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Investigating the Correlation between Semi quantitative HBeAg and HBV DNA Levels: Validating HBeAg Quantification as a Marker in Asymptomatic HBeAg-Positive Carriers

Dr. (Major) Samia Mahmood[1*], Dr. (Major General) Nishat Jubaida[2], Dr. (Colonel) Md. Monirul Hoque[3], Dr. Md. Rezaul Islam[4]

[1]Classified Specialist, Department of Microbiology, Armed Forces Institute of Pathology, Dhaka, Bangladesh
[2]Commandant, Department of Microbiology, Armed Forces Institute of Pathology, Dhaka, Bangladesh
[3]Classified Specialist, Department of Microbiology, Armed Forces Institute of Pathology, Dhaka, Bangladesh
[4]Specialist, Department of Emergency, United Hospital, Dhaka, Bangladesh

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***Corresponding author:** Dr. (Major) Samia Mahmood
Classified Specialist, Department of Microbiology, Armed Forces Institute of Pathology, Dhaka, Bangladesh

Abstract **Original Research Article**

Introduction: Hepatitis B virus (HBV) infection remains a significant global health challenge, annually contributing to approximately one million deaths from complications such as liver failure, cirrhosis, and hepatocellular carcinoma (HCC). ***Methods:*** This observational, descriptive, cross-sectional study was conducted at the Armed Forces Institute of Pathology (AFIP), Dhaka Cantonment, Dhaka, Bangladesh, from 3rd March 2020 to 2nd September 2020. Inclusion criteria required patients to be HBsAg positive for at least six months, resulting in a total of 101 patients included in this study. HBsAg and HBeAg were detected using ELISA, while HBV DNA levels were quantified by real-time PCR. ***Result:*** The study found that 47% of the patients belonged to the 26-45 years age group, with 62% being male and 38% female (male-to-female ratio 1.63:1). Among HBeAg-positive cases, 86.84% had high viral loads (>10^5 copies/ml), while 13.16% exhibited medium viral loads (10^3 – 10^5 copies/ml). Among HBeAg-negative cases, 6.35% showed detectable HBV DNA with low viral loads (<10^2 copies/ml). Higher sample-to-cut-off ratios (S/CO) indicated elevated HBV DNA levels in HBeAg-positive chronic hepatitis B patients compared to lower ratios in HBeAg-negative patients. ***Conclusion:*** Chronic hepatitis B represents a serious viral disease that, without careful monitoring, can lead to the development of HCC and cirrhosis in a significant proportion of patients. Regular surveillance and effective management strategies are essential in mitigating these risks.

Keywords: Chronic hepatitis B, HBsAg, HBeAg, HBV DNA, Real-time PCR.

INTRODUCTION

The hepatitis B virus (HBV) infection has remained a significant public health problem worldwide, accounting for around one million annual deaths from HBV-associated complications of liver failure, cirrhosis and hepatocellular carcinoma (HCC) [1]. It is endemic in Asia, sub-Saharan Africa, the South Pacific Region, Australia, New Zealand and in some populations of South America and the Middle East.[1] Globally over 2 billion people have been infected with HBV and 350 to 400 million are chronic carriers and 15% - 40% of them develop complications. 5-10% of adults and up to 90% of infants will become chronically infected and 75% of these in Asia, where hepatitis B is the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma [2].

According to the World Health Organization report, the prevalence of HBV infection in the South Asian region ranges from 2 to 8%. As a South Asian country Bangladesh is considered as intermediate endemic for hepatitis B infection, where the life risk of acquiring HBV infection is 20% to 60% [3]. Various studies from Bangladesh have shown that HBV is responsible for 31.25% cases of acute hepatitis, 76.3% cases of chronic hepatitis, 61.15% cases of cirrhosis of liver and 33.3% cases of hepatocellular carcinoma [4]. HBV prevalence in our healthy adult population appears to be on the decline. A study carried out by M N Islam in 1984 reported HBV prevalence in healthy Bangladeshi adults to be 7.2% [5]. A similar figure i.e. 7.5% was revealed by another study carried out by Mobin Khan and N Ahmed among healthy adult job seekers in 1996 [6]. In a recent study carried out by Zaman *et al.*, the

figure was dropped to 5.5%.[4] Several factors may have played a role, including introduction of HBV vaccination in the Expanded Program of Immunization (EPI) schedule, public awareness, health and family screening, mandatory screening of blood and blood products before donation, after which vaccination of negative individuals against HBV has increased [7].

The probability of becoming chronically infected is higher in individuals infected perinatally (90%) or during childhood (20-30%) when considering standing before an immature immune system comparing to immune competent individuals infected in adulthood (<1%). Between 15 and 40% of chronically infected individuals may develop severe liver disease and hepatocellular carcinoma, while the remaining becomes inactive carriers [8]. Diagnosis largely depends on laboratory investigations. Routine hepatitis B serology includes tests for the detection of HBsAg, HBeAg and their corresponding antibodies, anti-HBs, anti-HBe and anti-HBc [9]. Following infection, the first virologic marker HBsAg becomes detectable in serum between 8-12 weeks, remains detectable during symptomatic phase of acute hepatitis B and beyond. HBsAg becomes undetectable 1-2 months after the onset of jaundice and then anti-HBs becomes detectable in serum and remains indefinitely thereafter. Persistence of HBsAg beyond six months after acute infection is accepted as evidence of chronic infection [10].

HBeAg is an important biological indicator of virus replication status, a patient's infectivity, and extent of ongoing liver injury. HBe Ag positive result indicates early phase or active HBV infection. Therapeutic intervention with anti-viral agents can suppress HBeAg expression to undetectable levels [11]. The advent of molecular biology-based techniques has added a new dimension to the diagnosis and treatment of patients with chronic HBV infection. Viral load tests to quantify HBV in peripheral blood are currently the most useful and widely used method. High sensitivity molecular assays are clearly important for the diagnosis of HBeAg negative CHB and occult HBV, where viral loads can be quite low. Serum HBV DNA is the most important and reliable marker for monitoring hepatitis B viral replication and its detection and measurement is essential for the diagnosis, decision to treat and subsequent monitoring of the patients [12].

Objectives

General objective:

- To detect and quantify HBV DNA by real time PCR in Chronic HBV carriers.

Specific Objectives:

- To detect serum HBsAg for the diagnosis CHB.
- To detect serum HBeAg to identify the replicating phase of Hepatitis B virus in chronic patients.
- To establish relationship between semi quantitative HBeAg and HBV DNA levels to determine whether quantification of HBeAg could serve as a useful marker in asymptomatic HBeAg-positive carriers.

METHODOLOGY & MATERIALS

This observational, descriptive, cross-sectional study was conducted at the Armed Forces Institute of Pathology (AFIP), Dhaka Cantonment, Dhaka, Bangladesh from 3rd March 2020 to 2nd September 2020. A non-random (convenient) sampling method was employed, with a sample size of 101 participants. The research materials included ELISA materials such as ELISA plates, positive and negative controls, dilution buffer, conjugate, substrate, and stop solution. Additionally, a real-time PCR machine (QIAGEN Rotor-Gene-Q) was used. Data were collected using a preformed structured questionnaire. The main outcome variables were HBV DNA, HBsAg, and HBeAg. Inclusion criteria required patients to be HBsAg positive for at least six months. Exclusion criteria included hepatitis B infection for less than six months, co-infection with HIV, Hepatitis delta virus, or Hepatitis C virus, previous antiviral treatment, hepatocellular carcinoma, chronic hepatitis from other causes, connective tissue disorders, immune compromised patients, and unwilling patients. Ethical clearance was obtained from the Ethical Committee, nominated by the Directorate General of Medical Services (DGMS). Informed consent was obtained from patients or their legal guardians, and patient confidentiality was strictly maintained. Data were collected using a semi-structured questionnaire, patient history sheets, and investigation reports. HBsAg and HBeAg were detected via ELISA from fresh blood samples at AFIP's microbiology laboratory. HBV DNA was detected using real-time PCR as per the QIAGEN Rotor-Gene-Q machine instructions. Samples were excluded if error messages were displayed, no signal was detected with quantitation standards, or if the internal control of a negative sample showed weak or no signal. Demographic data (age, sex, marital status, occupation, past medical history) and clinical data (history of jaundice, needle stick injuries, exposure to infected blood, etc.) were collected. Laboratory results included HBsAg, HBeAg, and HBV DNA levels. Data were analyzed using SPSS version 22, with a p-value of <0.05 considered statistically significant. Quality assurance strategies included collecting and processing samples by the researcher, regular calibration and monitoring of test accuracy and precision, verification of all test system components (assay conditions, reagents, etc.), repeating tests if necessary, and careful evaluation of reports to ensure reliability. Operational definitions for the study included defining hepatitis B infection as a viral infection that attacks the liver, causing acute and chronic disease, and chronic HBV carrier status as the persistence of HBsAg beyond six months after acute HBV infection. PCR was

defined as a technique used to amplify specific DNA segments, generating thousands to millions of copies of a particular sequence.

RESULT

Table 1: Age distribution of the patients (n=101)

Age (years)	Number of Patients	Percent (%)
11-25	23	22.77
26-45	48	47.52
46-60	18	17.82
>60	11	10.89
Total	101	100

Table 1 shows the age distribution of our study patients. Maximum numbers of patients (47%) were between 26-45 years age group, next (22%) was between the age group of 11-25 years.

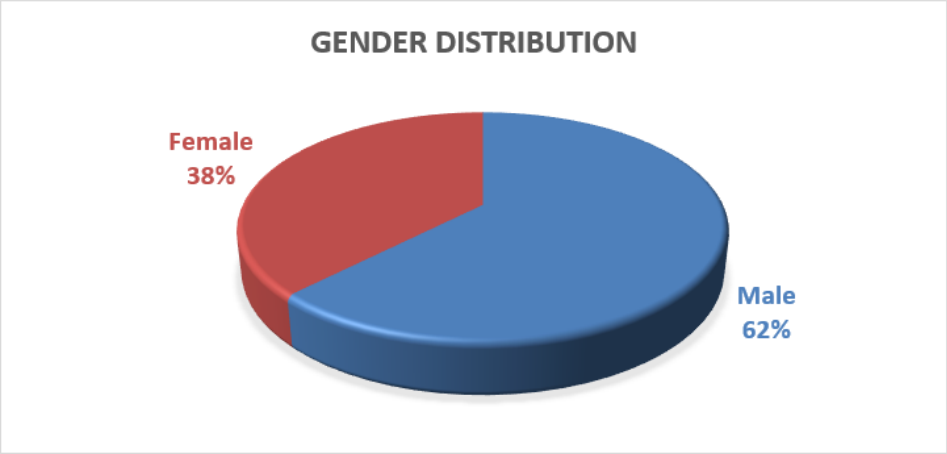

Figure 1: Pie Chart Showing Gender Distribution of Patients (n=100)

Figure 1 shows gender distribution of the patients. Out of 101 cases (62%) cases were male and (38%) were female. Male and female ratio was 1.63:1.

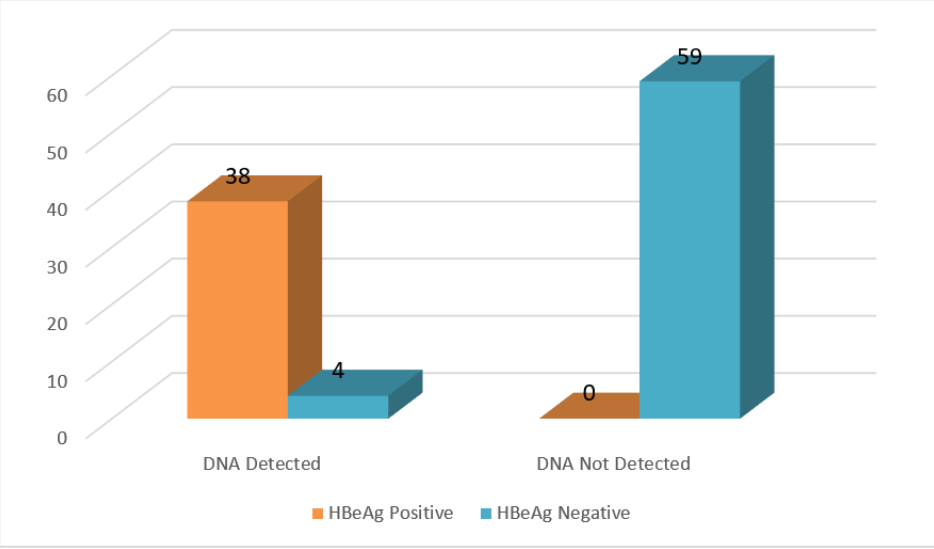

Figure 2: Bar Diagram showing association between HBeAg (ELISA) and HBV DNA (PCR)

Figure 2 shows association between HBeAg (ELISA) and HBV DNA (PCR). Among 101 study population, ELISA for HBeAg showed positive in 38 (37.62%) cases and 63 (62.38%) were HBeAg negative. Out of 101 chronic hepatitis B patients tested by real time PCR, DNA was detected in 42 (41.58%) patients and 59 (58.42%) patients were undetectable. Among 42 PCR positive patients, DNA load were 10^3 -10^5 copies/ml in

cases 05 (13.16%) and above 10^5 copies/ml in 33 (86.84%) cases. Association of HBV DNA status with HBeAg is shown in bar chart. All HBeAg positive cases had detectable DNA. Among 63 HBeAg negative chronic hepatitis B patients only 04 (6.35%) had detectable DNA by real time PCR. Statistically no significant difference between HBeAg and HBV DNA status was found.

Table 2: Association between HBeAg Status and HBV DNA Levels (n = 101).

HBeAg Status	HBV DNA				TOTAL
	LOW	MEDIUM	HIGH	NOT DETECTED	
Positive	00 (0.0)	05 (13.16)	33(86.84)	00 (0.0)	38
Negative	04(6.35)	00 (0.0)	00(0.0)	59(93.65)	63

Table 2 showed the association between HBeAg status and HBV viral load. Majority of the HBeAg positive cases (86.84%) had higher viral load (>10^5 copies/ml) while 13.16% of cases had medium viral load (10^3 -10^5 copies/ml). 6.35% HBeAg negative cases had detected HBV DNA with low viral load (<10^2 copies/ml).

Table 3: Frequency distribution of positive cases of HBeAg and HBV DNA in chronic hepatitis B patients

Parameter	Number of Positive cases	P- Value
HBeAg	38	0.133
HBV DNA	42	

Table 3 shows the distribution of positive cases of HBeAg and HBV DNA in chronic hepatitis B patients. Since P>0.05, so there was no significant difference between HBeAg and HBV DNA positive chronic hepatitis B patients and level of significance was 95%.

Table 4: Correlation among positive cases of HBeAg and HBV DNA in chronic hepatitis B patients

Correlation between	Pearson's correlation Coefficient (r)	Significance
HBeAg & HBV DNA	0.429	Positive correlation

Table 4 shows correlation coefficient (r) between HBeAg & HBV DNA was 0.429 which indicates a positive correlation.

Table 5: Comparison of sample to cut off ratio (S/CO) among HBeAg positive and HBeAg negative chronic hepatitis B patients

HBeAg Positive		HBeAg Negative	
Sample	Log S/CO	Sample	Log S/CO
1.571	1.020	0.096	-0.189
1.966	1.124	0.130	-0.062
2.028	1.130	0.105	-0.154
1.780	1.074	0.058	-0.412

Table 5 shows comparison between sample to cut off ratio in HBeAg positive and negative chronic hepatitis B patients. If S/CO ratio ≥ 1, it indicates high HBV DNA.

DISCUSSION

Hepatitis B Virus (HBV) remains an important cause of acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma in endemic areas. Although the incidence of new infection has decreased after the introduction of vaccination program, HBV infection is still a significant problem in the world. It is estimated that over 350 million persons have chronic hepatitis and more than one million individuals die of HBV-related chronic liver disease annually [1,2]. More than 70% of patients with complications of cirrhosis and hepatocellular carcinoma are negative for hepatitis B e antigen (HBeAg). As such, although the disease may become quiescent in some patients after HBeAg seroconversion, the disease can progress, and most disease related deaths occur in these patients [8]. Early diagnosis and prompt treatment of these patients are crucial to reduce morbidity and mortality. The sensitivity and specificity of different tests tend to show variation in different demographic characteristics or geographical background due to different levels of immunological responses [10]. This study conducted in a tertiary center, showed that there is positive correlation between HBV DNA and HBeAg for appropriate evaluation of chronic hepatitis B carriers.

In the present study, 101 serologically diagnosed CHB patients were enrolled for the detection

of HBeAg by ELISA and quantitation of HBV DNA by Real time PCR method. Among 101 study population 48 patients (48.48%) and 23 patients (23.23%) in the age group of 26-45 years and 11-25 years age group, that is the young adult group. A study carried out in India revealed the analogous result, 84% patients were in the 19-44 years age group [13]. Similar findings were also observed in Dhaka by Shamima *et al.,* and Majid *et al.,* [7,14] 22-38 years were the prevalent age group also found in a study done in Egypt [15]. All these observations support that younger populations are more affected by chronic hepatitis B infection which may be due to their greater exposures and interaction in society as compared to children and aged persons or probably due to the number of patients involved in this study were less than 40 years old. Chathuranga *et al.,* found that there was no significant difference between age and immune response to hepatitis B [16].

The current research observed that among 101 populations group 62% were male and the male female ratio was 1.63:1, that matched with the study of Nurulita *et al.,* [13]. A study by Shamima *et al.,* and Majid *et al.,* also observed male predominance in Bangladesh with a male to female ratio of 4.08:1 and 3.76: 1 respectively [7,14]. This difference could be due to men's lifestyles such as smoking, which can inhibit the response to the hepatitis B vaccine [17].

In the present study, 62.38% cases were found to be negative for HBeAg. Similar HBeAg negative cases were found by Shamima *et al.,* and Majid *et al.,* in Dhaka which were 78.8% and 88.57% respectively [7,14]. Due to mutation in core promoter and precore regions, HBeAg negativity occurs in CHB patients, which decrease or prevent the synthesis of HBeAg but do not impair viral replication [7]. Among 38 HBeAg positive cases, all were found to be positive for HBV DNA by Real time PCR and viral load of these patients were relatively higher ($>10^5$ copies/ml). Similar findings were observed in a study carried out in Brazil in 2009 [18]. In a similar study, out of 50 'HBV DNA PCR positive chronic hepatitis B patients, 48 were positive for HBeAg [19]. In another study, Rabbi, *et al.,* reported that 92.85% 'HBeAg positive' patients were found with active HBV virus replication (HBV DNA) [9].

Among 63 HBeAg negative patients in the present study, 04 (6.35%) had detectable HBV DNA while 59 (93.65%) had undetectable HBV DNA by Real time PCR. A study by Shamima *et al.,* and Majid *et al.,* was also found 31.2% and 33.33% undetectable HBV DNA [7,14]. The presence of circulating HBV DNA in HBeAg negative patients may be due to mutation in the precore region of HBV genome. The most commonly described mutation involves a base substitution in the second last codon of the pre-core genome resulting in the introduction of a stop codon and the failure of synthesis of the HBe protein. However, the replicative competence of the virus remains intact. The presence of serum HBV DNA in these cases was due to temporary reactivation of viral replication, part of the natural course of the chronic carrier state.

The present study observed a higher viral load among HBeAg positive patients 86,84%. Similar finding was observed in study from Bangladesh showing high DNA load 84.6% and 96% HBeAg positive patients compared to HBeAg negative patients [7]. A study from China also showed that HBV DNA levels were high in HBeAg positive patients (83.6%), low in HBeAg negative patients (16.4%). The presence of HBV DNA in HBeAg negative patients may be due to still active HBV replication in HBeAg negative patients [20]. Different results from various studies may be attributed due to the fact in different phases of HBV infection. Correlation between HBeAg levels and HBV DNA levels in chronic hepatitis B patients depended on different phases of HBV infections i.e., the early replicative phase characterized by the presence of HBeAg and high levels of serum HBV DNA and the non-replicative phase which is characterized by loss of HBeAg and marked decrease in serum HBV DNA.

The current study showed a positive correlation between HBeAg and HBV DNA, which was 0.429. Study by Nurulita *et al.,* also found a very strong correlation between HBeAg levels (r = 0.77) against HBV DNA Viral Load [13]. Similarly, study in Dhaka shows correlation coefficient(r) between HBeAg & HBV DNA was 0.591 which indicates a positive correlation [7].

The average log HBeAg (S/CO) values were 1.087($\geq$ 1) and these patients had relatively higher viral loads. Similar results were found in Ping Chen *et al.,* study [21]. However, Patients with detected HBV DNA but negative HBeAg had S/CO ratio <1.

In this study, we examined the correlation between semi quantitative HBeAg and HBV DNA levels in a large number of HBeAg positive HBV carriers. We also investigated whether quantification of HBeAg can indicate the relative HBV DNA level. Another finding was the strong correlation between semi quantitative HBeAg and HBV DNA levels, and this correlation appeared to be tighter at the highest HBeAg levels. Our results suggested that a high HBeAg level effectively indicates the relative HBV DNA level. Our results are consistent with those of previous studies, and a clinical utility of this finding is that we can use high HBeAg levels to estimate corresponding HBV DNA levels without qPCR detection of HBV DNA. This is particularly useful in the setting of rural hospitals where quantitative analysis of HBV DNA levels cannot be performed.

Chronic hepatitis B is usually associated with high morbidity due to improper diagnosis and maltreatment. Majority of patients suffer as primary or

secondary health care centers lack the facilities for early detection of viral replication due to unavailability of molecular assays. In HBeAg negative patients, detection of HBV DNA by RT-PCR is mandatory for the better outcome. This study has showed that there is a positive correlation among HBeAg and HBV DNA in chronic HBV carriers, though there is also some discordance observed which is negligible.

Limitations of the study

The sample size was small; a large-scale study is needed to reach a definitive conclusion. It was a single-center study, conducted only at the Armed Forces Institute of Pathology (AFIP). Therefore, the results may not fully reflect the overall situation of the country, although they align with other studies conducted in similar socio-economic contexts.

CONCLUSION

Chronic hepatitis B is a serious viral disease; in the absence of careful monitoring, it can lead to the development of HCC and cirrhosis in more than one-third of the patients. HBV DNA load varies between the different clinical stages. Hence, patients should be followed up regularly. HBV DNA viral load testing is a crucial tool to monitor and manage CHB patients and to correlate the results with HBeAg. Quantitative PCR assay enables accurate determination of the viral DNA levels in these patients. The ultimate outcome of this study will help many patients and the physicians to diagnose CHB carriers at an early stage, to understand the complex interaction between HBV and infected host as well as to decide the treatment accordingly to prevent further complications.

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Ethical approval: The study was approved by the Institutional Ethics Committee.

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