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Categorization of Hepatitis B Infected Patients Attending a Tertiary Care Centre, Puducherry

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Abstract

Hepatitis B infection is a common disease worldwide. Hepatitis B is one of the leading cause of malignancy and cirrhosis of liver. The diagnosis of Hepatitis B Virus (HBV) infection is mainly made through detection of serological markers. Our study aimed to detect presence of Hepatitis B Precore Antigen (HBeAg) and Antibodies to Hepatitis B core Antigen (HBcAg) among Hepatitis B Surface Antigen (HBsAg) positive samples detected in Microbiology laboratory during the study period. HBeAg, Total Anti HBc and Anti HBcIgM was detected using ELISA (DIA.PRO - ITALY) and patients were categorized based on presence of HBeAg, Total Anti HBc and Anti HBcIgM. Out of 180 samples tested positive for HBsAg, majority belonged to the age group of 41-60 years. With regard to gender, males were found to be majority and four percent were antenatal women. HBeAg was found in 20.6% patients indicating high infectivity. Out of 180 samples, 9.45% were found to have acute infection and 90.55% were with chronic infection. Among the patients with acute infection, 58.8% had high infectivity whereas in patients with chronic infection 16.56% had high infectivity. HBV Screening and categorization of positive patients are important to prevent chronic hepatitis, its complications among infected patients and to reduce the transmission of HBV in the community.

Keywords: Hepatitis, Infectivity, Antibody

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INTRODUCTION

Hepatitis B infection is a common disease worldwide. Hepatitis B is one of the leading cause of malignancy and cirrhosis of liver. Worldwide nearly 254 million people are living with Chronic Hepatitis B.[1] Based on the prevalence of infection, countries are categorized as three groups. They are low, intermediate and high endemicity zone.[2,3] When the prevalence of Hepatitis B infection is greater than eight percentage (8%), it is categorized as high endemicity zone, prevalence of 2-7% as intermediate and less than 2% as low endemicity zone. India is categorized in intermediate zone with average prevalence range of 4%.[3]

Hepatitis B virus (HBV) comes under Hepadnaviridae family. The DNA structure of HBV is partially double stranded.[4] The mode of transmission includes parenteral, sexual, vertical transmission. Risk factors for acquiring Hepatitis B infection include patients on hemodialysis, intravenous drug users, close contacts with HBV infected persons, persons having multiple sexual partners, exposure to contaminated body fluids, children born to mothers affected by HBV.[2]

Five phases of HBV Infections are Immunotolerant phase, Immune reactive HBeAg positive phase, Inactive HBV carrier state, Chronic hepatitis B with HBeAg negative, HBsAg negative or Resolution phase.[5] In Immunotolerant phase, Hepatitis B Precore antigen (HBeAg) is positive with high levels of HBV DNA, normal Alanine transaminase levels. There is no or minimal inflammation of liver. In Immune reactive phase, HBeAg will be positive, increased alanine transaminase, high HBV DNA levels and there will be active liver disease. In inactive HBV carrier stage, Hepatitis B Surface antigen levels are low, normal alanine transaminase levels, low HBV DNA levels, HBeAg will also be negative. In chronic hepatitis B with HBeAg negative, antibodies to Hepatitis B precore antigen (Anti HBe) will be positive, HBV DNA levels will be high and fluctuating Alanine transaminase levels. In HBsAg negative phase, HBV DNA replication is low.[6] The serological diagnosis for HBV are done by detection of Hepatitis B Surface antigen (HBsAg), Hepatitis B Surface Antibody (Anti HBs), Hepatitis B Precore Antigen (HBeAg), Antibodies to Hepatitis B core antigen (Anti HBc IgM, Anti HBc IgG), Antibodies to Hepatitis B Precore Antigen (Anti HBe). Molecular marker includes HBV DNA and other nonspecific markers includes Serum bilirubin, liver enzymes.

Main goal of treatment of Hepatitis B Infection is to reduce the mortality and morbidity by reducing development of chronic carrier state, cirrhosis and malignancy of liver. Treatment options includes Interferon alpha (INFα), Pegylated Interferon Alpha (PEG INFα) and Antiviral drugs. Nucleotide and Nucleoside analogues are used. The nucleoside analogues include Lamivudine, Emtricitabine, Telbivudine, Entecavir. Nucleotide analogues includes Adefovir, Tenofovir.[2] Prevention for Hepatitis B infection is mainly made through vaccination. WHO recommends all children should be vaccinated within 24 hours of birth. All health care workers at risk of acquiring this infection are need to be vaccinated with full course.

Screening of other serological markers of Hepatitis B among HBsAg positive patients is needed to reduce the mortality and morbidity of the infected patients and to reduce the community transmission. Our study aims to differentiate between acute and chronic infection among HBsAg positive patients by detecting other serological markers of Hepatitis B.

Aims and objectives

1. To detect the serological Markers Hepatitis B Precore Antigen (HBeAg) and antibodies against Hepatitis B Core Antigen (HBcAg) among Hepatitis B Surface Antigen (HBsAg) positive patients.
2. To categorize the HBV infected patients based on presence of HBeAg, antibodies against HbcAg
3. To report the presence of HBsAg, HBeAg and antibodies against HBcAg with respect to age and sex.

MATERIALS AND METHODS

Our study was a cross sectional descriptive study among HBsAg positive patients attending a tertiary care centre, Puducherry (India). All HBsAg positive serum samples detected by Immuno Chromatographic Test (ICT) as a part of routine investigation in our Microbiology laboratory for routine diagnostics and management purpose during the study period of 18 months

(January 2018- June 2019) were included in the study irrespective of their clinical details. Basic demographic details (Age, Sex) of all participants were recorded. No separate sample was collected from the participants for the study. No repeat sample was collected from same person.

After getting ethical approval and written consent, these samples were further analysed for the presence of Hepatitis B e Antigen (HBeAg) and antibodies against Hepatitis B Core Antigen (HBcAg) – Total Anti HBc and Anti HBcIgM by Enzyme Linked Immunosorbent Assay (ELISA) – by DIA.PRO - ITALY and results were interpreted as per the kit manufacturer s instruction.

HBsAg positive patients with HBeAg will be categorized as HBV infection with high infectivity and those with HBeAg negative as HBV infection with Low infectivity. HBsAg positive patients with Anti HBcIgM or HBeAg as Acute Hepatitis B infection and those with Total Anti HBc and absence of Anti HBc IgM as Chronic Hepatitis B infection.

The statistical data were entered in Microsoft Excel 2016 and was analyzed using Statistical Package for Social Science (SPSS) statistical version 16. The dependent (HBeAg and Antibodies against HBcAg) and independent variables (Age, Sex) were summarized using percentage and ratios. The correlation between the categorical variables (HBeAg, Antibodies against HBcAg, Sex) were analysed using chi square test and Fisher's exact tests were used for the analysis between numerical (Age) and categorical variables.

RESULTS

During the study period, totally 180 HBsAg positive samples were collected at our laboratory.

Participant Details

Majority of the infection were in the age group between 41-60 years and least number of infections among the age group between 10-20 years. Table 1 shows the age wise distribution of HBsAg Positive samples. Minimum age of individual affected with Hepatitis B is 14 years. Maximum age of individual affected with Hepatitis B is 72 years. Majority of the infection were seen in Male patients (60.55%) and only 39.45 % were female patients. Hepatitis B infection of about 59% were seen in Inpatients. Among the total patients, four percent (7 patients) were found to be antenatal women.

Table 1. Age wise distribution of HBsAg Positive samples

S. No.	Age wise distribution	Total Number (%) n=180
1	10-20 years	4 (2.2)
2	21-40 years	67 (37.2)
3	41-60 years	85 (47.2)
4	>60 years	24 (13.3)

Presence of Serological Markers among HBsAg Positive Patients

Among the total cases, 20.6% of patients were Positive for HBeAg, 6.1% for Anti HBc IgM and remaining for Positive for Total Anti HBc as shown in table 2. Among total 180 HBsAg positive cases, 17 patients were found to have acute infection, out of which majority of patients had acute infection with High infectivity (58.82%) and the remaining had acute infection with Low infectivity. Among Chronic Hepatitis B infection, only 16.56% of the individuals had High infectivity (Table 2). On statistical analysis, Age of the patients were significantly associated with Positivity of Anti HBc.

DISCUSSION

The present study was done in HBsAg positive patients to detect HBeAg and Antibodies against HBcAg. During the study period, totally 180 samples were tested positive for HBsAg. Among the study group, Males were found to be majority with 109 patients (60.55%). As male gender is more prone for developing alcoholic hepatitis and cirrhosis of liver which is a known risk factor for developing hepatitis, would be the cause for this difference in gender distribution in study population. This increased HBV infections in Males have been observed in several other studies also.[7-10] Majority of about 107 patients (59%) of our study group belonged to inpatients.

Among the study population, 4% (7 Patients) were antenatal women. However, percentage of HBV infection among antenatal

Table 2. Presence of serological markers among HBsAg Positive Patients

S. No.	HBsAg	HBeAg	Anti HBc IgM	Total Anti HBc	Total Number (n=180)	%
1	Positive	Positive	Positive	Positive	4	2.22
2	Positive	Positive	Negative	Positive	27	15
3	Positive	Negative	Negative	Positive	136	75.55
4	Positive	Positive	Negative	Negative	6	3.33
5	Positive	Negative	Positive	Positive	7	3.88

mothers was too small in our study population to arrive at a conclusion when compared to other studies on HBV infection among Pregnant mothers.[11,12] When the pregnant mother is HBsAg positive, risk for newborn acquiring infection is higher. HBV infection in Pregnant women was found to be 1.09% by Mishra *et al.* in Madhya Pradesh in 2017.[11] In another study from Tamil Nadu in the year 2016 showed HBV infection in Antenatal women was found to be 5.86%.[12] When pregnant mother with HBV infection is HBeAg positive, the risk is much more higher for the newborn to acquire the infection as proven by other studies.[13-18] Risk of newborn acquiring Hepatitis B infection is around 90% when HBsAg positive mother is HBeAg positive and risk is only 10% when the Antenatal mother is HBeAg negative.[16-18] In our study population, among the HBV infected antenatal women, only one patient (14.2%) was found to be HBeAg positive and the remaining were HBeAg negative. Whereas in a study among Nigerian pregnant women reveals two (6.5%) out of 31 HBV infected Antenatal Mothers were HBeAg positive.[14] An Allahabad study in 2011, reported 57% HBeAg positivity among pregnant women.[19] HBeAg positivity among pregnant women is very high compared to our study. Larger sample size and the study population was only antenatal mothers could be the cause for this difference. Alexander *et al.* in their study show HBeAg positivity of 16.1% among pregnant women.[20] A study in China in 2017 on Hepatitis B carrier mothers revealed caesarian section could greatly reduce the chance of vertical transmission of HBV from mother to child.[21] Alexander *et al*. emphasized and proved determination of HBeAg status of HBV infected pregnant women would help in reduction of Perinatal transmission of HBV to the newborn.[20] So, once the pregnant women is found to be positive for HBV, screening other serological markers especially HBeAg is more important to prevent the newborn from acquiring HBV infection.

When a person is infected with HBV and when that individual is positive for HBeAg, the patient is considered highly infectious.[5,8,14,22-24] In our study, among the total HBsAg positive samples, 37 (20.6%) patients were positive for HBeAg indicating high infectivity among these individuals. These results are in concordance with the results from previous studies as the positivity of HBeAg from previous studies were 17.14% in a study conducted at Kozhikode, India.[25] In another study from Haryana done in 2015, the positivity of HBeAg among HBsAg positive individuals were found to be 13.3%.[26] Rajani *et al*. conducted a study on clinically suspected acute hepatitis in New Delhi in 2008 revealed HBeAg positivity of 29.1% among HBsAg positive patients.[27] From the study done in Chennai during 2018 revealed HBeAg positivity of about 49.42%,[10] which could be attributed to study period was only 6 months and sample size was less. A study from West Bengal in 2015 by Sarkar *et al*. showed 51.44% with HBeAg positivity.[28] The individuals with HBeAg are likely to transmit the infection to others at a much higher rate (19%) than other HBV infected individuals who are negative for HBeAg (1-9%) which was proven by study done by Supran *et al*.[13] Price *et al*. in 2017 in Uganda showed prevalence of HBeAg among HBsAg positivity of 37%.[8] In our study, majority of HBeAg positivity 72.97% was seen in patients with chronic infection.

When an individual is positive for Hepatitis B Surface antigen (HBsAg) and Antibodies of IgM against core antigen (Anti HBc IgM) then that individual is said to be suffering from acute HBV infection. Anti HBc IgM is positive in all cases (100%) suffering from Acute infection which was revealed by Marcus *et al*.[29]

In our study, in a total of 180 Hepatitis B patients, 17 (9.45%) patients were found to have acute infection. The prevalence of acute infection in our study was in concordance with results of other studies also. Study from Haryana by Pandey *et al*. in 2015, showed acute infection of 9.13% among HBsAg positive patients.[26] Sarkar *et al*. from West Bengal in 2015 showed 6.12% of acute infection,[28] where as in study by Rajani *et al*. in 2008 from New Delhi showed 75%[27] of acute infection as study population was only from clinically suspected acute hepatitis patients. In our study, Out of the 17 patients suffering from acute infection, 4 patients (23.5%) were positive for Anti HBcIgM, Total Anti HBc, HBeAg and 6 patients (35.29%) were found to be positive for HBeAg but Negative for antibodies to Core Antigen. These 10 patients (58.8%) have acute infection with high infectivity. Similar rate of Acute infection with high infectivity was also noted in study by Prabina *et al*. from Chennai in 2018 as 62.5%.[10] In our study, six patients (35.29%) were positive for HBsAg and HBeAg but showed negative results for Total Anti HBc and Anti HBc IgM. Similarly, in an Uganda study in 2017 showed HBsAg, HBeAg positive with antibodies negative in 18% of HBsAg positive patients.[8] Seven patients (41.17%) with acute infection were positive for Anti HBcIgM and Anti HBc but negative for HBeAg signifies acute infection with low infectivity. Study from Chennai in 2018 also showed 37.5%[10] of acute infection with low infectivity and 58.97% by Pandey *et al*.[26]

So whenever a person is found to be HBsAg positive, it is mandatory to screen for Acute marker Anti HBcIgM to prevent the further complications and also screen for HBeAg as these individuals could transmit the infection at a higher rate. When an individual remains positive for HBsAg more than 6 months and positive for IgG Antibodies against core antigen, the person is said to have chronic infection with Hepatitis B. In our study, in total 180 HBsAg positive samples, 163 patients (90.55%) were with chronic infection. In a study by Prabina *et al*, 72.41% were with chronically infection. Among the patients with chronic infection, 27 patients (16.56%) were found to be positive for HBeAg, that is 16.56% were chronically infected with high infectivity. The remaining 83.43% were negative for HBeAg indicating chronic infection with low infectivity. These results of chronic infection with high infectivity were similar to results of Pandey *et al*. from Haryana in 2015 showed 13.3% of chronic infection with high infectivity[26] and Sarkar *et al*. from West Bengal in 2015 showed 18.94% of chronic infection with high infectivity.[28]

In our study, HBeAg positivity in patients with Acute infection is three times higher when compared to HBeAg positivity in patients with Chronic infection. Majority of the patients had Chronic infection.

CONCLUSION

HBV infection was common in Males. Majority of infection was seen in age group between 41-60 years. Patients with acute infection had high infectivity than patients with chronic infection. HBV infected Antenatal women with HBeAg positivity are at higher risk of transmitting infection to their newborn. HBV Screening is important to prevent Chronic Hepatitis and its complications. When an individual is found to be HBsAg positive, further analysis of other serological markers to be done routinely to categorize the patients as Acute and Chronic Infections and also to determine the risk of Infectivity of that individual so as to improve the mortality and morbidity associated with HBV infection and to prevent the transmission of the disease in the community.

Limitations

Our study was a Single institution study. Sampling on large population could give us more details on the HBV disease. Determination of other markers Anti HBe, HBcAg, Anti HBs, HBV DNA and liver function tests were not done. The Risk factors of acquiring HBV infection like medical history, life style, socioeconomic status, vaccination status and further follow-up among the study population was not assessed.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India, with reference number PG DISSERATATION /12/2017/124.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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