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Original Article

Detection of Hepatitis B Virus DNA in Chronic HBV Carriers and Correlation with HBeAg

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Abstract

Background: Chronic infection with hepatitis B virus causes a spectrum of diseases ranging from asymptomatic infective state to cirrhosis and hepatocellular carcinoma. The detection and quantification of HBV DNA plays an important role in diagnosing HBV infection as well as monitoring therapeutic responses. **Methods:** The aim of this study was to detect and quantited HBV DNA by real time PCR and to find out correlation HBeAg with HBV DNA in chronic carriers: The present study carried out among 61 previously diagnosed chronic hepatitis B patients. **Results:** The mean age of study population was 29.20 years. Among the 61 cases, 13(21.3%) were HBeAg positive and 48(78.7%) were HBeAg negative. By real time PCR, DNA detected in 45(73.8%) patients and 16(26.2%) patients were undetected. Association of HBeAg and HBV DNA was observed in 13 HBeAg positive cases, where 12(92.3%) had detectable DNA and in 48 HBeAg negative patients, 33(68.8%) had detectable DNA while 15(31.2%) were undetected. The present study observed a higher viral load (10^5copies/ml) among HBeAg positive patients (84.6%) than HBeAg negative patients (12.5%). **Conclusion:** The present study observed that, there was positive correlation among HBeAg and HBV DNA in chronic HBV carriers. However, there was some discordance observed among HBeAg and HBV DNA. Therefore, in addition to HBeAg, HBV DNA should be assessed for appropriate evaluation of CHB carriers.

Key words: Chronic hepatitis; HBeAg; ALT; HBV DNA.

INTRODUCTION

HBV (Hepattis B Virus) infection is highly prevalent worldwide as a cause of morbidity and mortality through its consequences such as acute hepatitis, chronic active or persistent hepatitis, cirrhosis of liver and primary hepatocelluler carcinoma. Globally over 2 billion people have been infected with HBV and 350 to 400 million are chronic carriers. Approximately 15% - 40% of them develop complication, namely cirrhosis of liver or hepatocelluler carcinoma and among them, around 660,000 die annually due to consequences of this infection[1-2]. Of the estimated 50 million new cases of HBV infection diagnosed annually, 5-10% of adults and up to 90% of infants will become chronically infected, 75% of these in Asia, where hepatitis B is the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma[3].

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There are well-known geographical differences in the prevalence of HBV infection. The dynamics of chronic HBV infection differ considerably between the East (Prevalence >10%) and the West (Prevalence <1%)[4]. In most low prevalent areas (Developed countries of America, Western Europe and Australia), HBV infection is acquired mainly during adolescence and mid adulthood and horizontal transmission is predominate, whereas early childhood and perinatal transmission is the main route in high prevalence (Developing countries of Asia, Africa and the Pacific Islands).

According to the World Health Organization report, the prevalence of HBV infection in the South Asian region ranges from 2 to 8%[5]. 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%[6]. Various studies from Bangladesh 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[7]. HBV prevalence in our healthy adult population appears to be on the decline. A study in 1984 reported HBV prevalence in healthy Bangladeshi adults to be 7.2% [8]. A similar figure i.e. 7.5%, was revealed by another study carried out among healthy adult job seekers in 1996[9]. The figure has dropped to 5.5%[7]. Several factors may have played a role, including introduction of HBV vaccination in the Expanded Programme of Immunization (EPI) schedule and equally importantly, public awareness, media-physician partnership, health and family screening, mandatory screening of blood and blood products before donation, after which vaccination of negative individuals against HBV has become a popular exercise.

Most of the healthy adults (90%) who are infected with hepatitis B virus usually recover and develop protective antibodies against further 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 cannot get rid of the virus and develop a chronic infection. Nearly all infants and most of the 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 and their corresponding antibodies, anti-HBs, anti-HBe and anti-HBc[10]. Following infection with HBV, the first virologic marker is HBsAg becomes detectable in serum between 8-12 weeks and 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.

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 become 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[11].

Most of the clinicians still depend on patients HBeAg / anti-HBe status and liver enzymes especially ALT for defining the degree of infectivity[12]. The advent of molecular biology-based techniques has added a new dimension to the diagnosis and treatment of patients with chronic HBV infection[13]. Viral load tests that quantify HBV in peripheral blood i.e serum or plasma are currently the most useful and most widely used. 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 HBV DNA detection and HBV DNA level measurement is essential for the diagnosis, decision to treat and subsequent monitoring of patients[13].

Traditionally seroconversion of HBeAg to anti-HBe coincides with the decrease or normalization of serum ALT concentration and a very low level of HBV replication[14]. But some studies have concluded that presence or absence of HBeAg may not necessarily reflect the serum HBV DNA concentration, particularly in persistent infection and thus absence of HBeAg poorly correlates with complete loss of HBV DNA from the serum. In some studies found that presence of HBV DNA in 83-100% of HBeAg positive carriers and in 26-64% of HBeAg negative cases[12].

So this study has been designed to detect HBeAg by Enzyme-link immunosorbent assay (ELISA) and correlate with HBV DNA by Polymerase Chain Reaction (PCR) method.

MATERIALS AND METHODS

The study was carried out in the Department of Microbiology, Chittagong Medical College and Chevron clinical laboratory, Chittagong, during the period of Jan'12 to Dec'12. Patients were selected from outdoor patients, department of Medicine, Chittagong Medical College Hospital, Chittagong.

Inclusion criteria: Patients who were HBsAg positive for at least 6 months.

Exclusion criteria:

1. Hepatitis B virus infection less than 6 months.
2. Patients co- infected with HIV, Hepatitis delta virus or hepatitis C virus.
3. Patients having previous antiviral treatment.
4. Those with hepatocellular carcinoma.
5. Patients with chronic hepatitis due to other causes.
6. Patient with connective tissue disorder.
7. Immunocompromised patient.

Samples were collected after taking informed consent from patients or his / her legal guardian.

Under all aseptic precaution, about 5 ml of venous blood were collected from the patients in a sterile vacutainer. After separation of serum (Blood centrifuged at 3000 rpm by using micropipette in eppendrope tube) ELISA were done for HBsAg and HBeAg detection and rest of serum were preserved at -70ºC immediately till further use for nucleic acid detection.

RESULTS

Among 61 study population, ELISA for HBeAg showed positive in 13 (21.3%) cases and 48 (78.7%) were HBeAg negative.

Among the total 61 CHB patients tested by real time PCR, DNA was detected in 45 (73.8%) patients and 16 (26.2%) patients were undetectable . Among 45 PCR positive patients, DNA load were 10^3-10^5 copies/ml in 24(53.3%) cases and above 10^5 copies/ml in 17(37.8%) cases. The mean viral load was 2.57 ± 9.96 [$\log_{10}$ (copies/ml)] and ranged between 405-6.27 × 10^8 [$\log_{10}$ (copies/ml)]. Association of HBV DNA status with HBeAg is shown in bar chart. Among 13 HBeAg positive cases, 12 (92.3%) had detectable DNA. Among 48 HBeAg negative CHB patients, 33 (68.8%) had detectable DNA and 15 (31.2%) had undetectable DNA by real time PCR. Statistically no significant difference between HBeAg and HBV DNA status was found (P 0.05).

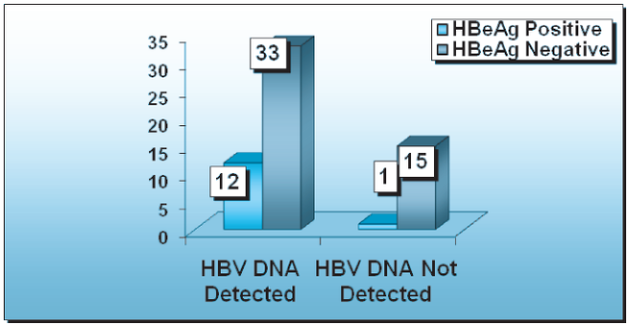

Figure 1 : Bar Chart, Association between HBeAg (ELISA) and HBV DNA (PCR)

Table I showed the association between HBeAg status and HBV viral load. Majority of HBeAg positive cases (84.6%) had higher viral load (10^5 copies/ml) while 47.9% of HBeAg negative cases had medium viral load (10^3-10^5 copies/ml).

Table 1 : Association between HBeAg status and HBV DNA levels (n = 61).

HBeAg Status	HBV DNA Levels				Total
	Low	Medium	High	Not Detected	
Positive	00 (0.0)	01 (7.7)	11 (84.6)	01 (7.7)	13
Negative	04 (8.3)	23 (47.9)	06 (12.5)	15 (31.3)	48

Table 2 shows correlation coefficient(r) between HBeAg & HBV DNA was 0.591 which indicates a positive correlation.

Table 2 : Correlations among HBeAg & HBV DNA in chronic hepatitis B carriers (n = 61).

Correlations Between	Pearson's Correlation Coefficient (r)	Significance
HBeAg & HBV DNA	0.591	Positive Correlation

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 programmes, 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. 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.

In the present study, 61 serologically diagnosed CHB patients were enrolled for the detection of HBeAg and quantitation of HBV DNA by Real time PCR method. The study found a male predominance (Male:female ratio of 4.08:1) among the study population. A study by Raihan (2010) and Majid (2011) also observed male predominance in Bangladesh with a male to female ratio of 4.8: 1 and 3.76: 1 respectively.

The mean age of the study population in the present study was 29.20 ± 9.76 years. Higher prevalence rate (52.4%) was observed in 21-30 years. In Bangladesh similar findings was also observed by Raihan (2010) and Majid (2011) in Dhaka, where mean age ranges were 30.51 ± 9.61 and 32.05 ± 12.99 years respectively. Similarly mean age ranges were also observed 33.1 ± 8.4 in India and 31.47 ± 7.81 in Egypt[15]. All these observations support that younger populations are more affected by CHB infection. It may be due to their greater exposures and interaction in society as compared to children and aged persons.

In the present study, 78.7% cases were found to be negative for HBeAg. Similar HBeAg negative cases were found by Raihan (2010) and Majid (2011) in Dhaka which were 63% and 88.57% respectively. 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.

Among 13 HBeAg positive cases, 12 were found to be positive for HBV DNA by Real time PCR while 1 case had undetectable HBV DNA. Similar finding was reported by Majid (2011) in Bangladesh and Lahiri et al. (2007) in India, they found 1 case and 3 cases which were HBeAg positive but were negative for HBV DNA by PCR respectively. It has been reported that HBeAg may be synthesized and secreted in the absence of an intact virus assembly and this phenomenon may be related to the integration of viral DNA into the host genome with loss of the replication stage. Alternatively, the discordance may have been due to HBV DNA clearance prior to HBeAg clearance, as reported in a follow-up study[16].

Among 48 HBeAg negative patients in the present study, 33(68.8%) had detectable while 15(31.2%) had undetectable HBV DNA by Real time PCR. A study by Majid (2011) was also found 66.67% had detectable HBV DNA and 33.33% undetectable HBV DNA. 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 (84.6%) than HBeAg negative patients (12.5%). Similarly another study from Bangladesh showed high DNA load in 96% HBeAg positive patients compared to HBeAg negative patients[17]. 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 high HBV DNA in HBeAg negative patients may be due to still active HBV replication in HBeAg negative patients (Shao et al. 2007)[18].

CONCLUSION

It may be concluded that, detection of HBeAg and HBV DNA among chronic HBV carriers has positive correlation with some discordance observed among HBeAg and HBV DNA. For appropriate evaluation of CHB carriers, HBV DNA may require independent determination in chronic carriers who show discordance between HBeAg and HBV DNA. However, this study has limited by lack of genotyping for HBV and determining the frequency of precore/core promoter mutation among HBeAg negative patients.

DISCLOSURE

All the authors declared no competing interest.

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