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Original Article

Microbiology-Section

Hepatitis B Virus (HBV) Infection in Liver Disease Patients in Mumbai, India with Special Reference to Hepatitis B Surface Antigen (HBsAg) Mutant Detection

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ABSTRACT

Objectives: To determine the prevalence of Hepatitis B Surface antigen (HBsAg) in patients attending the Hepatology Out Patient Department (OPD) of a tertiary care hospital and to compare the routinely used HBsAg detection kit with the mutant detection kit to find out the presence of mutants in a given setting.

Materials and Methods: A cross-sectional study was carried out in adult patients with liver disease attending the Hepatology OPD, of a tertiary care hospital in Mumbai, India. Age, gender and clinical history of the patient were recorded. Blood specimen was tested for HBsAg (Microscreen™ ELISA, Span diagnostics, India) and HBsAg mutants (Hepanostika HBsAg Ultra™ ELISA, Biomerieux, France). The samples with discordant results between these two ELISAs were confirmed by Hepatitis B Virus (HBV) Deoxyribonucleic Acid (DNA) Polymerase Chain Reaction (PCR) (Cobas Taqman™, Roche Molecular Systems, USA).

Results: Seven hundred and eighteen patients were enrolled in the study. The mean age of patients in the study group was 41 years (range 17 to 69 years). Four hundred and ninety seven (69.22%) were males and remaining were females. The prevalence of HBsAg was found to be 17.4%. The positivity amongst the male population was 18.1% which was higher than the female population (15.8%). Of the 718 samples tested, 120 were positive for HBsAg by Microscreen™ ELISA and 132 were positive by Hepanostika HBsAg ultra™. Of the 12 discordant samples, HBV DNA was detected in five samples indicating 0.7% prevalence of mutants.

Conclusion: Hepatitis B is prevalent in liver disease patients. The mutant detecting assay is recommended in set-ups where missing HBsAg in patients would have tremendous impact on the outcome such as in blood donors, organ or tissue donors and antenatal screening of mothers. It is also helpful in chronic liver disease patients where the routine HBsAg detection test is negative and the other causes of chronic liver disease have been ruled out. However, it is not recommended for use in routine diagnostic set-ups where high false positivity would lead to over-diagnosis of the condition.

Keywords: HBV, Liver disease, HBsAg detection, HBsAg mutants

INTRODUCTION

Hepatitis B infection is one of the major global public health problems. It is estimated that more than 240 million people have chronic (long-term) liver infections and about 6,00,000 people die every year due to the acute or chronic consequences of hepatitis B [1].

The hepatitis B infection does not usually require treatment because most adults clear the infection spontaneously [2]. However, treatment of chronic infection may be necessary to reduce the risk of cirrhosis and Hepatocellular Carcinoma (HCC). Individuals who remain (HBsAg) positive for at least six months are considered to be hepatitis B carriers and have a high probability of developing chronic hepatitis B infection [3].

In India, (HBsAg) prevalence in general population ranges from 2% to 8%, placing India in intermediate HBV endemicity zone and the number of HBV carriers is estimated to be 50 million, forming the second largest global pool of chronic HBV infections [4]. For screening of HBV infection, HBsAg detection assays are the most commonly used assays in most of the public hospitals in India. However, HBV mutations have been recently reported which cannot be detected by the commercially available assays for HBsAg detection [5,6]. Failure to detect these mutants in blood donors will result in transfusion transmitted hepatitis B infection and will also delay diagnosis in patients with liver disease due to hepatitis B infection. The mutation rate is 1.4 to 3.2 × 105 substitutions per site per year in chronic infections and nearly 100-fold higher in the liver transplantation setting [7,8].

The present study was hence carried out to find out the prevalence of HBsAg in liver disease patients and to compare the routinely used HBsAg detection kit with the mutant detection kit to find out the presence of mutants in our setting.

MATERIALS AND METHODS

A cross-sectional study was carried out in adult patients with liver disease attending the Hepatology OPD, of a multi-specialty tertiary care hospital in Mumbai, India after obtaining institutional ethics committee approval over a period of one year from June 2009 to May 2010. All the consecutive patients during the study period who were above 18-years of age and gave written informed consent were included in the study. Age, gender and clinical history of the patient were recorded in the case record form after written informed consent. Five ml of blood was collected in a plain vacutainer, and the serum was used to perform HBsAg assay, HBsAg mutant detection and HBV DNA PCR. For HBsAg detection the sample was tested by Microscreen™ ELISA (Span diagnostics, India). For HBsAg mutant detection, samples were tested by Hepanostika HBsAg Ultra™ ELISA (Biomerieux, France). Microscreen assay for HBsAg can detect upto 0.1 ng HBsAg/ ml whereas Hepanostika HBsAg Ultra can detect upto 0.05 ng/ ml.

The samples with discordant results between these two ELISAs were confirmed by HBV DNA by Cobas Taqman™ HBV test (Roche Molecular Systems, USA). All the tests were performed along with internal and external controls following the manufacturer's instructions.

The concordant positive and negative results by the two ELISAs were considered as positive and negative respectively. For the discordant samples the results by DNA PCR were considered as final.

RESULTS

Seven hundred and eighteen patients were enrolled in the study. The prevalence of HBsAg was found to be 17.4%.

The mean age of infected patients in the study group was 43 years (range 17 to 69 years). Four hundred and ninety seven (69.22%) were males and two hundred and twenty one (30.78%) were females. The positivity amongst the male population was 18.1% which was higher than the female population (15.8%) but the difference was not statistically significant (p = 0.5226) [Table/Fig-1].

Of the 718 samples tested, 16.7% were positive for HBsAg by Microscreen™ ELISA and 18.4% were positive by Hepanostika HBsAg™ ultra. [Table/Fig-2] Of the 12 discordant samples, HBV DNA was detected in 5 samples indicating 0.7% prevalence of mutants.

Considering the results of HBV DNA PCR as gold standard the sensitivity, specificity, PPV and NPV of Microscreen™ ELISA was 96%, 100%, 100% and 99.16% respectively. The sensitivity, specificity, PPV and NPV of Hepanostika HBsAg™ ultra was 100%, 98.81%, 94.96% and 100% respectively.

	Number	Positive (%)
Age		
15-30	109	38(34.9)
30-45	310	47(15.2)
≥ 45	299	40(13.4)
Gender		
Male	497	90(18.1)
Female	221	35(15.8)

[Table/Fig-1]: Age and Gender wise distribution of patients

Test (n=718)	Positive	Negative
Microscreen	120	598
Hepanostika HBsAg Ultra	132	586

[Table/Fig-2]: Comparison of ELISA results

DISCUSSION

Viral hepatitis is the most common cause of chronic liver disease throughout the world [9,10]. In India, HBV is reported to be responsible for 70% of chronic hepatitis cases and 80% of cirrhosis of liver cases. About 60% of those cases with HCC are HBV marker positive [11]. HBsAg has been the principal target for laboratory testing to identify active infection by HBV as it is the first serological marker to appear during the course of HBV infection. There have been literature reports of HBsAg being missed by some assays due to the presence of surface mutants [5,6]. This is a cause of great concern as infection can still progress and be transmitted from such patients despite the apparent absence of HBsAg.

The mean age of the infected subjects in the present study was 43 years which is in concordance with a study published by Arora and Mann [12]. Nayak et al., in their study have suggested that 30% of chronic carriers get infected vertically and remaining get infection horizontally from those who got it vertically [13]. The chances of horizontal infection occurring through close contact with carriers, use of unsafe injections, and an association with a number of socio-cultural practices increases with advancing age. Hepatitis B vaccination became available in 1981 and has been included in the Universal Immunization program in India as late as 2007- 2008. This has contributed significantly in reducing the prevalence there after [14]. However in the present study the prevalence of Hepatitis B infection gradually decreased with age from 34.9% in 15-30 years age group to 13.4% in patients older than 45 years of age. This possibly could be due to the other causes of chronic liver disease in older age group [Table/Fig-1].

HBsAg prevalence in men was higher (18.1%) as compared to women (15.8%) in the present study. A similar higher prevalence in men has also been reported by Abdool Karim et al., [15]. Though no specific reasons can be attributed to the higher prevalence in men, it could possibly be due to a higher exposure risk in this population or because more male population seeks health care [16,17].

In our study, 17.4% of patients with liver disease had hepatitis B infection which is similar to the study by Kumar et al., (17.34%) [18]. Other studies in India have reported HBsAg detection rate varying between 12.2-51% in liver disease patients [11,12,18,19]. The difference in the prevalence might be because in these studies various viral markers such as Anti HBc, HBeAg, and antibodies to HBeAg were detected whereas in the present study only HBsAg testing was done. Also the prevalence rate in a given region is dependent on the degree of endemicity in that region. The higher prevalence in the present study as compared to that in the general population is expected since the patients enrolled were liver disease patients attending the Hepatology OPD with majority having symptoms like jaundice, anorexia, nausea, vomiting, and haematemesis.

HBV is a DNA virus; it contains a polymerase that lacks proofreading activity, so error frequencies are comparable to those seen for retroviruses and other RNA viruses. Owing to the low fidelity of the polymerase, the high replication rate, and overlapping reading frames, mutations occur throughout the HBV genome, including the sequence coding for the immunodominant “a” determinant of the viral envelope that produces HBsAg mutants [7,8]. For detecting hepatitis B virus HBV infection the routinely used immunoassays employ capture antibodies often having specificity for epitopes present on the antigenic (a) determinant of the HBsAg and cannot detect the mutants.

Microscreen™ ELISA (Span Diagnostics) has a murine monoclonal antibody capable of recognizing HBsAg. Hepanostika HBsAg™ Ultra (Biomerieux) however has a unique mixture of polyclonal and monoclonal antibodies (human and murine), that are capable of recognizing the various subtypes of HBsAg, including one binding to a previously unidentified epitope outside the major immunodominant region (called the “a” determinant) [20].

The false negativity (96% sensitivity) using Microscreen in the present study may be attributed to two major reasons; presence of mutants that are not detected by the assay and patients receiving antiviral treatment. In the present study, as the patients were not on antiretroviral therapy, lower positivity using Microscreen was most probably due to the presence of mutants. Similar results have also been described in various other studies [21,22]. In situations where Hepatitis B infection is suspected, but the routine HBsAg testing is negative due to the presence of mutants, anti-HBc testing in combination with HBsAg testing is recommended for HBV diagnosis by Moerman et al., [23]. Anti-HBc testing is usually not performed in any public hospitals in India for the routine diagnosis and follow-up of patients with Hepatitis B infection. In the present study also this testing was not performed and is a limitation of the study.

Clinically, conditions favouring outgrowth of mutants include passive immunization with Hepatitis B Immune Globulin (HBIG) of HBV infected individuals, low level persistent HBV replication, reactivation of resolved Hepatitis B, treatment with inhibitors of HBV DNA polymerase, and possibly failed active vaccination of HBV infected individuals [24]. In the present study, of the five patients with a positive HBV DNA result, two had a previous HBsAg positive report with Microscreen, probably indicating either an occult infection or infection with a mutant strain.

Studies in literature have shown that mutants develop spontaneously, although less frequently during the course of a chronic HBV infection [25,26]. It has been reported that one fourth of HBV related chronic liver disease in Asian Indians is attributable to mutant HBV forms [27].

HBV DNA assay is considered as the gold standard for confirmation of doubtful and occult HBV infection where HBsAg in the serum is characteristically absent [17, 28-29]. In the present study, HBV DNA was detected in 5 of the 12 discordant samples giving a 0.7% prevalence of mutants. This is low as compared to a study by Malik et al., who in their study have determined HBV surface, core promoter, precore/core region mutations and genotypes using PCR and direct sequencing [30].

CONCLUSION

Hepatitis B contributes significantly to chronic liver diseases in India. Mutant detection assay is recommended in set-ups where missing HBsAg in patients would have tremendous impact on the outcome such as in blood donors, organ or tissue donors and antenatal screening of mothers. It is also helpful in chronic liver disease patients where the routine HBsAg detection test is negative and the other causes of chronic liver disease have been ruled out. However, it is not recommended in routine diagnostic set-ups where high false positivity would lead to over-diagnosis of the condition.

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