Hepatitis B Vireamia in Hepatitis B Surface Antigenemic Patients in Bangladesh

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Abstract

Objectives: This study evaluates the distribution of hepatitis B vireamia in patients with hepatitis B virus (HBV) infection. Methods: HBV-infected patients were enrolled in this study. HBV DNA tests were carried out using Smart Cycler II to detect HBV DNA level in serum samples of all HBsAg-positive patients. Results: The distribution of HBV DNA level was found significantly related to age groups (p<0.05), gender group (p<0.05), ALT group (p<0.05), and HBeAg group (p<0.05). The HBV DNA level was recorded to be significantly higher in the HBeAg-positive group (p<0.05) in compared to the HBeAg-negative group. Conclusions: A low level of viral replication may perseverance in chronic HBV-infected patients who are HBeAg-negative, and the level of HBV DNA was higher in the HBeAg-positive group.

Keywords: Hepatitis B, HBV DNA level, HBeAg, ALT

1. Introduction

The hepatitis B virus (HBV) is one of several viruses known to cause hepatitis in humans. Until the 1970s, laboratory tests were not available to distinguish any of these clinically similar infections. HBV is completely unrelated to the viruses that cause hepatitis A, C, or other non-A, non-B (NANB) hepatitis [1].
HBV is usually transmitted by contact with the blood, semen or vaginal secretions of an infected (HBV DNA or HBsAg-positive) person. Because of the high concentration of virus in blood, an extremely small inoculum is sufficient to transmit infection. The virus must be introduced through broken skin or come into contact with mucous membranes for infection to occur. HBV may also be found in saliva and other body fluids [2].

HBV transmission patterns and the seroprevalence of chronic HBV infection vary markedly worldwide, although seroprevalence studies in many countries are limited, and the epidemiology of hepatitis B is changing. Approximately 45% of persons worldwide live in regions in which HBV is highly endemic (i.e., where prevalence of chronic HBV infection is ≥8% among adults and that of resolved or chronic infection [i.e., anti-HBc positivity] is >60%) [3]. Historically, >90% of new infections occurred among infants and young children as the result of perinatal or household transmission during early childhood [4]. Infant immunization programs in many countries have led to marked decreases in incidence and prevalence among younger, vaccinated members of these populations. Countries of intermediate HBV endemicity (i.e., HBsAg prevalence of 2%-7%) account for approximately 43% of the world's population; in these countries, multiple modes of transmission (i.e., perinatal, household, sexual, injection-drug use, and health-care-related) contribute to the infection burden. Regions of the world with high or intermediate prevalence of HBsAg include much of Eastern Europe, Asia, Africa, the Middle East, and the Pacific Islands [3, 5]. In countries of low endemicity (i.e., HBsAg prevalence of <2%), the majority of new infections occur among adolescents and adults and are attributable to sexual and injection-drug-use exposures. However, in certain areas of low HBV endemicity, prevalence of chronic HBV infection is high among indigenous populations born before routine infant immunizations.

Hepatitis B virus (HBV) infection is one of the major causes of chronic liver diseases in the world and also in Bangladesh. HBV causes a broad spectrum of liver diseases ranging from acute self-limited hepatitis to fulminant hepatitis, chronic hepatitis, and asymptomatic infection. It is also one of the main causes of liver cirrhosis and hepatocellular carcinoma (HCC) [6-9]. Infection with hepatitis B virus (HBV) is a major public health problem, affecting about 350-400 million people worldwide. It is endemic in Asia, sub-Saharan Africa, the South Pacific Region, Australia, and New Zealand and in some populations of South America and the Middle East [10, 11]. There are well-known geographical differences in the prevalence of HBV infection. By virtue of its morbidity and mortality HBV infection is of definite concern in the East (Asia-Pacific region) where it reflects an important public heath problem. The progressive of chronic HBV infection vary significantly between the East (prevalence >10%) and the West (prevalence <1%) [12]. The prevalence of HBV infection ranges from 2 to 8%, in the South Asian region according to the report of World Health Organization. As per WHO Bangladesh in the ranked of moderate to high risk group of countries for HBV infection in 2001 [13]. The evolution of chronic hepatitis B completely depends upon the geographic location of the host, age and mode of acquisition of virus and predominant type of virus. In high endemic regions perinatal infection or horizontal infection early in childhood are the main routes of HBV transmission, whereas hepatitis B is primarily a disease of adolescents and adults as a result of high risk sexual activity and injection drug use [14]. In low endemic areas, such as Western countries, The possibility 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 immunocompetent individuals infected in adulthood (<1%) [10,14]. Between 15 and 40% of chronically infected individuals may develop severe liver disease and hepatocellular carcinoma (HCC), while the remaining become inactive carriers [10]. During acute phase almost all the infants and most adults who progress to chronic infection have no symptoms. So, diagnosis largely depends on laboratory investigations. Routine hepatitis B serology includes tests for the
detection of HBsAg, HBeAg, their corresponding antibodies anti-HBs, anti-HBe and anti-HBc (total) and anti-HBcIgM. Subsequent infection with HBV, characteristically HBsAg becomes detectable in serum during the incubation period of 3-5 weeks before emergence of clinical symptoms and after elevation of transaminase level it persists for 2-4 weeks. As the patient recovers it disappears in 2-6 months and after a window period, protective anti-HBs antibody appears. After acute infection the existence of HBsAg beyond six months is accepted as evidence of chronic infection [15].

HBeAg persists detectable for many months and usually for years in chronic hepatitis B virus infection. Detection of HBeAg has modest value in typical cases of acute hepatitis. In acute infection HBeAg generally becomes detectable in the serum when HBsAg first appears but disappears after several weeks when acute hepatitis resolves. On the other hand, HBeAg is an essential marker of viral replication, infectivity and ongoing liver injury in chronic infection. The presence of anti-HBe is associated with likelihood of spontaneous resolution of acute infection and the Antibody to HBeAg appears as HBeAg disappears from the serum. In chronic hepatitis B virus infection the loss of HBeAg and attainment of anti-HBe tends to be associated with biochemical and histological enhancement [16].

For defining the grade of infectivity the clinicians still depend on patients HBeAg/Anti-HBe status and liver enzymes especially alanine aminotransferase (ALT) [17]. ALT flares reflect a high level of virus replication in chronic HBV carriers if they coincide with related clinical, biochemical, serological and histological alterations [18].

During acute phase of infection, anti-HBc of IgM class predominates. Within six months as the infection evolves anti-HBc IgM levels gradually decline and often become undetectable [19].

The detection of HBV DNA and HBV DNA polymerase gives a measure of active viral replication in plasma and is important in detecting HBV infection in sero-negative cases with viremia infected with mutant virus. The value of HBV DNA and HBV DNA polymerase is important in the selection of cases for treatment and in monitoring response to treatment. Currently available methods for detection of HBV DNA are direct hybridization or competitive polymerase chain reaction which is not routinely practiced in most hepatitis testing laboratories [20, 21]. The presence of HBeAg is accepted as a classical indicator of replication, it has been shown that in many populations, especially in Mediterranean region, anti-HBe and HBV DNA can be found positive together at the same time [22, 23, 24]. In a similar fashion, another point mutation at region 1897 induce development of another stop codon (TGA), or the transformation of start codon ATG into ACG hamper the translation of precore region [25]. Finally, during the natural course of HBV infection, the synthesis of HBeAg is interrupted during viral replication as a result of different mutations and only HBV DNA remains as a marker of viremia accompanied with anti-HBe [26].

2. Materials and methods

Blood samples were collected aseptically from 538 HBsAg positive patients prescribed for diagnosis of HBV infection in Private Diagnostic Centre Ltd, Bangladesh. Samples were analyzed to determine the levels of some biochemical parameters including bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST); and to determine the presence of some immunological parameters including HBsAg, HBeAg and Anti-HBe. Dry Biochemistry Random Access Multibatch Analyzer (Vitros 350, J&J, USA) and Vitros TBIL, ALT and AST slides were used to determine total serum bilirubin, ALT and AST levels, consecutively. All the experiments were carried out according to the manufacturer’s instruction (J & J, USA).

To assess the immunological parameters, Multi Batch Immunoassay Analyzer (ARCHITECT i2000SR, Abbott, USA) was used for qualitative determination of HBsAg, HBeAg and anti-HBeAg.

HBV DNA tests were carried out using molecular probe hybridization method (Digene
hybrid capture system) to detect HBV DNA level in serum samples of all HBsAg-positive patients. In probe hybridization method, DNA value >300 virus copies/ml was considered as detectable DNA level in all serum samples.

The HBV DNA level was compared with HBeAg/anti-HBe status and the level of liver enzyme ALT. In this cross sectional study, data analysis was done by using SPSS (Statistical Package of Social Sciences).

3. Results and discussion

In this study, 538 hepatitis B surface antigenemic patients were recruited and evaluated for serological and biochemical profile of patients. The patients’ age ranged from 10 - 65 years with a mean of about 33.05 years. 28.1 % the total (i.e. 151 patients) were found in <25y group followed by ≥41y (24.3% of the total, i.e. 131 patients), 25-30y (19.7% of the total, i.e. 106 patients), 31-35y (17.7% of the total, i.e. 95 patients), and 36-40y (10.2% of the total, i.e. 55 patients) groups respectively, as shown in figure 1.

![Age groups frequency](image)

**Figure 1.** % Frequency of HBV positive cases are classified according to age groups.

In figure 2, row factors and column factors were statistically significant (*p=0.0484 and ***p=0.0003 respectively).

76.4% HBeAg negative patients have highest HBV DNA copies per ml of blood samples.

Column factor analysis found very strong relationship between HBeAg negative and HBeAg positive cases, p value was <0.0001. The results are shown in figure 3.
**Figure 2.** Two-way ANOVA (α=0.05) analysis in age groups and HBV DNA groups of patients showed significant findings. Patients number and highest HBV DNA copies found in <25y age group.

**Figure 3.** Patients are stratified with HBe antigen in association with HBV DNA copies/ml of blood serum. The distribution of HBV DNA copies in two groups found significant by row and column factor analysis in Two-way ANOVA analysis. Figure 4 describe the frequency of respondents varies with different specifications.
Figure 4. Number frequency of respondents varies with different specifications, [A] being with HBV DNA copies (ml) in ALT (IU/L), in [B] with gender, in [C] with gender and HBeAg positivity, in [D] with gender and ALT, in [E] with age and gender and in [F] with gender and HBsAg.

In this study, hepatitis B viremia in hepatitis B surface antigenic patients was investigated and various conditions on sex, age, HbeAg (+/-) and serum ALT were also considered. Two age groups such as <25y and >40y were the dominant respondents in this study and male respondents were found highest frequency.

4. Conclusions

Bangladesh is a developing country with a population of 160 million. Like most advanced countries in Asia, significant increases have been accomplished for containment of different infectious diseases during last 30 years. Hepatitis
B and Hepatitis C Virus infections remain a major health issue in end-stage renal disease (ESRD) population having important effect on overall morbidity as well as mortality. The chronic HBV infection can characteristically start with an acute infection, as indicated by the presence of HBsAg positivity for HBeAg, a marker of ongoing viral replication, higher viral load and heightened infectivity may also develop and remain for months or years [27]. The age at acquiring of HBV has a large impact on the likelihood of the disease becoming chronic. The presence of HBeAg in serum correlates with the presence of viral replication in the liver [29]. Monitoring ALT level is of value in assessing hepatocellular destruction in patients with chronic hepatitis B virus infection [29]. HBeAg-positive patients with or without raised ALT level were found with active HBV virus replication (HBV DNA). This study does not agree with the other study's results [29] that many HBeAg-positive cases with normal ALT level were found infectious with detectable HBV DNA level. In contrast, two HBeAg positive patients had undetectable level of DNA. This might be the limitation of probe hybridization method. Though the probe hybridization assay allows measurement of viral load (quantitative detection), this method cannot measure a very small quantity of HBV DNA value <0.5 pg/ml. Traditionally sero-conversion of HBeAg to anti-HBe coincides with the decrease or normalization of serum ALT concentration and a very low level of HBV replication [30]. But some studies have concluded that presence or absence of HBeAg/anti-HBe may not necessarily reflect the serum HBV DNA concentration, particularly in persistent infection and thus absence of HBeAg and presence of anti-HBe poorly correlates with complete loss of HBV DNA from the serum [31]. Further evaluation of HBeAg/ anti-HBe assays by HBV DNA has been recommended in assessment of possible infectivity and chronic liver disease in the HBsAg positive patients [30]. The distribution of HBV DNA level was found significantly related in age groups (p<0.05), gender group (p<0.05), ALT group (p<0.05), and HBeAg group (p<0.05). The HBV DNA level was documented to be significantly higher in the HBeAg-positive group (p<0.05) in compared to the HBeAg-negative group.

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