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HBeAg/anti-HBe, alanine aminotransferase and HBV DNA levels in HBsAg positive chronic carriers

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Abstract

Serum samples from a total of 72 chronic hepatitis B virus carriers were analysed by serological, biochemical and molecular assays. The aim was to evaluate the relationship of the serological and biochemical parameters with molecular markers in order to assess the infectivity of virus. Out of 72 chronic HBsAg positive carriers, 28 patients were HBeAg positive and anti-HBe negative, 38 patients were HBeAg negative and anti-HBe positive, only 3 patients were positive for both HBeAg and anti-HBe and the rest 3 patients were negative for both markers. Detectable HBV DNA level was found in 92.86% HBsAg-positive/anti-HBe negative patients along with raised alanine aminotransferase (ALT) level (67.86%) compared with HBeAg-negative/anti-HBe positive carriers (36.84%) (p value = 0.02) and out a total of 38 HBeAg-negative/anti-HBe positive carriers, 12 (31.58%) patients had detectable level of HBV DNA. Among the 14 HBeAg-negative/anti-HBe positive patients with elevated ALT level, 8 (57.14%) had detectable HBV DNA whereas out of 24 HBeAg-negative/anti-HBe positive patients with normal ALT level only 4 (16.66%) had detectable HBV DNA level. Significantly high rate of detection of HBV DNA was seen among anti-HBe positive patients with raised ALT level compared with the patients with normal ALT level (p value = 0.01).

Introduction

About 400 million people worldwide have chronic hepatitis B virus (HBV) infection with varying degree of liver damage. There are well-known geographical differences in the prevalence of HBV infection. This is of particular concern in the East (Asia-Pacific region) where it posses an important public health problem by virtue of its morbidity and mortality. The dynamics of chronic HBV infection differ considerably between the East (prevalence >10%) and the West (prevalence <1%)[1]. According to World Health Organization report, the prevalence of HBV infection in the South Asian region ranges from 2 to 8%[2]. In 2001, the WHO ranked Bangladesh in the moderate to high risk group of countries for HBV infection[3].

The evolution of chronic hepatitis B depends upon the geographic location of the host, age and mode of acquisition of virus and predominant type of virus. Early childhood and perinatal transmission of HBV is common in the East whereas adulthood and horizontal transmission predominate in the West[1]. Most healthy adults (90%) who are infected with hepatitis B virus recover and develop protective antibodies against future HBV infections. A smaller number of infected adults (5-10%) become chronically infected with HBV. Unfortunately, 90% of infants and up to 50% of young children infected with HBV can not get rid of the virus and develop a chronic infection[4].

Nearly all infants and most adults who progress to chronic infection have no symptoms during the acute phase. 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-HBc IgM. Following infection with HBV, classically HBsAg becomes detectable in serum during the incubation period of 3-5 weeks before appearance of clinical symptoms and persists for 2-4 weeks after elevation of transaminase level. It disappears in 2-6 months as the patient recovers and after a window period, protective anti-HBs antibody appears. Persistence

of HBsAg beyond six months after acute infection is accepted as evidence of chronic infection[5].

In chronic hepatitis B virus infection, HBeAg may remain detectable for many months and usually for years. In typical cases of acute hepatitis, detection of HBeAg has little value. HBeAg usually becomes detectable in the serum when HBsAg first appears but disappears within several weeks as acute hepatitis resolves. However in chronic infection, HBeAg is an important marker of viral replication, infectivity and ongoing liver injury. Antibody to HBeAg is detectable as HBeAg disappears from the serum and the presence of anti-HBe is associated with likelihood of spontaneous resolution of acute infection. In chronic hepatitis B virus infection the loss of HBeAg and acquisition of anti-HBe tends to be associated with biochemical and histological improvement[6].

Most of the clinicians still depend on patients HBeAg/Anti-HBe status and liver enzymes especially alanine aminotransferase (ALT) for defining the degree of infectivity[7]. ALT flares reflect a high level of virus replication in chronic HBV carriers if they coincide with related clinical, biochemical, serological and histological alterations[8].

During acute phase of infection, anti-HBc of IgM class predominates. As the infection evolves, anti-HBc IgM levels gradually decline and often become undetectable within six months. If anti-HBc IgM is negative, the probability of acute infection in HBsAg-positive cases is nil[9].

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[10, 11].

Although 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[12, 13]. The persistence of viremia despite of anti-HBe sero conversion is explained by mutations at precore region. As an example, G→A mutation at nucleotide 1896 region, induce transformation of codon 28 from TGG (triptophan codon) into TAG (stop codon)[14]. 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[15]. 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[16].

Materials and Methods

Serum samples from 72 known chronic HBsAg carriers were collected and tests were carried out at the Medinova Medical Services Laboratory, Dhaka. Of them 56 males and 16 females between age ranges of 4 to 80 years were included. This wide distribution of the patients by age and gender was due to deliberate selection concerned with serological criteria. In this study, HBsAg-positive, anti-HBc IgM negative and anti-HBc (total) positive cases were considered as chronic HBsAg carriers. All the patients were tested for serological markers, HBeAg and anti-HBe, biochemical parameter ALT level and molecular marker HBV DNA. ALT was done to assess the infectivity of HBV. HBV DNA level was measured to assess viral load of circulating HBV, hence infectivity in serum of chronic hepatitis patients.

In this study, all the serological tests were done by Abott AXYM automated immunoassay analyzer using test kits from Abbott laboratories. Biochemical tests were done by DADE BEHRING Dimension® clinical chemistry system. 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 >0.5 pg/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).

Results

Out of 72 chronic HBsAg carriers, 28 were HBeAg-positive/anti-HBe negative, 38 were HBeAg-negative/anti-HBe positive, 3 patients were HBeAg-positive/anti-HBe positive and 3 were HBeAg-negative/anti-HBe negative (Table I).

Table I. Detectable HBV DNA level in chronic HBV carriers (n=72)

Patient	HBV DNA		
	≥0.5 pg/ml	<0.5 pg/ml	Total
HBeAg-positive/anti-HBe negative	26	2	28
HBeAg-negative/anti-HBe positive	12	26	38
HBeAg-positive/anti-HBe positive	3	0	3
HBeAg-negative/anti-HBe positive	2	1	3

Among 28 patients who were HBeAg-positive and anti-HBe negative, 26 (92.86%) patients had HBV DNA level ≥0.5 pg/ml, and only 2 (7.14%) patients had undetectable HBV DNA level (Table II). Out of 38 patients who were HBeAg-negative and anti-HBe positive, 12 (31.58%) patients had detectable HBV DNA levels. These patients were thought to have precore mutant virus. Remaining 26 (68.42%) patients did not have detectable HBV DNA level (Table II).

Table II. Serum HBV DNA and ALT level in HBeAg-positive /anti-HBe negative and HBeAg-negative/anti-HBe positive patients

Patient	HBV DNA		
	≥0.5 pg/ml	<0.5 pg/ml	Total
HBeAg-positive/anti-HBe negative			
ALT level (>72 U/L)	18	1	19
ALT level (<72 U/L)	8	1	9
HBeAg-negative/anti-HBe positive			
ALT level (>72 U/L)	8	6	14
ALT level (<72 U/L)	4	20	24

All the 3 patients, positive for both of HBeAg and anti-HBe had detectable HBV DNA level. Among the 3 patients who were negative for both of HBeAg and anti-HBe, 2 patients had detectable HBV DNA level and 1 had undetectable HBV DNA level (Table I)

Out of 28 HBeAg-positive/anti-HBe negative patients 19 (67.86%) had raised ALT and 9 (32.14%) had normal ALT level. In case of 38 HBeAg-negative/anti-HBe positive patients 14 (36.84%) were with elevated ALT level and 24 (63.16%) were with normal ALT level. So, elevated ALT levels were found in significantly higher percentage of HBeAg-positive/anti-HBe negative carriers (67.86%) in comparison to HBeAg-negtaive/anti-HBe positive carriers (36.84%) (p value = 0.02).

Total 19 (67.86%) HBeAg-positive/anti-HBe negative carriers were with elevated ALT level among which 18 (94.74%) had detectable HBV DNA. Only one (5.26%) was found without detectable level of HBV DNA. Nine (32.14%) HBeAg-positive/anti-HBe negative carriers had normal ALT level among which 8 (88.89%) were found with detectable HBV DNA level. Only 1 (11.11%) had not detectable level of HBV DNA (Table II).

Among 14 (36.84%) HBeAg-negative/anti-HBe positive patients with elevated ALT level 8 (57.14%) patients had detectable HBV DNA level and 6 (42.86%) patients were without detectable level of HBV DNA, whereas, out of 24 (63.16%) HBeAg-negative/anti-HBe positive patients with normal ALT level only 4 (16.66%) had detectable DNA level and 20 (83.33%) were found without detectable HBV DNA level. Detectable HBV DNA level was significantly more common in HBeAg-negative/anti-HBe positive patients with elevated ALT levels than patients with normal ALT level (p value = 0.01).

All of the 3 HBeAg-positive/anti-HBe positive patients had elevated ALT level and all of them had detectable HBV DNA. Only one HBeAg-negative/anti-HBe negative patient had elevated ALT level and was found with detectable level of HBV DNA. The rest two HBeAg-negative/anti-HBe negative patients had normal ALT level one of whom had detectable level of DNA.

Discussion

The presence of HBeAg in serum correlates with the presence of viral replication in the liver[6]. Monitoring of ALT level is of value in assessing hepatocellular damage in patients with chronic hepatitis B virus infection[5]. In a study, out of total 50 HBV DNA PCR positive hepatitis B virus carriers who had elevated serum ALT level, 48 samples were positive for HBeAg. Based on the results it was recommended that detectable HBeAg should be taken as a surrogate marker for HBV DNA in hepatitis B virus carriers with raised serum ALT in case of non availability of facility to conduct HBV PCR (testing)[5]. But in the present study, out of 28 HBeAg-positive/anti-HBe negative patients, 19 patients were with elevated ALT level and 9 patients were with normal ALT level. Among these 19 HBeAg-positive patients with elevated ALT level 18 patients had detectable HBV DNA, only one escape HBV DNA detection. Out of the 9 HBeAg positive patients with normal ALT, 8 patients had detectable HBV DNA. We found no difference in the rate of detection of HBV DNA in HBeAg-positive patients irrespective of raised or normal ALT level. Thus a total of 26 (92.85%)

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[5] as 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 can not 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[8]. 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[17]. 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[8]. Data from studies suggest presence of HBV DNA in 83-100% of HBeAg-positive/anti-HBe negative carriers and in 26-64% of HBeAg-negative/anti-HBe positive cases[7, 18] and this later group is usually infected with precore mutant strain which is common in Asia and Mediterranean[19, 20]. Detectable level of HBV DNA in 12 (33.33%) HBeAg-negative/anti-HBe positive patients of this study might have precore mutant virus and need DNA sequencing for confirmation but it was not possible.

The presence of HBV DNA in circulation and its correlation with ALT level and anti-HBe indicates an unpredictable relationship[7]. But in a significant number of studies, presence of HBV DNA has been associated with raised ALT level even in presence of anti-HBe[21, 22]. Such a picture has been reflected in this study. 57.14% of anti-HBe positive patients with raised ALT had detectable level of HBV DNA compared with 16.67% with normal ALT. So, all anti-HBe positive patients should be considered infectious irrespective of their ALT status and those with raised ALT should be given special attention regarding infectivity.

Some of HBV infected patients may present with unexpected serological patterns[6]. Low titre of antigens, formation of antigen + antibody immune complexes may be the reason of atypical serum profile of HBeAg-negative/anti-HBe negative patients. In these cases the antigens become undetectable by the available commercial tests[23]. The simultaneous determination of both indicators may be the result of either breakage of immune complexes during experiment and determination of antigens and antibodies separately or less commonly a new infection by a different strain of HBV[24].

From the above discussion, it may be suggested that traditional hepatitis B virus serology results should be evaluated together with serum ALT levels and all HBsAg positive patients who are positive for anti-HBe with elevated ALT levels should be considered infectious until molecular tests for infectivity like PCR, hybridization for detection of HBV DNA is done.

Authors Contribution

FJR: Concept development, analysis of laboratory works and preparation of manuscript.

MKR: Helps in development of objective: relatively cheaper laboratory tests could guide physicians in diagnosis, methodology and result writing.

TS: Helps in data analysis and improvement of manuscript.

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