

# Hepatitis B Surface Antigen Serum Titer: Correlation with Hepatitis B Virus DNA and Alanine Aminotransferase Levels Among Hepatitis B e Antigen-Negative Chronic Hepatitis B Patients

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## Abstract

**Background and Aims:** The precise identification of true inactive hepatitis B carrier is difficult due to frequent fluctuations in Hepatitis B virus (HBV) DNA and serum transaminase levels, needing serial determinations for a period of at least one year. Hence we correlated the hepatitis B surface antigen (HBs Ag) titer of untreated Hepatitis B e Antigen (HBe Ag)-negative patients with their corresponding HBV DNA and alanine aminotransferase (ALT) levels, classified these patients as either inactive carrier or patients in the reactivation phase using the American Association for the Study of Liver Diseases (AASLD) guidelines and finally determined if there was a significant difference in HBs Ag titer between these groups.

**Methods:** A cross sectional retrospective study was done. All HBe Ag- negative Chronic hepatitis B (CHB) patients who had their HBs Ag titer, HBV DNA and ALT done at National Kidney and Transplant Institute (NKTi) were obtained and clinical information was abstracted from their case records. A total of 40 patients were included in the study.

**Results:** The mean HBs Ag titer among untreated HBe Ag negative CHB patients was 3037.04 IU/mL (SD +/- 8718.94 IU/mL). Using Spearman's coefficient of correlation, HBs Ag was found to be directly correlated with HBV DNA ( $R = 0.821$ ,  $p = 0 < 0.05$ ) and serum ALT ( $R = 0.654$ ,  $p = 0 < 0.05$ ). Moreover, using Mann Whitney T Test, the mean difference in HBs Ag titer between inactive carrier group (mean 103.72 IU/mL, SD +/- 144.25) and reactivation phase group (mean 5690.99 IU/mL, SD +/- 11517.39) was significant ( $p$  value =  $0 < 0.05$ ).

**Conclusion:** HBs Ag titer was found to be directly correlated with HBV DNA and ALT. To our knowledge, this is the first local study done that supports the concept that HBs Ag titer can provide complementary information in differentiating patient as true inactive carrier from those in the reactivation phase.

**Keywords:** Inactive hepatitis B carrier; Hepatitis B virus; Hepatitis B Reactivation; ccc DNA; HBs Ag quantification

## Introduction

Chronic hepatitis B infection (CHB) is a global health problem affecting more than 350 million people worldwide.<sup>1</sup> Hepatitis B is a major public health problem in the Philippines with an estimated seven million people or around 10-20% of the population chronically infected with the virus.<sup>2</sup> Prolonged and persistent liver inflammation caused by active infection with HBV may result in progression to liver fibrosis, cirrhosis and ultimately hepatocellular carcinoma (HCC) and even death.

During the initial phase of chronic HBV infection, serum HBV DNA levels are high and HBe Ag is present. The majority of carriers eventually lose HBe Ag and develop antibody to HBe Ag (anti-HBe). After spontaneous HBe Ag seroconversion, 67% to

80% of carriers have low or undetectable HBV DNA and normal ALT levels with minimal or no necroinflammation on liver biopsy, this is called the "inactive carrier" state. Roughly 10% to 20% of inactive carriers may have reactivation of HBV replication and exacerbations of hepatitis after years of quiescence, this is characterized by HBV DNA levels >2000 IU/mL and continued necroinflammation in the liver, the so-called "reactivation" state. Therefore, serial testing is necessary to determine if an HBs Ag-positive, HBe Ag-negative carrier is truly in the "inactive carrier state" and lifelong follow-up is required to confirm that the inactive state is maintained.<sup>3</sup>

HBe Ag-negative HBV carriers represent the great majority of cases of HBV infection, having become more prevalent during the last decade because of the aging HBV-infected population.<sup>4</sup> Clinical conditions associated with chronic HBe Ag-negative HBV infection are variable, ranging from inactive carrier status to reactivation chronic HBV infection. The inactive carriers have no or mild liver histological lesions and survival is comparable with the non-infected population, while patients in reactivation has more severe progression

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of the disease with frequent development of cirrhosis.<sup>5</sup> According to the American Association for the Study of Liver Diseases (AASLD) guidelines, the differentiation between the inactive carriers and reactivation phases of HBe Ag-negative CHB is based on an HBV DNA cut-off set up at 2000 IU/ml and an ALT more than the upper normal limit.<sup>3</sup>

Current antiviral treatments may slow disease progression in cirrhotic patients and lead to cure of active chronic HBV infection if identified before the development of cirrhosis.<sup>6</sup> Thus, an early diagnosis of chronic HBV infection may allow earlier consideration of initiating antiviral therapy. However, differentiating inactive chronic HBV infection and reactivation status is problematic because HBe Ag-negative chronic HBV infection is characterized by wide fluctuations in viral replication, with intermittent reductions of HBV DNA serum levels below the inactive carrier cut-off <2,000 IU/mL and spontaneous normalization of ALT. Therefore, to ensure an accurate diagnosis between active and inactive chronic HBV infection, it is mandatory to monitor serum HBV DNA by sensitive and quantitative polymerase chain reaction assays and ALT over a period of at least one year.<sup>7</sup> However, such an approach is costly in resource-constrained countries like the Philippines.

HBs Ag has been used as the hallmark for the diagnosis of HBV infection.<sup>8</sup> The interest in HBs Ag quantification started with the possible observation of its association with the level of covalently closed circular (ccc) DNA, the template for viral replication inside the nuclei of hepatocytes and is a hallmark of infected hepatocytes.<sup>9</sup> The quantification of HBs Ag has been present for quite some time, but initial tests were labor intensive and thus restricted to research setting. Studies with newly available automated quantitative assays recently have shown that serum HBs Ag levels vary significantly during the different phases of chronic HBV infection and are inversely correlated with the immune control of HBV.<sup>10</sup> The higher the immune control, the lower is the HBs Ag level. Recently, HBs Ag serum levels were shown to be directly correlated with intrahepatic ccc DNA levels and more importantly, to HBV replication and overall burden of infection in HBe Ag positive population. HBs Ag serum levels decline during therapy, and is associated with long-term sustained virologic response.<sup>11</sup>

The identification of inactive carrier status is not always straightforward due to wide fluctuations in viral replication and biochemical activity. With the recent correlation of HBs Ag level with disease activity, these findings suggest that quantification of HBs Ag might help to characterize the phase of HBV infection in HBe Ag-negative carriers. Extensive literature search on studies correlating HBs Ag level with HBV DNA titer and ALT level among HBe Ag-negative CHB showed

dearth of local studies. Hence the question remains, can HBs Ag titer of untreated HBe Ag-negative CHB patients be correlated with their corresponding HBV DNA and ALT levels?

Several foreign studies have already correlated HBs Ag level with disease activity. Moreover, these studies have consistently showed that HBs Ag levels were higher in patients with active HBe Ag-negative CHB than in inactive carriers. A longitudinal study made by Chan et al. of Hong Kong reported on 68 HBe Ag-negative CHB patients predominantly infected with genotypes B and C HBV and was followed for over eight years. The researchers concluded that patients with an inactive disease tended to have lower HBs Ag levels than those who had active disease;  $2.24 \pm 1.61$  log IU/ml vs.  $2.98 \pm 0.88$  log IU/ml, respectively ( $p = 0.054$ ).<sup>12</sup> In Taiwan where genotype B and C HBV are prevalent, a cross sectional study published by Su et al. on 251 CHB patients showed that mean serum HBs Ag level was higher in the immune clearance phases (3.81 log IU/ml), significantly decreased in the inactive carrier state (2.25 log IU/ml) and re-elevated in the reactivation phase (2.77 log IU/ml).<sup>13</sup> A prospective cohort study by Brunetto et al. of Italy studied 209 HBV carriers with genotype D. Their findings showed that serum HBs Ag levels were significantly lower in inactive (56 carriers) than in active infection (153 carriers). Moreover, among inactive carriers, HBs Ag serum levels were lower in asymptomatic carriers whose viremia remained persistently below 20,000 IU/ml than in asymptomatic carriers with fluctuations >20,000 IU/ml. They concluded that the combination of a single measurement of HBs Ag <1000 IU/ml and HBV DNA <2000 IU/ml allowed identifying "inactive carriers" with a PPV 87.9% and NPV 96.7%, which is comparable with that of long-term tight monitoring.<sup>14</sup> Thus it can be hypothesized that among untreated HBe Ag-negative CHB patients at NKT, HBs Ag serum titer will be directly correlated with their corresponding HBV DNA and ALT levels.

In addition to the absence of local studies, the prevalence of HBe Ag-negative CHB patients seems to be increasing worldwide, including the Philippines, because of the aging of infected population.<sup>15</sup> The accurate differentiation of inactive HBV carrier and active CHB needs serial determinations of serum ALT and HBV DNA levels and HBs Ag quantification may bridge this gap in accurately identifying "true" inactive carriers. The clinical utility of HBs Ag quantification lies in the management of HBe Ag-negative CHB patients, in order to better identify those that could benefit from therapy and increase survival. Better identification of "true inactive carriers" who have a good survival prognosis and need less frequent monitoring, will reduce the costs of medical expenses.

## Objectives

### General:

To correlate HBs Ag titer of untreated HBe Ag-negative CHB patients at NKTl with their corresponding HBV DNA and ALT levels

### Specific:

- 1) To determine the HBs Ag titer of untreated HBe Ag-negative CHB patients at NKTl
- 2) To classify HBe-Ag-negative CHB patients as either inactive carrier or in the reactivation phase based on their HBV DNA and ALT levels using the AASLD 2009 clinical practice guidelines
- 3) To determine if there is a significant difference in the HBs Ag titer among HBe Ag-negative CHB in the inactive carrier state and reactivation phase

## Methodology

### Research Design

This is a cross sectional retrospective study

### Study Population

Inclusion criteria were as follows:

1. All in-patient and out-patient HBe Ag-negative CHB patients who were tested for HBs Ag titer at NKTl laboratory from 2012-2014
2. HBV DNA and ALT levels done within one month of HBs Ag titer quantification
3. Age 18 years and above

Exclusion criteria were as follows:

1. HBe Ag-negative CHB patients with previous treatment
2. Concurrent Hepatitis C infection
3. Signs of other infection or disease activity, decompensated congestive heart failure, uncontrolled hypertension, uncontrolled diabetes mellitus

### Data Collection

Data were collected from NKTl laboratory from 2012-2014. HBs Ag had been quantified by Elecsys HBs Ag II Quant assay (Roche Diagnostic), which has an automatic on-board dilution with a range of HBs Ag measurement from 0.05 to 52,000 IU/ml. Data from patients who had their HBV DNA and ALT levels determination done within one month of the titer was then collected also from NKTl laboratory. HBV DNA levels was quantified by COBAS TaqMan assay, sensitivity 12 IU/ml with a dynamic range 6.0–1.10 × 10<sup>8</sup> IU/ml, while serum ALT level was detected by commercially available immunoassays with upper normal limit value of 41 U/L. Patient demographics, including comorbidities and maintenance medications

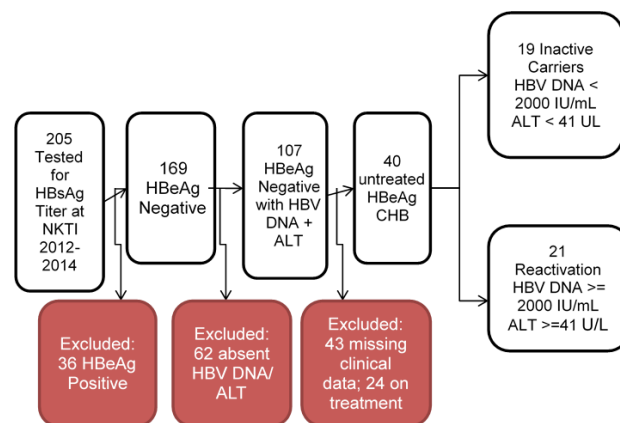


Figure 1: Schematic diagram of data collection

were then obtained by abstraction from their case records.

A total of 205 CHB patients were tested for HBs Ag titer at NKTl from the period of 2012-2014, of which, only 40 (19.51%) satisfied the inclusion/exclusion criteria (Figure 1). Excluded from the study were the following: 36 patients were positive for HBe Ag; 62 patients either did not have HBV DNA/ALT result or had their HBV DNA and ALT done more than one month from HBs Ag titer quantification; 43 patients had incomplete clinical data and 24 patients were already on treatment at the time of testing. Using the AASLD 2009 clinical practice guidelines, patients were classified based on their biochemical and virologic profiles as: (1) inactive carriers have serum HBV DNA levels <2000 IU/mL and normal ALT (less than the upper normal limit); (2) reactivation patients have HBV DNA serum levels >2000 IU/mL and/or elevated ALT (more than the upper normal limit).

During data review, analysis and publication, patient confidentiality and anonymity was maintained at all times. Patient's name and other information not related to the study proper was not disclosed. This was achieved by assigning study codes on each patient record and creating a separate document linking the study code to subject's identifying information such as name and address. The document was locked in a secure location and was made accessible only to the primary investigator.

### Data Processing and Statistical Analysis

All categorical variables such as gender and hepatitis B carrier status were summarized using frequency counts with corresponding percentages. All the other continuous variables such as age, HBs Ag titer, HBV DNA, and ALT levels were summarized using mean and standard deviation. The Spearman coefficient of correlation test were used to calculate the correlations between HBs Ag serum titer, ALT and

HBV DNA. Significant correlation was set at a p-value of less than or equal to 0.05. In order to determine if there is a significant difference in HBs Ag serum titer of those classified as inactive carrier and reactivation, Mann Whitney T test was used. p-value of less than or equal to 0.05 was considered significant. Statistical analysis was performed using SPSS version 20.0 software package.

## Results

A total of 40 (19.51%) untreated HBe Ag-negative CHB patients were included in the study, of whom, 30 (75%) were males and 10 (25%) were females with a mean age of 43.08 years (SD +/- 14.07). Their mean HBs Ag titer was 3,037.04 IU/mL (SD +/- 8718.94 IU/mL). Among these, 19 (47.50%) patients had HBV DNA <2000 IU/mL and ALT <41 U/L and thus were classified as inactive carriers (Figure 2). Among the inactive carriers, 13 (68.42%) were males and six (31.58%) were females with a mean age of 45.58 years (SD +/- 16.66) (Table I). While twenty-one (52.50%) patients had HBV DNA >2,000 IU/mL and ALT >41 U/L and thus were classified as patients in the reactivation phase (Figure 2). Of the patients in the reactivation group, 17 (80.95%) were males and four (19.05%) were females with a mean age of 40.81 years (SD +/- 11.18) (Table I).

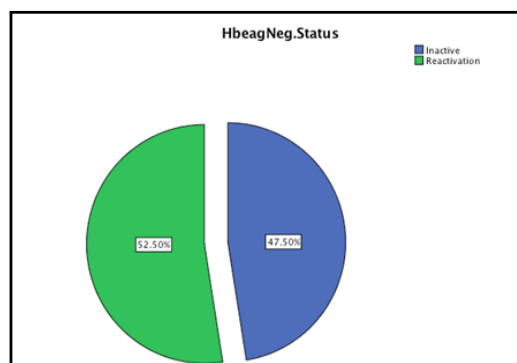


**Figure 2: Proportion of inactive carrier and reactivation phase among HBe Ag Negative CHB**

**Table I: Demographics, HBs Ag, HBV DNA, ALT Serum Levels according to Virologic**

|                          | Inactive Carrier Group | Reactivation Group         |
|--------------------------|------------------------|----------------------------|
| Population (%)           | 19 (47.5%)             | 21 (52.5%)                 |
| Age (SD), years          | 45.58 (+/- 16.66)      | 40.81 (+/- 11.18)          |
| Male/Female (%/%)        | 13/6 (68.42/31.58)     | 17/4 (80.95/19.05)         |
| HBs Ag Titer (SD), IU/mL | 103.72 (+/- 144.25)    | 5690.99 (+/- 11517.39)     |
| HBV DNA (SD), IU/mL      | 206.95 (+/- 388.98)    | 14425697 (+/- 35205369.56) |
| ALT (SD), U/L            | 29.28 (+/- 8.16)       | 101.66 (+/- 78.60)         |

The mean HBs Ag titer in the inactive carrier group was 103.72 IU/mL (SD +/- 144.25), while in the reactivation group, it was 5,690.99 IU/mL (SD +/- 11,517.39). (Table I). The mean HBV DNA level was 206.95 IU/mL (SD +/- 388.98) in the inactive carrier group while it was 14,425,697.24 IU/mL (SD +/- 35,205,369.56) for the reactivation group. The mean ALT level was 29.28 U/L (SD +/- 8.16) for the inactive carrier group while it was 101.66 IU/mL (SD +/- 78.60) for the reactivation group. Using Spearman's coefficient of correlation, HBs Ag titer was found to be directly correlated with HBV DNA ( $R = 0.821$ ,  $p = 0 < 0.05$ ) (Figure 3). HBs Ag titer was also directly correlated with ALT ( $R = 0.654$ ,  $p = 0 < 0.05$ ) (Figure 4). Moreover, using Mann Whitney T Test, the mean difference in HBs Ag titer between inactive carrier group and reactivation was significant ( $p$  value =  $0 < 0.05$ ).

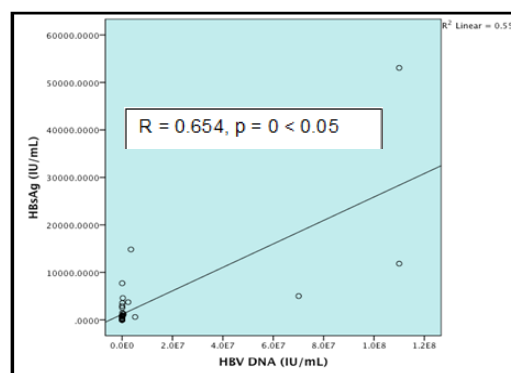


**Figure 3: Correlation of HBs Ag titer with HBV DNA**

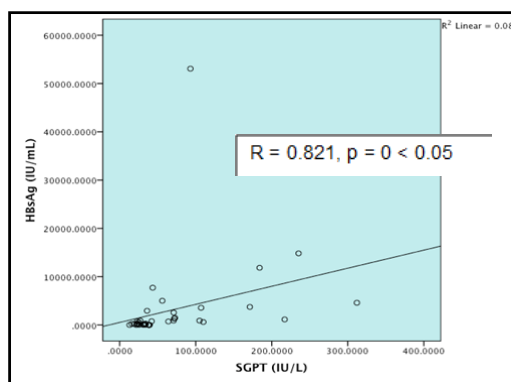


**Figure 4: Correlation of HBs Ag titer with ALT**

## Discussion

Studying retrospectively a group of untreated HBe Ag-negative CHB patients at NKTI, we found that HBs Ag titer was directly correlated with HBV DNA ( $R = 0.821$ ,  $p = 0 < 0.05$ ) and with ALT ( $R = 0.654$ ,  $p = 0 < 0.05$ ). Moreover, HBs Ag level was significantly lower in inactive carrier than those in the reactivation phase (103.72 IU/mL vs 5690.99 IU/

mL,  $p < 0.05$ ). As HBs Ag titer rises, HBV DNA and ALT also proportionately increases. This reflects the close relation of HBs Ag serum levels to ccc DNA, the template for viral replication inside the nuclei of hepatocytes and a hallmark of infected hepatocytes. It was also found to be associated with HBV replication and overall burden of infection. The levels decline during therapy, and is associated with long-term sustained virologic response.<sup>16</sup> However, HBe Ag-negative chronic HBV infection is known to have wide fluctuations in viral replication and biochemical activity, with intermittent reductions of HBV DNA serum levels below the inactive carrier cut-off <2,000 IU/mL and spontaneous normalization of ALT lasting from a few weeks to several months.<sup>7</sup> As was done by Brunetto et al. in their prospective study among genotype D HBV carriers,<sup>14</sup> serial monitoring of HBs Ag with HBV DNA and ALT must be done to further validate this correlation.

Differentiating between inactive carrier and reactivation is fraught with problems due to wide fluctuations in viral replication and biochemical activity, necessitating regular monitoring of HBV DNA and ALT. Brunetto et al., in their prospective cohort study of HBe Ag-negative CHB Genotype D, showed that single point measurement of HBs Ag titer <1,000 IU/mL with HBV DNA <2,000 IU/mL can accurately identify inactive carrier state (94.3% diagnostic accuracy, 91.1% sensitivity, 95.4% specificity).<sup>14</sup> Tseng et al. in their study of CHB genotype B/C, were also able to identify inactive carrier state confidently with HBs Ag titer <100 IU/mL and HBV DNA <2,000 IU/mL (NPV 98.6%; PPV 45.5%).<sup>17</sup> Our study found that HBs Ag level was significantly lower in inactive carrier than those in the reactivation phase (103.72 IU/mL vs 5,690.99 IU/mL,  $p < 0.05$ ). This concurs with the results of several international studies which also report a significant correlation of HBs Ag level with disease activity.<sup>12-14</sup> Difference in HBs Ag level set point for inactive carrier status among the cohorts may be attributable to different HBV genotypes and absence of a uniform criteria in classifying the inactive carriers. HBV genotypes exert a major influence on HBs Ag secretion as suggested by several reports both in vivo and in vitro, where the highest levels have been observed for genotype A.<sup>17</sup> A study among Filipinos by Lontok et al. showed that genotype A is the predominant genotype among Filipinos.<sup>18</sup> A more recent study by Batoctoy et al. showed that genotype A and C are equally predominant among Filipinos.<sup>19</sup> Therefore, a genotype-specific study of HBs Ag level quantification among HBe Ag-negative CHB Filipinos may be needed to further validate the findings of our study. Moreover, our data further strengthens the evidence of using HBs Ag titer in accurately identifying true inactive carriers and is of diagnostic value for

the characterization of the phase of HBV infection as compared with monitoring HBV DNA and ALT only. Several genotype-specific studies already showed that single point HBs Ag quantification combined with HBV DNA provides a very accurate and cost efficient means for virologic characterization.<sup>13,14</sup>

## Conclusion

Among untreated HBe Ag-negative CHB patients at NKTl, the mean HBs Ag titer was 3,037.04 IU/mL (SD +/- 8,718.94 IU/mL). Among patients in the inactive carrier state, the mean HBs Ag titer was 103.72 IU/mL (SD +/- 144.25) while for the reactivation group, the mean HBs Ag titer was 5,690.99 IU/mL (SD +/- 11,517.39 IU/mL). Our study showed that there was a significant direct correlation between HBs Ag titer and HBV DNA ( $R = 0.821$ ,  $p = 0 < 0.05$ ) and ALT ( $R = 0.654$ ,  $p = 0 < 0.05$ ) levels. Furthermore, our finding confirms that there was a significant difference in HBs Ag titer between inactive carrier and reactivation group (103.72 IU/mL vs 5,690.99 IU/mL,  $p < 0.05$ , respectively).

To our knowledge, this is the first local study that supports the concept that HBs Ag titer can provide complementary information in the accurate differentiation of HBe Ag-negative CHB patients as true inactive carrier from those in the reactivation phase.

## Recommendations/Limitations

HBs Ag kinetics is greatly influenced by HBV genotype. To further validate the results of our study and for a generalized use of HBs Ag titer in clinical practice, we therefore recommend conduct of a HBV genotype-specific study among CHB patients at different phases of infection. It is also recommended that a larger study population and a more stringent criteria in classifying HBV carriers by doing serial determination of HBV DNA, ALT and HBs Ag titer (e.g. one year of monthly monitoring) be done to allow a precise characterization of virologic profile and avoid risk of misclassification caused by fluctuations of viral load.

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