Original Article

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Profile of Hepatitis B 'e' Antigen and Antibodies to Hepatitis B 'e' Antigen in Hepatitis B Seropositive Patients at a Tertiary Care Hospital in Bengaluru, India

K S Rashmi[1], Syeda Misbah-Ul-Khair[2], K L Ravikumar[3]

[1]Associate Professor, Department of Microbiology, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India, [2]Postgraduate Student, Department of Microbiology, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India, [3]Emeritus Professor, Department of Microbiology , Central Research Laboratory, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India

Abstract

Introduction: Hepatitis B virus (HBV) infection is a disease of global public health significance due to its worldwide distribution and potentially life-threatening sequelae. Prognosis and risk of infectivity is related to the hepatitis B 'e' antigen (HBeAg) status of the infected person.

Purpose: In view of the disease burden caused by HBV, the present study was undertaken to assess the seroprevalence of HBeAg and antibody to HBeAg (anti-HBe) in hepatitis B surface antigen (HBsAg) seropositive individuals of both sexes and different age groups.

Materials and Methods: A total of 13560 individuals were screened for HBsAg using electrochemiluminescence immunoassay. HBsAg seropositive individuals were further tested for HBeAg and anti-HBe by enzyme-linked immunofluorescent assay.

Results: HBsAg was tested positive in 2.12% (287/13560) of the total individuals screened. Out of 287 (179 males and 108 females) HBsAg-positive individuals, 37 (12.89%) were HbeAg-positive, 283 (81.1%) were anti-HBe-positive and 16 (5%) were seronegative for both HBeAg and anti-HBe. Among 37 HBeAg-positives, male to female ratio was 1.3:1 (21:16) with higher seropositivity in individuals aged more than 50 years.

Conclusion: HBeAg seroprevalence of 12.89% indicates high infectivity among HBV-infected individuals with higher risk in the advanced age group. The observations in the present study emphasize the need for using various serological markers for diagnosis and screening of HBV infection. Compulsory childhood HBV vaccination programs, massive intervention activities, treatment and awareness programs should be strengthened to control the spread of HBV infection.

Key words: Anti-hepatitis B 'e', Chronic hepatitis, Hepatitis B 'e' antigen, Hepatitis B virus

INTRODUCTION

Hepatitis B virus (HBV) infection is a global health problem and as estimated by the World Health Organization (WHO), approximately 2 billion people have been infected worldwide, with serological evidence of past or present infection with HBV. More than 350 million (5-7% of the world's population) suffer from chronic HBV infection. Around 15-40% of patients infected with HBV will develop life-threatening liver consequences (including cirrhosis, liver failure, and hepatocellular carcinoma) resulting in 600,000-1.2 million deaths per year due to HBV.[1-3]

In the South-East Asia region, the estimated burden of chronic HBV infection is 100 million. HBV is the second most common cause of acute viral hepatitis after HEV in India. With 3.7% point prevalence, that is, over 40 million HBV carriers, India is considered to have an intermediate

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Corresponding Author: Dr. K S Rashmi, D1, Foot prints, Good Earth Malahar, Doddabele Road, Kambipura, Bengaluru - 560 060, Karnataka, India. Phone: +91-9845269790. E-mail: rashminaga05@gmail.com

level of HBV endemicity. Every year, 1 million Indians are at risk for HBV and about 100,000 die from HBV infection.[4] There is no ideal and specific cure for HBV infection. The burden of HBV infection is substantial because of high morbidity and mortality.[5] The silent nature of the disease coupled with significantly untimely death necessitates early, reliable, and affordable method of diagnosis.[6]

The diagnosis, severity and infectivity of HBV infection can be determined by the presence of serological markers such as hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), hepatitis B core antibody (anti-HBc [IgM and IgG]), hepatitis B envelope antigen (HBeAg), hepatitis B envelope antibody (anti-HBe), and hepatitis B viral DNA (HBV DNA). Though both HBsAg-only - and HBsAg-plus, HBeAg-positive persons are infective to others, additional HBeAg positivity indicates high viral replication and infectivity with HBV DNA levels of 10^7-10^9 IU/mL.[7]

The limited data available on the prevalence of HBeAg/anti-HBe positivity among HBV-infected individuals compels the need for determining the presence of HBeAg/anti-HBe, as it signifies the infectivity and prognosis of the HBV infection. Knowledge of the occurrence of HBeAg/anti-HBe seropositivity helps to understand the frequency of highly infective HBV carriers in the given region which in turn helps to design and implement preventive and control measures. Hence, the present study was designed to determine the frequency of HBeAg/anti-HBe seropositivity in hepatitis B individuals in our tertiary care hospital.

MATERIALS AND METHODS

This hospital based descriptive study was conducted over a period of 26 months (January 2012 to March 2014) at KIMS Hospital and Research Centre a 1000-bedded hospital in Bengaluru, India. A total of 13560 blood samples from patients of all age groups were collected after obtaining the informed oral consent from the patients. Briefly, 2-3 ml of blood was collected from each patient using strict aseptic precautions and serum was obtained using standard methods. The samples were screened for HBV infection by detecting HBsAg in serum by electrochemiluminescence immunoassay using Roche diagnostics Elecsys 2010 immunoassay. HBsAg-negative blood samples were excluded from the study. The samples found to be positive for HBsAg after repeated screening, were further tested for the presence of HBeAg/anti-HBe seropositivity by enzyme-linked immuno fluorescent assay using VIDAS HBe/anti-HBe (BIOMERIEUX, SA France).

HBsAg testing: Roche diagnostics Elecsys 2010 immunoassay was used for detection of HBsAg in serum. Roche diagnostics Elecsys 2010 immunoassay is an automated random access multichannel analyzer for immunological tests intended for *in vitro* quantitation of various analytes including HBsAg. Using electrochemiluminescent technology, the Elecsys HBsAg II test uses biotinylated monoclonal anti-HBsAg antibody and polyclonal anti-HBsAg antibodies labeled with ruthenium complex treated with a serum to form a sandwich. This is attached to streptavidin micro-particles which are electrically initiated to produce chemiluminescence which is read by photomultiplier. Results are obtained automatically by the Elecsys software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cut-off value previously obtained by HBsAg calibration. Samples with cut-off index <0.90 are non-reactive and those with ≥1.0 are considered reactive.

HBeAg/Anti-HBe Testing

VIDAS HBe/anti-HBe (BIOMERIEUX, SA France) was used for qualitative detection of HBeAg and anti-HBe in serum. VIDAS HBe/anti-HBe is an automated qualitative test for use on VIDAS family instruments, for detection of HBe/anti-HBe in serum or plasma by enzyme-linked fluorescent assay technique. All the steps are carried out automatically in the instrument. The solid phase receptacle (SPR) serves as a solid phase. After dilution in the instrument, the sample is cycled in and out of SPR. Meanwhile, the HBeAg present in the sample will bind simultaneously to the specific monoclonal antibody fixed to SPR and to another monoclonal specific antibody conjugated with biotin. The presence of biotin is detected by incubation with streptavidin conjugated with alkaline phosphatase. The conjugate enzyme catalyzes the hydrolysis of the substrate into a fluorescent product, whose fluorescence is measured at 450 nm. The intensity of fluorescence is proportional to the concentration of antigen present in the sample. Results are analyzed automatically.

Descriptive statistical analysis was used for the study to calculate the percentage.

RESULTS

Out of 13560 individuals tested during the study period, 287 individuals tested positive for HBsAg with a prevalence of 2.12% (Table 1). In the total of 287 HBsAg individuals tested, 179 were males, and 108 were females with the ratio of 1.6:1.

All HBsAg-positive samples were further tested for HBeAg and anti-HBe.

HBeAg Prevalence

37/287 samples were positive for HBeAg with the prevalence of 12.89% (Table 2). Out of 37 positives, 21 were male (56.8% cases), and 16 were females (43.2% cases) with the ratio of 1.3:1.

Anti-HBe Prevalence

234/287 were seropositive for anti-HBe with a prevalence of 81.5% (Table 2). Out of 234, 148 were males (63.25% cases), and 86 were females (36.75% cases) with a male:female ratio of 1.7:1.

The rate of seropositivity was characterized based on age group, highest HBeAg seroprevalence of 23.52% was found among patients with age >50 years (Figure 1).

DISCUSSION

HBV infection with its associated sequel is a disease of major public health importance, being the 10th leading cause of death globally.[8] Chronic hepatitis B infection constitutes more than 50% of the chronic hepatitis cases in the India. In milieu of a large population, absence of a compulsory national immunization program and increasing burden of infection and liver disease due to HBV, India may soon have the largest HBV infection pool in the world, emphasizing the relevance of its HBV epidemiology not only nationally but also internationally.[9]

Table 1: HBsAg prevalence

Year	Total tested	HBsAg positive
2012	5310	113
2013	6965	147
2014	1285	27
Total	13560	287

HBsAg: Hepatitis B surface antigen

Table 2: Seroprevalence of HBeAg and anti-HBe

Year	HBeAg-positive	Anti-HBe-positive	HBeAg-negative, anti-HBe-negative
2012	14	93	6
2013	16	122	9
2014	7	19	1
Total	37	234	16

HBeAg: Hepatitis B 'e' antigen, HBe: Hepatitis B 'e'

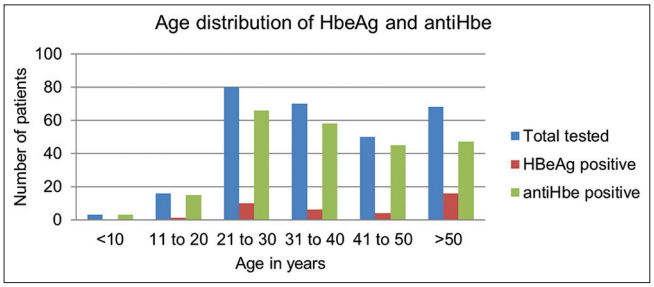

Figure 1: Age wise distribution of seroprevalence of Hepatitis B 'e' antigen and anti-hepatitis B 'e'

With the availability of an effective vaccine for over two decades, the national infant immunization program focuses on blocking mother-to-child transmission of hepatitis B with relatively insufficient attention to older age groups, especially adults.[5,9] Vaccine, though available on request, is recommended only for health care workers and other high-risk groups. HBV transmission has become an important health concern among adults, mainly because of difficulties in risk identification and in program implementation.[5]

In contrast to many other viral infections, chronic HBV infection passes through different phases, each of which is in dynamic equilibrium with the other, determined by a closely integrated interaction between the virus and the host immune system.[9] The first asymptomatic immunotolerant phase characterized by seropositivity of HBsAg and HBeAg with high levels of HBV DNA in the serum. The second immunoactive phase, associated with loss of tolerogenic effect, a decrease in HBV DNA concentration, increased ALT levels and increased histologic activity, reflecting immune-mediated lysis of infected hepatocytes. This phase has a variable duration from months to years. The third inactive carrier state phase is of non-replication stage, which occurs after seroconversion from HBeAg positivity to antibody to HBeAg, a marked reduction of serum HBV DNA levels, normal ALT levels and resolution of liver necroinflammation. Inactive HBsAg carriers form the largest group of chronic HBV infected patients. This phase may last for a lifetime. During this stage, HBV DNA may still be detectable by polymerase chain reaction (PCR) in serum and more often in the liver. In rare cases of immunosuppression, as with cancer chemotherapy or after organ transplantation, HBV can be reactivated with the reappearance of HBeAg and high levels of HBV DNA. The patients with inactive HBsAg carrier state may be grouped into three categories (i) HBeAg-positive and anti-HBe-negative, (ii) HBeAg-negative and anti-HBe-positive and (iii) HBeAg and anti-HBe-negative. Majority of our Indian patients belong to the second group.[10]

The present study was performed to assess the seroprevalence of HBeAg/anti-HBe in HBsAg-seropositive individuals in our hospital.

HBsAg is routinely detected qualitatively in HBV infection. HBsAg is also used as a potential marker for monitoring therapeutic responses. Furthermore, the role of serum HBsAg quantification in distinguishing inactive carriers from the subjects having an active form of the disease has also been implicated.[11] The prevalence of HBsAg in

the general population of Asia, Africa, Southern Europe and South America ranges from 2% to 20%.[11] HBsAg seropositivity of 3-4% is reported in the Indian population. There is wide variation in the prevalence in different regions of the country with the highest prevalence in Andaman and Arunachal Pradesh.[9] Various studies across India reports HBsAg seroprevalence ranging from 1.6% to 5.7% in South India,[5,8,12,13] 1.6-3% in North India[14] and 2.97% in West Bengal.[9] HBsAg seropositivity of 2.12% (287/13,560) was observed in our study.

Detection of HBeAg is of little value in typical cases of acute hepatitis. 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 biomarker of viral replication, infectivity and on-going liver injury.[15]

Further analysis of HBsAg-seropositive samples in the present study revealed 12.89% (37/287) positivity for HBeAg with the higher prevalence in males (56.7%) compared to females (43.2%) in the ratio of 1.3:1. HBeAg seropositivity was also found to be high in patients above 50 years of age. The results are consistent with other studies.[8,16] HBeAg seroprevalence of 15-45% has been observed in other studies from south India.[12,13,17] Higher incidence in older age group may be associated with higher prevalence of acute hepatitis in old age. Compared to younger individuals, adults older than 50 years of age are at 1.5 to the twofold higher risk of having chronic HBV infection with the fourfold higher prevalence of HBeAg seropositivity. Furthermore, physiological changes associated with aging, such as diminished immune response ("immune senescence"), metabolic derangements, nutritional deficiencies, and greater cumulative exposure to environmental hepatotoxins may also contribute to worse outcomes of viral hepatitis in the elderly.[18]

In the natural history of HBV infection, the most important event is HBeAg seroconversion characterized by loss of HBeAg and development of antibody to HBeAg (anti-HBe). This generally occurs years after replicative phase and indicates a transition to a low/non-replicative phase with a potential for resolution of infection and improvement of necro-inflammation in the liver. Age of acquisition of the virus, the immune competence of the host and the strength of immune response to the viral antigens are some of the determinants of timing and efficiency of seroconversion. The prognosis of chronic HBV infection is dependent on the amount of inflammation, necrosis and fibrosis in the liver at this point of seroconversion. If the significant liver damage is already present at this point, then the prognosis after seroconversion, spontaneous or treatment related is unlikely to be good, despite suppression of viral replication. On the other hand, if the seroconversion had occurred early and is maintained, then the long-term prognosis is excellent.[9]

In our study, anti-HBe antibody was found in 81.5% of the study population with higher prevalence in males and patients with age more than 50 years. Several studies have documented 53-90% seroprevalence of anti-HBe antibodies.[17,19,20] We also observed that 5% of HBsAg-positive individuals were seronegative for both HBeAg and anti-HBe antibodies. These patients may be in the early phase of seroconversion.

In a subset of persons, despite anti-HBe positivity, active viral replication persists due to emergence of mutants in the pre-core and basal core promoter regions of HBV. This state, characterized by continuing viral replication despite anti-HBe positivity has been termed as HBeAg-negative hepatitis. Response to anti-viral therapy and outcome of HBeAg-negative hepatitis is different from that of the HBeAg-positive phenotype. It has been increasingly recognized that HBeAg-negative hepatitis is progressively increasing in prevalence globally. In India also, the majority of HBV infected persons are HBeAg-negative, although the exact frequency and prevalence of HBeAg-negative Hepatitis has not been estimated. It would therefore be important to delineate the molecular character, viral load and response to therapy in HBeAg-negative hepatitis in India.[9]

The present work is limited by the scarcity of data regarding the correlation of serological parameters of HBV infection with serum ALT and HBV DNA levels.

CONCLUSION

The HBsAg seroprevalence of 2.12% was observed in our study of whom 12.89% were seropositive for HBeAg, indicating the presence of highly infective HBV transmission pool among HBV-infected individuals. Hence, testing for various serological markers should be done as a primary test to identify persons with chronic HBV infection. All HBsAg-positive patients who are positive for anti-HBe with elevated ALT levels are considered to be infectious until molecular tests for infectivity like PCR, hybridization for detection of HBV DNA are done to rule out viral core promoter mutant forms of chronic HBeAg-negative viral hepatitis.[6,15] Susceptible adults whose HBV markers are all negative should have repeat test or catch-up immunization, especially those individuals who are in high-risk group.[17,18] Multicenter studies involving the seroprevalence of HBeAg, HBV DNA levels and detection

of viral core promoter mutants are recommended to plan and implement appropriate medical care, vaccination and control strategies.

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