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Original Article

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HBeAg Status among Chronic Hepatitis B Patients at a Referral Hospital of Bangladesh

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Abstract

Background: HBeAg status in chronic hepatitis B patients is important for outcome and treatment **Objective:** The purpose of the present study was to see the status of HBeAg Chronic Hepatitis B (CHB) patients. **Methodology:** This cross sectional study was conducted in the Department of Virology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka between July 2010 to June 2011. Serologically diagnosed CHB patients were enrolled for the study. The HBV DNA was quantified. Samples were tested for HBeAg with ELISA kit. **Results:** A total of 200 serologically diagnosed CHB patients were enrolled for the study. Among the total study population, HBeAg positive CHB patients were 74(37%) cases and HBeAg negative patients were 126 (63%) cases. Among the HBeAg negative patients, viral load was less and patients were significantly older. The mean viral load of HBeAg positive and HBeAg negative was 6.40±2.042 and 2.83±2.55 respectively. HBV DNA was a more reliable indicator of the presence of virus than HBeAg, and was detected in 98.65% (73/74) HBeAg positive carriers, and in 66.67% (84/126) HBeAg negative patients. **Conclusion:** HBeAg negativity is more prevalent among the CHB patients in Bangladesh. [J Shaheed Suhrawardy Med Coll, 2014;6(2):60-63]

Keywords: HBV DNA, CHB, HBeAg, Real time PCR

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Introduction

Hepatitis B virus (HBV) causes a spectrum of liver diseases including acute hepatitis, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Hepatitis B e antigen negative chronic hepatitis (e-CHB) with detectable levels of HBV DNA in serum has been reported in cases from Asia[1]. Globally, over 2 billion people are infected with Hepatitis B virus (HBV) and 370 million people are living with chronic HBV[1]. Among them, around 660,000 die annually due to consequences of this infection[1]. Bangladesh is a densely populated country with intermediate endemicity for chronic hepatitis B (CHB) infection[2]. Studies 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 in Bangladesh[3-5]. The hepatitis B surface antigen (HBsAg) positivity among the healthy adult population of Bangladesh was 7.2%-7.5%[6-7]. Serologic assays for HBV is the mainstay diagnostic tool for HBV infection. However, the advent of molecular biology based techniques has added a new dimension to the diagnosis and treatment of patients with chronic HBV infection[8]. Viral load tests that quantify HBV in peripheral blood like serum or plasma is 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[9].

HBeAg-negative CHB is recognized as an important form of chronic hepatitis, where HBeAg negativity is due to mutations in pre-core and core promoter regions[10]. In HBeAg-negative Asian CHB patients, 45 to 57% have pre-core mutations and 41 to 70% have core promoter mutations[11-12]. Most studies believe that in Asian carriers, precore mutations are not responsible for disease progression[13], whereas, core promoter mutations may have some role in the development of cirrhosis-related complications[13-14]. Around 50% and 70% of patients clear HBeAg within 5 years and 10 years of diagnosis

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respectively[15]. In general, patients who clear HBeAg have a better prognosis than patients who remain HBeAg positive for prolonged periods of time[16]. It was thought that seroconversion from HBeAg to HBeAb is accompanied by cessation of HBV replication and remission of liver disease. The prevalence of HBeAg negative CHB were 39.7 to 51.3% from Bangladesh[17-18], whereas, in Iran, Hong Kong and Korea the prevalence were 65%, 69% and 19.6%, respectively[19,11,20]. In Europe, the prevalence of HBeAg negative variants in patients with CHB was 72% to 90% from France[21], Italy[22] and Greece[23]. Therefore the present study was undertaken to see the status of HBeAg chronic hepatitis B (CHB) patients.

Methodology

This cross sectional study was carried out among chronic HBV infected patient during the period of July 2010 to June 2011 for a period of 1(one) year. All the serologically diagnosed chronic hepatitis B patients at any age with both sexes who were referred to the Department of Virology at Bangabandhu Sheikh Mujib Medical University (BSMMU) were included as study population. Samples were selected by non probability convenience sampling method. Blood were collected using aseptic venipuncture technique. Approximately 5 mL venous blood were collected and placed into EDTA anticoagulant containing tube. The HBV DNA was quantified with a commercially available kit (RoboGene HBV DNA Quantification Kit, Lot no- 009, Germany) according to the manufacturer's instructions. Specimens were tested for HBeAg with a commercially available ELISA kit (Bio-Quant, Inc. UK) according to the manufacturer's instructions. Results were expressed as mean ± standard deviation (SD) or percentage. Statistical analysis of HBV-DNA value was performed after log10 conversion. Fisher's Exact test and Mann-Whitney U test were used for comparison. Statistical analysis was made using SPSS 17.0 software, and p-value of <0.05 considered as significant.

Results

In the present study 200 CHB patients were included. The mean age with SD of the study population was 32.05±12.99 years old with a range of 7 to 65. The male was predominat than female with a ratio of 3.76:1. Among the total study population, 74(37%) cases were HBeAg positive and 126(63%) cases were HBeAg negative CHB patients. HBV DNA levels were 4.15±2.6 by Real time PCR and ALT was 83.63 ± 146.23 U/ml (Table 1).

Table 1: Baseline characteristics of study population (n=200)

Variables	Findings
Age*	32.05±12.99
Gender (male: female)	3.76 : 1
HBeAg	
*Positive	74
*Negative)	126
Serum ALT*	83.63 ± 146.23
HBV DNA load*	4.15 ± 2.93

*Mean±SD

The study populations were divided into 2 age groups of <40 and >40 years. Among all the CHB patients, 153(76.50%) were below 40 years of age. Of them, 63(41.18%) were HBeAg positive and the remaining 90(58.82%) were HBeAg negative. In contrast, 47(23.50%) patients were above 40 years. In this group, the majority 36 (76.60%) were HBeAg negative, while 11 (23.40%) were HBeAg positive. HBeAg positivity rate in younger patients were comparatively higher than older patients which was statistically significant (p<0.05) (Table 2).

Table 2: Relation between age and HBeAg status (n=200)

Age group	HBeAg status		Total	p value
	Positive	Negative		
<40yrs	63(41.18)	90(58.82)	153(76.5)	
>40yrs	11(23.40)	36(76.60)	47 (23.5)	0.037*
Total	**74 (37)**	**116 (63)**	**200 (100)**	

*Fisher's Exact test was done; P<0.05 indicates significant

In this study HBeAg positive CHB patients were 74(37%) cases and HBeAg negative patients were 126(63%) cases. Among all the HBeAg positive and negative patients, mean viral load was 6.40±2.04 [log10 (copies/ml)] and 2.83±2.55 [log10 (copies/ml)] respectively. Viral load of HBeAg positive patients was comparatively higher than HBeAg negative patients and this difference was highly significant (p=0.00). The mean ALT level was 134.35±193.03 (U/L) for HBeAg positive patients and 53.83±99.33 (U/L) for HBeAg negative patients. Overall, HBV DNA and ALT level were significantly higher in HBeAg positive patients (p<0.05) (Table 3).

Table 3: HBV DNA by Real time PCR and ALT levels in relation to HBeAg status (n=200)

Variables	HBeAg status		P value
	Positive	Negative	
HBV DNA	6.40±2.04	2.83±2.55	0.0001
ALT (U/L)	134.35±193.03	53.83±99.33	0.0001

*Data are expressed as Mean±SD; Mann-Whitney U test was done; P<0.05 indicates significant; HBV DNA [log10 (copies/ml)]

Discussions

An estimated 350 million individuals in the world have chronic HBV infection. Although positive for HBsAg, most of them are HBeAg negative. HBeAg positivity is highly prevalent only in younger age groups of HBsAg carriers[24-25]. In current community-based studies from different parts of the world, the prevalence of HBeAg negativity in chronic HBV infection has been found to range between 70% and 100%[26-30]. The loss of HBeAg is usually associated with biochemical and histologic remission of hepatitis and with significant suppression in HBV replication[24-31]. Thus, the great majority of HBeAg-negative subjects have normal ALT levels and undetectable serum HBV DNA by the classic hybridization methods. However, with very sensitive techniques as the polymerase chain reaction (PCR) and the nested PCR assay, residual amounts of HBV DNA can be detected in the serum of most HBeAg-negative subjects[32].

The present study observed a significantly higher age among HBeAg negative patients. Another study shows that, the age of

patients with HBeAg-negative CHB ranges between 40 and 55 years[33]. A study from the Department of Hepatology, BSMMU also was reported similar findings[2]. Previous studies were reported that only a few countries had more HBeAg-negative than HBeAg-positive CHB patients[24]. It is apparent that there is a worldwide increase in the prevalence of HBeAg-negative CHB. In Italy, 41% of patients with CHB were HBeAg negative during the period 1975 to 1985, but in the last decade this increased to 90%[34]. 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[35]. In this present study, prevalence of HBeAg negative cases was 63%. The HBeAg negative CHB was found 88.57% at the Department of Virology at BSMMU in 2010. Studies from the Department of Hepatology, BSMMU found 51.3% HBeAg negative CHB[36] and 39.7% HBeAg negative CHB[37]. These results indicate a significant increase of HBeAg negative CHB among Bangladeshi population. This probably indicates that the majority of HBV positive patients in this region has been infected for a long time and has developed mutations in the pre-core region[38].

The mean viral load of HBeAg positive and HBeAg negative patients in the present study was 6.40±2.04 [log10 (copies/ml)] and 2.83±2.55 [log10 (copies/ml)] respectively. Previous studies show that in comparison to HBeAg positive CHB patients, HBeAg negative patients have lower serum HBV DNA and have more advanced disease as evidenced by liver histology[39]. Similar result was also observed in the present study. A study from Iran showed that HBV DNA levels were higher in HBeAg-positive patients, where 87% patients were negative for HBeAg[40]. In a study from Korea, it was observed that the median serum HBV DNA for HBeAg negative patients was approximately two log lower than HBeAg positive patient, regardless of ALT level[41]. A lower alanine aminotransferase level was reported among HBeAg negative patients from Italy[42]. A 6 years study conducted by the Department of Hepatology, BSMMU was showed that ALT and HBV DNA levels were significantly lower in HBeAg negative subjects[2]. Another study from Bangladesh showed high DNA load in 96% HBeAg positive patients compared to only 54.1% among HBeAg negative patients[43]. In this study, HBV DNA and ALT level were also significantly lower in HBeAg negative patients. Another study was showed that the viral load in HBeAg-positive patients was higher than in HBeAg-negative individual[38]. The mean ALT was significantly higher in HBeAg-positive than in HBeAg-negative patients, which could be due to a higher degree of inflammation.

Conclusion

In conclusion HBeAg negativity is more prevalent among the CHB patients in Bangladesh. Careful evaluation for the histological activity of HBV, functional status of the liver and presence of cirrhosis or hepatocellular carcinoma in HBeAg negative patient may be helpful for their further treatment. Patients who are HBeAg negative have lower viral load and have significantly older. ALT level is raised in patients with detectable HBV DNA. However, this study is limited by lack of determining the frequency of precore/core promoter mutation among HBeAg negative CHB patients. This aspects need to be evaluated further in future studies with large number of CHB patients.

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