study. We stratified the patients into four groups; group A with HBeAg positive result and ALT > 2 x UNL; group B with HBeAg positive result and ALT < 2 x UNL; group C with HBeAg negative result and ALT > 2 x UNL; and group D with HBeAg negative result and ALT < 2 x UNL. We studied the correlation between HBsAg level and serum HBV DNA level in each groups using Pearson correlation. We also evaluated the accuracy of HBsAg titers for predicting HBV DNA levels in each groups using receiver operating characteristic (ROC) curve analysis. For ROC analysis, HBV DNA levels were divided into HBV DNA ≥ 20,000 IU/mL and HBV DNA < 20,000 IU/mL in group A and B. In group C and D, HBV DNA levels were divided into HBV DNA > 2,000 IU/mL and HBV DNA < 2,000 IU/mL.

Laboratory Assays

We conducted several laboratory assays including hepatitis B surface antigen assay, serum ALT and AST assays and serum HBV DNA assay. We used Vidas HBsAg ultra (Biomerieux, Lyon, France) reagent for HBsAg assay. The lowest level start from 0.12 ng/mL. Serum HBV-DNA assay**;** HBV DNA was extracted by Cobas amplicor HBV monitor test (Roche diagnostics) according to the manufacturer's instructions. HBV DNA was quantified by Cobas Amplicor HBV monitor test. The range of HBV DNA detection was from 60 to 3.8 x 10^4 IU/mL (315 copies/mL to 2 x 10^5 copies/mL). Serum ALT assay: alanine aminotransferase, Cobas Integra 400, cat. no. 20764957 (Roche diagnostics). The method was according to the International Federation of Clinical Chemistry (IFCC) recommendation, but without pyridoxal-5'-phosphate. We expected ALT values of 31 U/L in female and 41 U/L in male. Serum HBeAg assay: Vidas HBe/Anti-HBe REF 30 305 (Biomerieux France) was the reagents used in this study. We combined the principle of an enzyme immunoassay method with final fluorescent detection (ELFA) method.

Statistical Analysis

Statistical tests were performed by SPSS version 13.0. HBV DNA were expressed as logarithmic rather than the IU/mL for analysis. Data were analyzed by using Kruskall-Wallis test and Post-Hoc (Mann-Whitney) test. The Spearman's correlation coefficient was tested to demonstrate the correlation between HBV DNA and HBsAg for four groups stratification. Statistical significance was defined as a value of $p < 0.05$. All statistical measurements were 2-sided tests.

RESULTS

Eighty nine patients fulfilled the criteria were recruited. There were 63 (70%) male and 26 (30%) female. Mean age was 38.49 ± 11.21 years (range 11-60 years). Twenty eight (31.5%) patients were HBeAg positive, and 61 (68.5%) were HBeAg negative. Mean value of HBsAg was 22.65 ± 6.5 IU/mL (range 0.74-38.67 IU/mL). The mean value of HBV DNA was 4.70 ± 1.65 log 10 IU/mL (range 1.86–7.78 log 10 IU/mL), ALT level median 46.5 (range 11-2,301 IU/mL) (table 1).

There were 16 (18%), 12 (13.5%), 11 (12.4%), 50 (56%) patients in group A, B, C, D, respectively. Detailed clinical features in four difference groups are presented in table 1. The result of all anti HCV test were negative. Approximately 80% patients had abdominal ultrasonography examinations and the result were normal. Moreover, 8 patients had fatty liver, and one patient had early chronic liver disease.

Table 1. Clinical features, HBV DNA levels and HBsAg titers in four different groups

Characteristic	A n = 16	B n = 12	C n = 11	D n = 50
Sex (male/female)	12/4	7/5	10/1	33/17
Age (years)	31.3 ± 11.3	31 ± 11.2	40.2 ± 11.7	41.9 ± 9.4
HBsAg level (IU/mL)	21.9 ± 7.9	23.2 ± 4.7	22.1 ± 6.0	22.8 ± 6.8
HBV DNA (log IU/mL)	5.98 ± 1.22	6.84 ± 0.5	5.02 ± 1.46	3.68 ± 1.11
ALT (median, range)	155 (82-2,116)	41 (17-62)	170 (82-2,301)	29 (11-73)

Note: Kruskall-Wallis test: age p = 0.001; HBsAg level p = 0.95

Pearson's correlation test of HBsAg titers and HBV DNA level in HBeAg positive patients showed weak positive correlation but statistically, it was not significant (r = 0.28; p = 0.149). Similar result was also found in HBeAg negative patients (r = 0.22; p = 0.083) (figure 1).

There were weak positive correlations in group A, C, and D but statistically, they were not significant (group A, r = 0.40; p = 0.12; group C, r = 0.28; p = 0.40; group D, r = 0.26; p = 0.07). In group B the correlation was negative (r = 0.40; p = 0.10) (figure 2).

There were 15 cases in group A, which had serum HBV DNA level < 20,000 IU/mL and only one case had serum HBV DNA level < 20,000 IU/mL. The area under the ROC curve for baseline HBsAg level for predicting serum HBV DNA was 1.00 (95% CI = 1.00-1.00; p = 0.107) (figure 3). At HBsAg cut off level 7.91 IU/mL, there were 100% sensitivity and 100% specificity of serum HBV DNA level ≥ 20,000 IU/mL.

There were 12 cases B group with serum HBV DNA ≥ 20,000 IU/mL, and zero case with serum HBV DNA level < 20,000 IU/mL. The area under the ROC curve of this group could not be performed. There were 9 cases in group C with serum HBV DNA < 2,000 IU/mL, 2 cases with serum HBV DNA level < 2,000 IU/mL. The area under the ROC curve for baseline HBsAg level for predicting serum HBV DNA level was 0.67 (95% CI = 0.17-1.16; p = 0.48) (figure 4). At HBsAg cut off level 13.51 IU/mL, the sensitivity and specificity for serum HBV DNA level > 2,000 IU/mL were 100% and 50% respectively.

There were 29 cases in group D with serum HBV DNA ≥ 2,000 IU/mL and 21 cases with serum HBV DNA level < 2,000 IU/mL. The area under the ROC curve for baseline HBsAg level for predicting serum HBV DNA was 0.59 (95% CI = 0.43-0.75; p = 0.30) (figure 5). At HBsAg cut off level 21.38 IU/mL, the sensitivity and specificity for serum HBV DNA level > 2,000 IU/mL were 72% and 62% respectively.

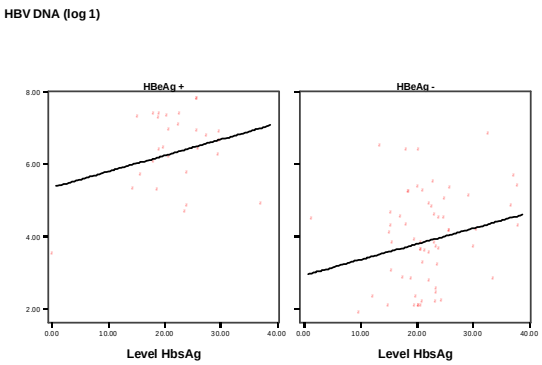

Figure 1. Correlation HBsAg level and HBV DNA level among HBeAg positive and HBeAg negative patients. In group HBeAg positive r = 0.28; p = 0.149, and in group HBeAg negative r = 0.224; p = 0.083

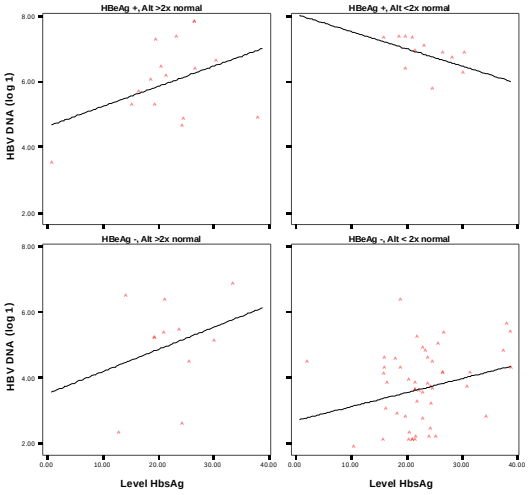

Figure 2. Correlation between HBsAg and serum HBV DNA levels among four different groups

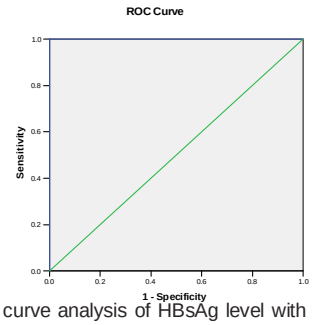

Figure 3. ROC curve analysis of HBsAg level with serum HBV DNA level ≥ 20,000 mL in HBe positive and ALT level > 2 x ULN. The area under the ROC curve of HBsAg for predicting serum HBV DNA level was 1.00 (95% CI = 1.00-1.00; p = 0 .107)

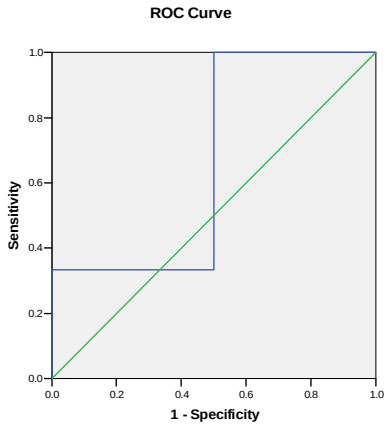

Figure 4. ROC curve analysis of HBsAg level with serum HBV DNA level ≥ 2,000 mL. The area under ROC curve for HBsAg level for predicting serum HBV DNA level was 0.67 (95% CI = 0.17-1.16; p = 0.48)

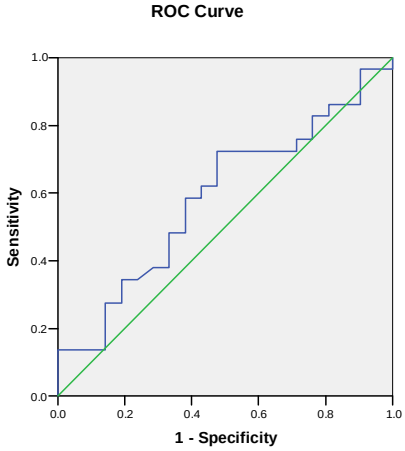

Figure 5. ROC curve analysis of level HBsAg with serum HBV DNA level < 2,000 mL in HBeAg negative group and ALT < 2 x ULN. The area under ROC curve for HBsAg level for predicting serum HBV DNA level was 0.59 (95% CI = 0.43-0.75; p = 0.30)

DISCUSSION

In our study there were weak positive correlation between serum HBsAg level and serum HBV DNA level in both HBeAg positive and HBeAg negative patients. However, it was statistically not significant. Chen et al, found a good correlation between HBsAg and HBV DNA levels in asymptomatic hepatitis B virus carriers. It was found both in HBeAg positive and HBeAg negative patients with normal ALT level.[1] Another study by Kohmoto et al, showed similar result in lamivudine-treated patients. In contrast, a retrospective study by Kuhn et al failed to find a correlation between HBsAg and HBV DNA level in untreated blood donors.[5,6] Yoshikawa et al also found no significant correlation among asymptomatic blood donors in Japan.[6] Galli et al found weak correlations (r = 0.177). The subject were 54 patients with HBeAg negative chronic inactive hepatitis B, 47 patients with HBeAg negative chronic hepatitis B and 12 patients with HBeAg positive chronic hepatitis B.[6]

When we stratified the subject into four groups, there were weak positive correlations between serum HBsAg level and HBV DNA level in group A, C and D patients; however, the correlation was not significant. Chan et al did not find any correlation between serum HBsAg level and HBV DNA level in HBeAg positive patients with high ALT level,[2] which is similar to group A in our study. Lu et al who studied chronic hepatitis B in patients treated with antiviral therapies showed a significant correlation between serum HBV DNA levels and HBsAg levels (29 HBeAg negative patients and 57 HBeAg positive patients). They showed that the correlation still significant at the end of follow-up period.[7]

In group B patients, we found a weak negative correlation between HBsAg level and serum HBV DNA level, but statistically it was not significant. This group is similar to the HBeAg positive and normal ALT level subgroup (16 cases) in Chen's study. They found that mean log HBsAg titer in this group were significantly higher than the other group which had HBeAg negative result with normal ALT and low or high HBV DNA level.[2] Lu et al showed that the HBsAg serum level and intra hepatic cccDNA were significantly lower in HBeAg negative patients than HBeAg positive patients in chronic hepatitis B patients.[7] Our study found no significant differences of HBsAg titers between group A, B, C an D. In group B, patients may be in low immune response stage (immune tolerance). Furthermore, the group B in our study and Chen's study showed relatively younger age patients.

In group C, there was a weak positive correlation but statistically it was not significant. This group is similar to a study conducted by Moucari, i.e. a group of patients with HBeAg negative result and high ALT level.[6] They found that pre-treatment serum HBsAg levels were correlated significantly with baseline serum HBV DNA levels (Spearman rank correlation 0.45).[2]

Group D was similar to a subgroup in Chen's study, the HBeAg-negative patient with normal ALT level. But, unlike the Chen's study, we eliminated patients without countable HBV DNA levels (HBV DNA < 60 IU/mL).[2] In this group, the age was significantly older than HBeAg-positive patients. In contrast, Mahtab et al who studied 88 chronic hepatitis B (CHB) patients aged between 8-22 years old, concluded that HBeAg negative CHB is an entity that can also be found in young population. In subgroup patients with HBeAg negative, they found 38.2% serum ALT level increased (cut-off level: 42 IU/L), 26.5% had high HBV DNA level (> 10^5 copies/mL), 17.6% had significant fibrosis, and necro-inflammation was seen in 56% patients.[8]

In group A, we found 100% sensitivity and specificity in HBeAg-positive patient and high ALT level, at HBsAg cut-off level of 7.91 IU/mL that could predict serum HBV DNA level more than 20,000 IU/mL. Chen et al showed the best cut-off for HBsAg in differentiating group asymptomatic carriers HBeAg positive with high HBV DNA levels (> 2 x 10^7 copies/mL) with other asymptomatic carrier HBeAg negative (HBV DNA level < 10^3, 10^3 to 10^5 and > 10^5 copies/ml) was 15,000 IU/mL, with both sensitivity and specificity 100 %.[1]

In group C, at HBsAg cut-off level of 13.51 IU/mL, the sensitivity and specificity for HBV DNA serum level > 2,000 IU/mL were 100% and 50% respectively. We did not find other study that has similar characteristic to this group. In group D, there were patients with HBeAg-negative result and ALT level < 2 x UNL, and the baseline HBsAg level of 21.38 IU/mL was used for predicting serum HBV DNA level > 2,000 IU/mL, with 72% sensitivity and 62% specificity. Chen et al showed the best cut-off for HBsAg in differentiating group asymptomatic carriers HBeAg negative with low HBVDNA levels (< 10^3 copies/mL; PCR undetectable) to other asymptomatic carriers HBeAg negative with higher HBV DNA level (10^3 to 10^5 and > 10^5 copies/mL) was 1,600 IU/mL with a sensitivity of 69.4% and specificity of 66.7%.[1]

We used reagent Vidas HBsAg ultra (Biomerieux, Lyon, France), which may cause different results in our study. Most authors now use the architect HBsAg assay as utilized in studies conducted by Chen, Chan, Lu and Moucari.[1,2,3,9] Deguchi et al found that Architect HBsAg QT (Abbot Japan Corp) is a reliable, sensitive and specific assay for HBsAg detection and quantification.[10] Chen et al had mentioned in their study that the old-generation assay could not measure HBsAg titer accurately, and therefore, they used the Abbot Architect HBsAg assay.[11] However, Weber et al evaluated Vidas HBsAg Ultra kit for detecting HBsAg in 2006, which was compared to a well-established test (AxSYM HBsAg v2, Abbot diagnostics, Wiesbaden, Germany), and they concluded that Vidas HBsAG Ultra is a highly sensitive and specific tool and represents an improvement for detecting of HBsAg in routine diagnostic laboratories and the utilization of the reagent in such laboratories is to determine quantitative measurement of HBsAg titers.[12,13]

Other main limitations of this study include small sample size and we did not conduct HBsAg and HBV DNA simultaneously. Further study with consideration of these limitations is needed to bring a more conclusive result.

CONCLUSION

We found a weak-positive correlation between HBsAg titers and HBV DNA levels in HBeAg-positive patients, as well as in HBeAg-negative patients. However, it was not significant. There were weak positive correlations between HBsAg level and HBV DNA serum level, for group A, C and D patients, but again, they were not significant. Moreover, in group B, the correlation was negative. In group A, there was good accuracy to predict for high serum HBV DNA level. In other groups, HBsAg was likely did not show good accuracy for serum HBVDNA level.

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