Profile of Hepatitis B 'e' antigen and antibodies in Hepatitis B seropositive patients at a tertiary care hospital in Mathura, Uttar Pradesh India.

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Abstract

HBe Ag is a no particulate viral protein and also a marker of active replication in patients infected with Hepatitis B Virus (HBV). Hepatitis B 'e' antigen status is an important factor to know the prognosis and risk of infectivity in person infected with HBV. HBe Ag is an index of viral replication, infectivity, severity of disease and response to antiviral therapy. Seroconversion from HBe Ag positive to HBe Ag negative/anti HBe Ag positive phase usually heralds resolution of infection. Being an important milestone of chronic HBV infection HBe Ag status positive v/s negative is also a determinant of the mode of transmission of the virus. Development of chronic infection, following acute Hepatitis B, requires the expression of HBe Ag. Our aim was to know the seroprevalence of HBe Ag and antibody to HBe Ag (anti-HBe) in hepatitis B surface antigen (HBs Ag) seropositive individuals. 6000 individuals were screened for HBs Ag. Detection of HBe Ag and Anti-HBe antibody was done by Electrochemiluminescence immunoassay (Roche diagnostics) on COBAS e 411immunoassay analyzer. HBe Ag seroprevalence of 13.08 % indicates high infectivity among HBV-infected individuals. Present study emphasizes the need for using various serological markers for diagnosis and screening of HBV infection.

Keywords: Chronic hepatitis, Hepatitis B 'e' antigen, Biomarkers, ECLIA, anti-HBe antibody

Introduction

Despite immunization, Hepatitis B virus (HBV) is still prevalent Worldwide, approximately 20% of every two billion HBV infected cases continue to chronic stages, and causes nearly 900,000 deaths every year [1]. About 1 million Indians are at risk for HBV and about 100,000 die from HBV infection [2]. However, the majority of the infected population are unaware of their condition. It is also a serious threat to blood transfusion safety, especially in Endemic countries [3]. Hepatitis B virus (HBV) can cause acute to chronic liver diseases such as cirrhosis and hepatocellular carcinoma. Although HBs Ag can be detected from samples like urine, bile, tears, sweat, vaginal secretions, cerebrospinal fluid and synovial fluid but serum, saliva, and semen are reported to be infectious [4]. Several HBV biomarkers can be used for HBV diagnosis such as hepatitis B surface antigen (HBs Ag), hepatitis B surface antibody (anti-HB), hepatitis B e-antigen (HBeAg), and hepatitis core antigen (anti-HBc). The quantification of biomarkers level in the body fluids determines the infection level of HBV [5]. The hepatitis B e antigen is a product of the pre –C/C gene that has been found in hepatocytes during proliferation of hepatitis B virus and also an important diagnostic tool to determine the status of ongoing HBV infections [6,7]. Chronic HBV infection may have distinct clinical phenotypes can be divided into five phases. Phase1- High replicate, low infection state (HRLI) characterized by HBe Ag positivity, high viral load, normal or low levels of liver aminotransferases minimal necroinflammatory activity on biopsy. Phase 2- HBe Ag positive chronic hepatitis B phase with fluctuating aminotranferase levels, high HBV DNA and non-inflammatory on liver biopsy [8,9]. This phase sometimes leads to HBe Ag seroconversion. Phase 3- HBe Ag negative phase with low or undetectable levels of HBV DNA and normal aminotransferases. If this phase is prolonged, it can lead to lower rates of progression to cirrhosis and hepatocellular carcinoma (HCC) [10,11]. Phase 4- HBe Ag negative phase and represents a late immune reactive phase, with periodic fluctuating levels of aminotransferases and HBV DNA. HBV Virus may contain nucleotide substitutions in the pre-core or basal core promoter region explaining the lack of HBe Ag expression. Phase 5 is the HBs Ag negative phase where HBs Ag is lost and HBV DNA is usually undetectable in this phase, low levels of HBV DNA can albeit rarely persist in serum and the virus can be detected within the liver [8,9]. Usually HBe Ag can be detected when viral replication is high both in self-limited infections and in chronic hepatitis B; its presence for more than 10 weeks is indicative of a transition to persistant infection. 10HBe Ag can be detected in serum shortly after HBs Ag during acute HBV infections and usually disappears before HBsAg, when alanine aminotransferase (ALT) levels increases, followed by the presence of the corresponding

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antibody (anti-HBe). Therefore HBe Ag test is meaningful in association with the anti-HBe test for monitoring the course of HBV infection and the effect of treatment for chronic hepatitis B [11,12,13]. The Elecsys HBeAg assay uses monoclonal anti-HBe antibodies (mouse) for detection of HBeAg. Data on the prevalence of HBe Ag/anti-HBe positivity is very less, detection of HBe Ag/anti-HBe, should be done at a large scale as it is significant in the infectivity and prognosis of the HBV infection. Beside this, it is helpful 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 such as reduction in transmission, long-term complications and death.

Materials and Methods

This Prospective study was conducted over a period of 2 years (March 2021 to March 2023) at KD Medical College, Hospital and Research Center in Mathura, India. A total of 6000 blood samples were collected after obtaining the informed consent from patients of all age groups.

Inclusion and exclusion criteria- All those patients who were seropositive for HBs Ag were included in the study. Professional blood donors, high risk group like intravenous drug abuser and patients on antiretroviral therapy were excluded in this study.

Collection and storage of samples-3-5 ml of blood was collected from each patient using strict aseptic precautions and serum was separated by using centrifuge machine. These separated samples were stored at -20˚C for further investigation.

Processing of samples- All Serum samples were screened for HBs Ag by one step rapid visual assay HEPACARD manufactured by diagnostic enterprises for the qualitative detection. The method uses monoclonal antibodies immobilized on a nitrocellulose strip in thin line. The samples found to be positive for HBs Ag after repeated screening, were further tested for the presence of HBe Ag/anti-HBe. The Elycis kit was used for detection which is manufactured by Roche diagnostics. It works on the concept of Electrochemiluminescence immunoassay on the COBAS e 411 immunoassay analyzer. Total duration of assay was 18 minutes. In between two times incubation was done automatically. In 1st incubation: HBe antigen from 21 µL sample, a biotinylated monoclonal HBeAg-specific antibody, and a monoclonal HBeAg-specific antibody labeled with a ruthenium complex) form a sandwich complex. In 2nd incubation: After addition of streptavidin-coated micro particles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture was aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances were removed with ProCell II M. Application of a voltage to the electrode then induce chemiluminescent emission which was measured by a photomultiplier. Results were determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration

Calculation:-The analyzer automatically calculates the cutoff based on the measurement of HBEAG Cal1 and HBEAG Cal2. The result of a sample was given either as reactive or non-reactive as well as in the form of a cutoff index (signal sample/cutoff).

Result Interpretation- COI < 1.0 Non-reactive for HBe Ag COI ≥ 1.0 Reactive for HBe Ag.

Anti-HBe antibody was also detected in serum samples by Elycys on COBAS Machine according the kit manufacturers.

Results

Total 6000 individuals were screened during the study period, 382 individuals were detected positive for HBs Ag with a prevalence of 6.33%. Out of these 220 were males and 162 were females with the ratio of 6:4. Table 1, Figure1, Figure 2.

Table 1: *Showing Gender wise distribution of HBs Ag seropositivity among all screened samples.*

Sex	Number of screened sample	HBs Ag positive	Percentage among positive HBs Ag
Male	4290	220 (3.66%)	57.59%
Female	1710	162(2.79%)	42.41%
Total	6000	382 (6.33%)	

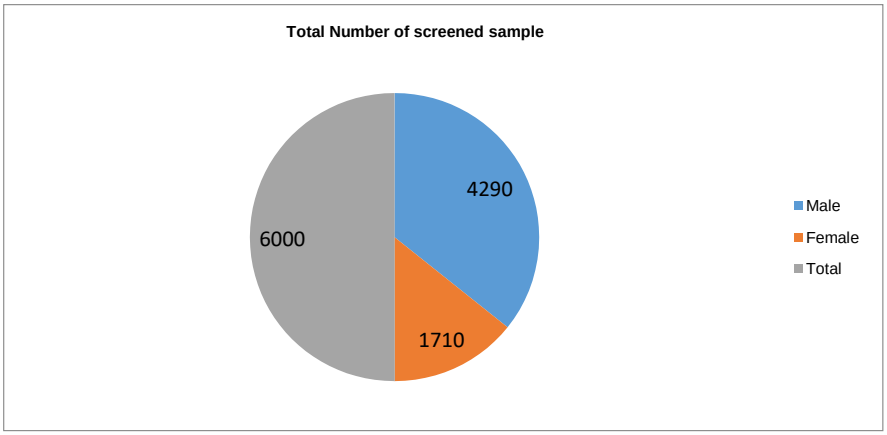

Figure 1: *Gender wise distribution of total screened samples.*

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All HBs Ag-positive samples were further tested for HBe Ag and anti-HBe antibody. Total 50 samples were found positive for HBe Ag with the prevalence of 13.08% (Table 2). Out of 50 positives, 32(64%) were male and 18 (36%) were females with the ratio of 0.8:0.45. 298/382 samples were seropositive for anti-HBe antibody with a prevalence of (78%).Out of 298, 168 (56.37%) were males and 130 (43.62%) were females with a male: female ratio of 4:3. (Table 2), Figure 3. The rate of seropositivity was characterized based on age group, highest HBs Ag seroprevalence of 36.64% was found among patients with age >50 years Table 3, Figure 4. On Department wise distribution highest sample were seropositive from Medicine Department (49.7%) followed by Surgery Department (20.94%). Table4, Figure 5

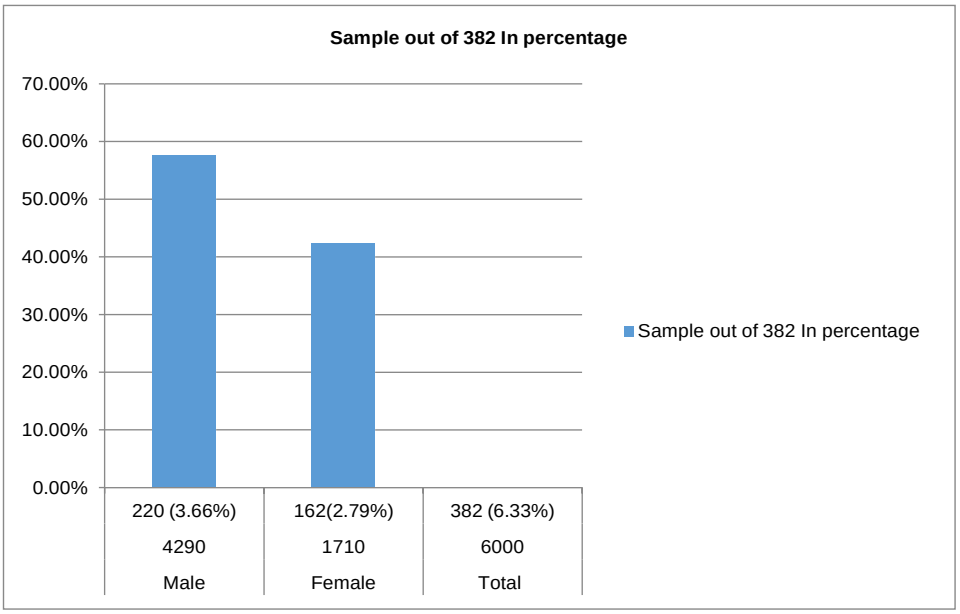

***Figure 2**: Gender wise distribution of HBs Ag Seropositivity among all screened samples.*

***Table 2:** Showing Seropositivity of HBe Ag and anti HBe antibody among HBs Ag Seropositive Individuals.*

	Total Positive Sample For HBs Ag	Positive samples for HBe Ag	Positive sample for anti HBe antibody	Seronegative For both HBe Ag and anti-HBe antibody
Male	220	32 (8.37%)	168 (43.97%)	20 (5.24%)
Female	162	18 (4.72%)	130 (34.03%)	14 (3.67%)
Total	382	50 (13.09%)	298 (78%)	34 (8.91%)

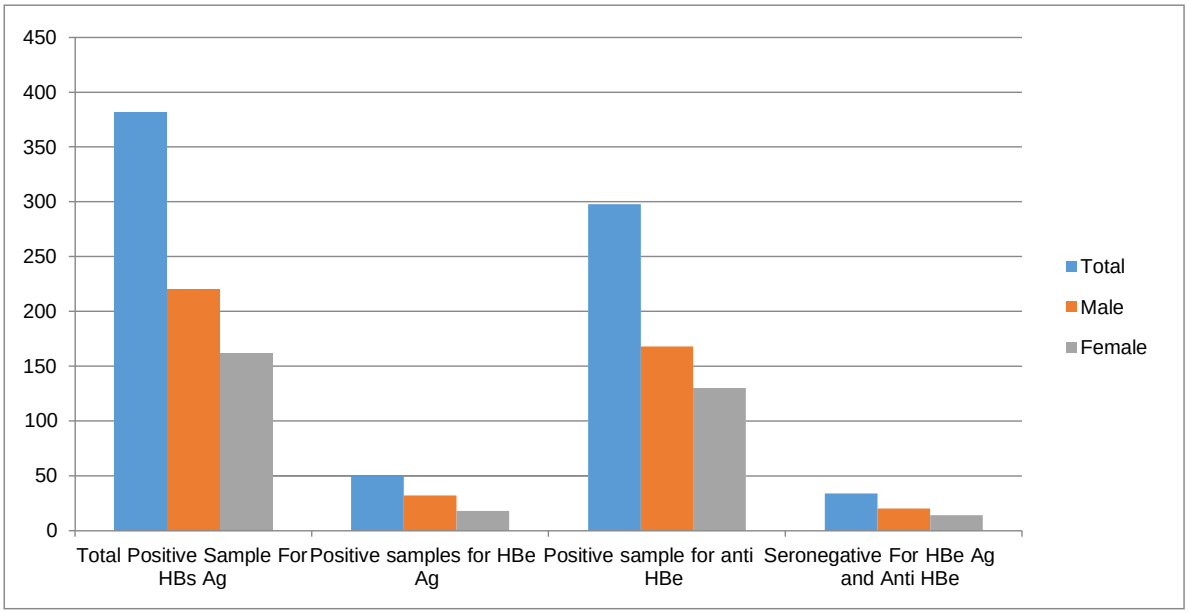

***Figure 3:** Seropositivity of HBe Ag and anti HBe among HBs Ag Seropositive Individuals.*

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Table 3: *Age wise Distribution of HBs Ag Seropositive Individuals.*

Age Group in years	Frequency	Prevalence of HBs Ag positivity
0-10	02	0.5%
11-20	18	4.7%
21-30	30	7.85%
31-40	45	11.78%
41-50	140	36.64%
51-60	110	28.79%
61-70	25	6.54%
71-80	08	2.09%
81-90	04	1.04%

Showing highest infectivity of Hepatitis B Virus in age group 41-50 years with a frequency rate 140 (36.64%) followed by 51-60, 110 (28.79%) and 31-40 yrs 45(11.78%).

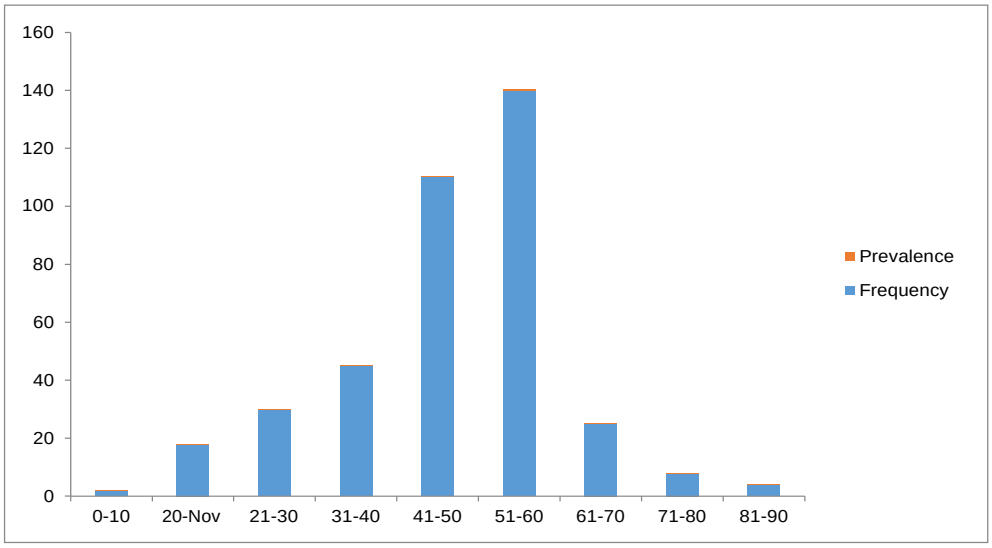

Figure 4: *Age wise Distribution of HBs Ag Seropositive Individuals.*

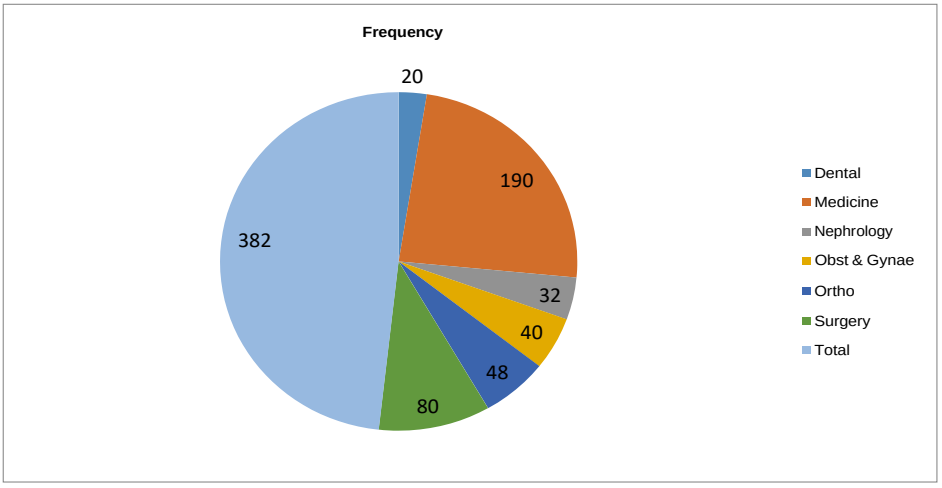

Figure 5: *Department wise distribution of HBs Ag Seropositive Sample.*

Table 4*: Department wise distribution of HBs Ag Seropositive Sample.*

Wards/Department	Frequency	Percentage
Dental	20	5.23%
Medicine	190	49.7%
Nephrology	32	8.37%
Obst & Gynae	40	10.47%
Ortho	48	12.56%
Surgery	80	20.94%
Total	382	

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Discussion

Present study was performed to assess the seroprevalence of HBeAg/anti-HBe in HBsAg-seropositive individuals. Totally 6.33% HBs Ag seroprevalence was observed out of these 13.08% were found seropositive for HBe Ag. It may be an indication of presence of highly infective and replicative phase among HBV-infected individuals [14,15]. Some studies have shown that HBe Ag is a biomarker of active viral proliferation in hepatocytes, infectivity, and transmission and is associated with an increased risk of hepatocellular carcinoma.16It has been declared in some studies that HBe Ag Expression also determines whether acute HBV infection develops into a chronic infection, thus it is necessary to prerequisite that the strains of HBV infecting an individual express HBe Ag [17,18,19]. Therefore, testing for the HBe Ag can aid in identifying individuals with a high risk of developing liver cancer and in planning patient management. Several factors which are associated with an increased risk of advanced liver diseases for patients with chronic hepatitis B (CHB) have been identified. These include age, male gender, repeated episodes of severe acute exacerbation, and HBV reactivation after HBe Ag seroconversion [20, 23]. Present study declares 13.08% (50/382) positivity for HBeAg with the higher prevalence in males (64%) compared to females (36%) in the ratio of 1.7:1. HBeAg seropositivity was found to be high in patients above 50 years of age. The results are consistent with other studies [21, 22, 26]. Many physiological changes have been found associated with age, such as diminished immune response, metabolic derangements, nutritional deficiencies and greater cumulative exposure to environmental hepatotoxins may also contribute to worse outcomes of viral hepatitis in the elderly.23, 24 In this study, anti-HBe antibody was found in 78% with higher prevalence in males and patients with age more than 40 years. Several studies with 53-90% seroprevalence of anti-HBe antibodies have been documented. HBe Ag seroconversion to anti-HBe suggests the end of active viral replication and is therefore associated with clinical resolution (self –limited) or remission (chronic disease), marking a transition from the immune active phase of the disease to the inactive carrier state [25, 26]. We observed that 8.9 % of HBsAg positive individuals were seronegative for both HBe Ag and anti-HBe antibodies. This type of situation may be seen in the early phase of seroconversion. Hepatitis B virus infection can occur without detectable HBe Ag due to infection with HBV variants containing precore stop codon mutants; while the virus can no larger produce HBeAg, disease activity is ongoing and anti-HBe may be present. In India, the majority of HBV infected persons are HBe Ag-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 HBe Ag-negative hepatitis B [27, 28]. In comparison with other studies, present work remains limited by the inadequacy of data in regard to the correlation of serological parameters of HBV infection with serum ALT and HBV DNA levels [29,18]. At last in this study HBV infected group was divided on the basis of several medical departments and the highest HBV prevalent rate was found from department of medicine (49.7%) followed by surgery (20.94%). Such type of distribution couldn't be found in any other study, this may be due to its irrelevancy to the HBV infections. In view 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 [31]. According to WHO's global hepatitis strategy, the aim of elimination and eradication of HBV globally could be accomplished by reducing new hepatitis infections by 90% and deaths by 65% between 2016 and 2030, through vaccination, diagnostic tests, medicines and education campaigns [30].

Conclusion

For global eradication of HBV by 2030, it is necessary that clinicians should understand the natural history of the infection, particularly the course of spontaneous HBeAg Seroconversion. Successful elimination of HBV infection depends on efforts to make a top priority on the public-health as we are now in the second decade of this new century.

Author Contributions

Conceptualization- Dr. Shama Tomar, Dr.Bichitananda Swain

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Conflicts of Interest

The authors declare no conflict of interest.

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