VIROLOGICAL SPECTRUM OF CHRONIC HEPATITIS B PATIENTS IN TRIPURA

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ABSTRACT

BACKGROUND

Hepatitis B is a global public health problem and India is in intermediate zone of endemicity. Detailed virological evaluation will provide a better idea regarding treatment plan and regular follow up. Hepatitis B Virus (HBV) is a global public health threat affecting about 350 million people across the world. Hepatitis B Virus (HBV) infection is a common cause of liver cirrhosis and Hepatocellular Carcinoma (HCC). The natural course of HBV infection is a dynamic interplay of complex interactions between virus replication and host immune response.

MATERIALS AND METHODS

716 HBsAg positive patients who attended liver clinic during a period of 4 years were analysed for epidemiological and virological status. In total, 716 blood samples that were known to be positive for HBsAg were randomly collected for this study during 2012 to 2015. The serum was separated and preserved at -20°C in sterile plastic containers. Each patient who attended the liver clinic provided informed consent to collect blood specimens and perform various serologic and biochemical assays.

RESULTS

Majority of the patients were between 20 and 40 years, which states a high prevalence among young individuals. More prevalent among male as compared to female population. HBeAg positive chronic hepatitis represented 15.3% of the cases. Hepatitis B viral load was substantially elevated in 28% of the study population. 20% of the study population had a family member positive for HbsAg, which is quite a significant number.

CONCLUSION

High prevalence of immunologically active patients increases the burden of therapy and high probability of complications in future.

KEYWORDS

Hepatitis B Virus, Virological Profile, HBeAg, HBV DNA.

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BACKGROUND

Hepatitis B Virus (HBV) is a global public health threat affecting about 350 million people across the world.[1] Hepatitis B Virus (HBV) infection is a common cause of liver cirrhosis and Hepatocellular Carcinoma (HCC). The natural course of HBV infection is a dynamic interplay of complex interactions between virus replication and host immune response.[2] In approximately 95% of adults, exposure to HBV leads to an acute infection, which usually gets resolved in about 6 months without long-term consequences, whereas the remaining 5-10% fails to control the viral infection leading to chronic illness.[3] Hepatitis B surface antigen (HBsAg) is an early seromarker of HBV infection.[4] India has a high proportion of HBV associated chronic carriers and extensive studies have been carried out on them.[5] In India, the prevalence rate of hepatitis varies from 1 to 13 percent with an average of 3.7%. With a population of more than 1.25 billion, India has more than 40 million HBV carriers and contributes a large proportion of global HBV burden. Every year, one million Indians are at risk for HBV and about 1,00,000 die from HBV infection.[6]

The diagnosis of HBV infection and differentiating each clinical state of the disease involve integrative interpretation of HBV serologic markers. These markers include the following-

- HbsAg- A coated lipoprotein particle that forms a part of the viral surface.
- Anti-HBs- An antibody to viral surface antigen, which provides protective immunity to the virus.
- Anti-HBc- An antibody to viral genome core; the core DNA particle is not detectable in serum, but rather in hepatocytes.
- HBeAg- One of the viral secretory proteins associated with high titre of HBV DNA and active liver disease.
- Anti-HBe- An antibody to HBeAg.

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- HBV DNA- Quantitative measurement of HBV DNA used to assess recovery from infection as well as candidacy for antiviral therapy.

In persons with chronic HBV infection, regular monitoring of disease activity should be performed as viral replication and degree of liver injury can vary throughout the course of disease.

The expression of HBeAg may vary depending on the genotype just as clinical outcome and response to antiviral treatment in different population groups have been associated with varying viral genotypes.[7] Several studies have shown that HBeAg is a biomarker of active viral proliferation in hepatocytes, infectivity and transmission and is associated with an increased risk of hepatocellular carcinoma.[8] Therefore, testing for the HBeAg can aid in identifying individuals with a high risk of developing liver cancer and in planning patient management. This can also provide information on the future burden of liver cancer associated with HBV.

CHB has been traditionally characterised into four phases[9] reflecting the dynamic relationship between viral replication and evolution and the host immune response. These phases are of variable duration and not every person infected with CHB will evolve through all phases. Given the dynamic nature of CHB infection, serial monitoring of HBV DNA and Alanine Aminotransferase (ALT) levels is important to characterise the phase of infection. A single ALT and HBV DNA level are insufficient to assign phase of infection and/or need for treatment. Of note, some persons will be in the "gray zones," meaning that their HBV DNA and ALT levels do not fall into the same phase.

1. Immune-tolerant phase- In this highly replicative/low inflammatory phase, HBV DNA levels are elevated, ALT levels are normal (<19 U/L for females and <30 U/L for males) and biopsies are without signs of significant inflammation or fibrosis.
2. HBeAg-positive immune-active phase- Elevated ALT and HBV DNA levels in conjunction with liver injury characterise this phase.
3. Inactive CHB phase- In this phase, HBV DNA levels are low or undetectable, ALT levels are normal and anti-HbE is present. Liver histology shows minimal necroinflammation.
4. HBeAg-negative immune reactivation phase- Among those who seroconvert from HBeAg to anti-HbE positive, 10%-30% continues to have elevated ALT and high HBV-DNA levels and roughly 10%-20% of inactive carriers may have reactivation of HBV replication and exacerbations of hepatitis after years of quiescence.

	ALT	HBV DNA	HBeAg
Immune-tolerant phase	Normal	Elevated, typically >1 million IU/mL	Positive
HBeAg-positive immune-active phase	Elevated	Elevated ≥20,000 IU/mL	Positive
Inactive CHB phase	Normal	Low or undetectable <2,000 IU/mL	Negative
HBeAg-negative immune reactivation phase	Elevated	Elevated ≥2,000 IU/mL	Negative

Acute hepatitis B is primarily a disease of adults and often occurs among members of high-risk groups. Less than 3% of acute hepatitis B cases occur among persons under 14 years of age, 28% individuals have had contact with a person with hepatitis B and 63% indicate no apparent source of infection.[10] In the latter instance, the most likely source of infection is a household member who is an HBV carrier or has an acute case of hepatitis B.

When an individual is found to have acute or chronic hepatitis B infection, an investigation of the circumstances surrounding transmission is warranted. Previous studies have shown that persons living in the same household as an HBV carrier have a 40% or higher likelihood of current or prior HBV infection.[11] Serologic testing of household members may detect other infected persons who require medical evaluation and counseling and will identify susceptible persons who may require prophylaxis.[12]

Aim- Despite the widespread HBV infection and its association with liver disease, limited literature about the burden of viral infectivity status is known where HBV is expanding. The present study aimed at describing the HBV virological profile in HBsAg positive patients who attended liver clinic during a period of 4 years and studied the prevalence of hepatitis B virus 'E' antigen (HBeAg) among individuals determined to be Hepatitis B Virus (HBV) surface antigen positive and analysed the gender/age category associated with more active HBV infection and to assess the relationship between HBV-DNA load, ALT levels, review the performance of serum ALT and HBV-DNA levels as the screening tool.

MATERIALS AND METHODS

In total, 716 blood samples that were known to be positive for HBsAg were randomly collected for this study during 2012 to 2015. The serum was separated and preserved at -20°C in sterile plastic containers. Each patient who attended the liver clinic provided informed consent to collect blood specimens and perform various serologic and biochemical assays.

HBsAg Testing- HBsAg ELISA was used for the detection of HBsAg in plasma. Briefly, HBsAg ELISA (analytical sensitivity- 0.2 ng/mL; assay sensitivity- 99.9%; specificity- 100%) is based on the double antibody sandwich method and detects HBsAg in plasma, which is a marker of active HBV infection.[13]

HBeAg Test- The OnSite HBeAg rapid test was used for the qualitative detection of HBeAg in serum/plasma. The OnSite

HBeAg rapid test is a lateral flow chromatographic immunoassay.[14]

HBV DNA- The tests use real-time Polymerase Chain Reaction (PCR) assay in which HBV DNA was extracted from serum by using spin columns and their concentrations were determined by using a programmable DNA high-speed thermal cycler.[15]

ALT- Kinetic method- The system monitors the change in absorbance at 340 nanometers.[16] Cut-off value set for ALT is 30 in males and 19 in females according to American Association for the Study of Liver Diseases.[9]

Age group for the study was taken in the interval of 20 years viz. less than 20 years, 20-40 years, 40-60 years, more than 60 years and subjects were grouped accordingly. Levels of ALT and HBV DNA were determined in HBeAg positive and negative individuals and grouped separately to determine number of individuals requiring active management. HBeAg-positive patients were considered as wild virus-infected hepatitis, HBeAg-negative patients with normal transaminases and viraemia less than 2000 IU/mL were considered as HBV inactive carriers, HBeAg-negative patients with elevated transaminases and viraemia higher than 2000 IU/mL were considered as HBeAg-negative hepatitis.[17]

Statistical Analysis- Comparisons of continuous variables between groups were done by unpaired t-test using the SPSS predictive analytics software. Categorical variables were analysed by the Chi-square test or Fisher exact test.

RESULTS

In total, 716 HBsAg-positive individuals participated in the study. Subjects were divided on the basis of presence or absence of HBeAg. The mean age is approximately same in both the groups. Hepatitis B virus 'E' antigen was positive in 80 individuals, which constitutes 15% of the total study population (Figure 1).

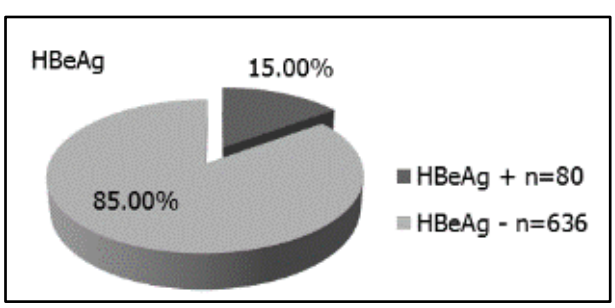

Figure 1. HBeAg Status in the Study Population

Mean ALT among male in HBeAg-negative category is 24.4 IU/mL. Mean ALT among female being 15.7 IU/mL. Mean ALT in HBeAg-positive category is on the higher side with 87.7 IU/mL in male and the mean ALT is female study population in 68.3 IU/mL. HBV DNA copies were quantified and found to be very high in HBeAg positive individuals as depicted in the Table 1.

Characteristic	HBeAg Negative, n=636 (85%)	HBeAg Positive, n=80 (15%)
Mean age (years)	35.5	33.4
M:F	522:114	67:13
Mean ALT (IU/L)	Male = 24.4 Female = 15.7	Male = 87.7 Female = 68.3
HBV DNA (copies/mL) (mean)	1,865.6	374, 378, 645.53

Table 1. Clinical Parameters of HBsAg Positive Patients

Age criteria selected for the study being at the interval of 20 years (Figure 2). Age distribution being <20 yrs. - 56 (8%), 21-40 yrs. - 433 (60%), 41-60 yrs. - 196 (28%), >60 yrs. - 31 (4%). It is understood that majority of individuals are in the age group of 20 to 40 years.

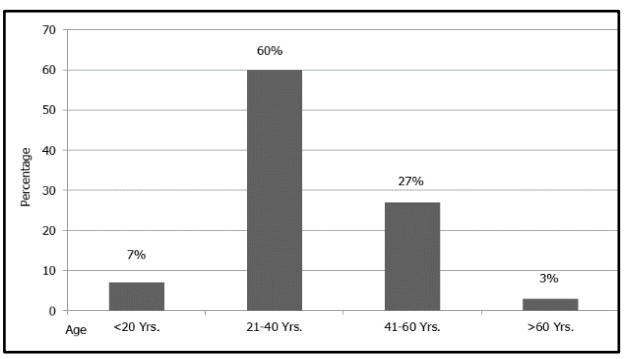

Figure 2. Age Distribution

Males (n=433) dominated females (n=283), which constitutes about 61% against 39% females. Of the participants tested for HBV e-antigen status, 80 were found to be positive (15.4%). Among the 80 individuals in the HBeAg-positive category, 67 were males, which account to 83.7% against 13 females constituting 16.3%, which is in the ratio of approximately 5:1. Among the HBeAg-negative individuals (n=636), male dominate with 522 individuals (82.1%) as against 114 female subjects (17.9%) (Figure3).

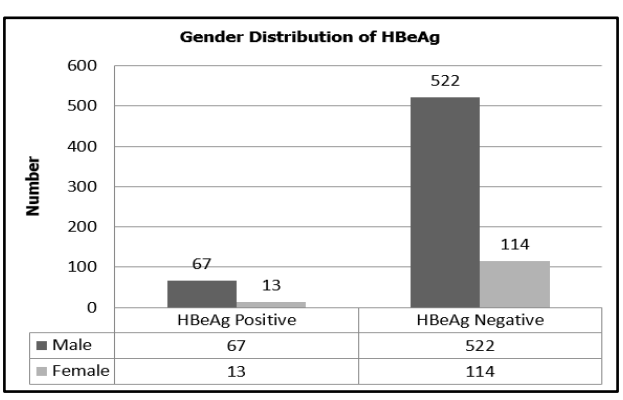

Figure 3. Gender Distribution of HBeAg

Family history of hepatitis B virus is positive in 144 individuals, which account for 20%, which is quite a significant number (Figure 4).

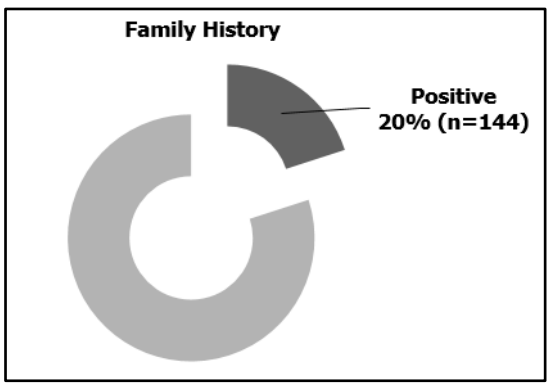

Figure 4. Family History Positivity

Considering the significance of HBV DNA and ALT in the further management and treatment of individuals with hepatitis B virus, a comparison has been made between ALT, HBV DNA in individuals with HBeAg status. According to AASLD (American Association for the Study of Liver Diseases) immune-active CHB is defined by an elevation of ALT >2 ULN (>60 U/L for males and >38 U/L for females) or evidence of significant histological disease plus elevated HBV DNA above 2,000 IU/mL (HBeAg negative) or above 1,00,000 IU/mL (HBeAg positive). Out of 636 HBeAg negative individuals, there were 406 patients whose HBV DNA is less than 2,000 copies/cu mm and ALT at the baseline (<30 U/L for male and 19 U/L for female), 49 HBeAg-negative patients were having HBV DNA of 2,000 to 20,000 copies/cu mm and baseline ALT (<30 U/L for male and 19 U/L for female). 85 individuals were noted to have HBV DNA between 2,000 to 20,000 copies/cu mm and elevated ALT (30-60 U/L in male and 19-38 U/L in female). 96 HBeAg negative individuals had HBV DNA more than 20,000 copies/cu mm with elevated ALT (>60 U/L in males and 38 U/L in females) (Table 2).

HBV DNA (Copies/cu mm)	ALT (U/L)	No. of Individuals
<2,000 (inactive CHB)	<30 males and <19 females	406 (63.9%)
2,000 to 20,000	<30 males and <19 females	49 (7.7%)
2,000 to 20,000	30-60 males and 19 -38 females	85 (13.3)
>20,000 (immune reactivation)	>60 males and >38 females	96 (15.1)

Table 2. HBeAg Negativity Status (n=636)

Among the 80 HBeAg-positive patients in the study, about 73 individuals had HBV DNA more than 20,000 copies/cu mm with ALT elevated (>60 u/L in males and 38 u/L in females), which is a significant number. Three individuals had HBV DNA >2000 copies/cu mm and ALT elevated (30-60 u/L male and 19 to 38 u/L female). Two individuals had HBV DNA >2000 copies/cu mm an ALT normal (<30 u/L male and 19 u/L female). Two individuals had HBV DNA <2000 copies/cu mm and normal ALT (<30 u/L male and 19 u/L female) (Table 3).

HBV DNA (Copies/cu mm)	ALT (U/L)	No. of Individuals
<2,000 (inactive)	<30 males and 19 females	2 (2.5%)
2,000 to 20,000	<30 males and 19 females	2 (2.5%)
2,000 to 20,000	30-60 males and 19-38 females	3 (3.7%)
>20,000 (immune active)	>60 males and >38 females	73 (91.3%)

Table 3. HBeAg Positivity Status (n=80)

DISCUSSION

HBV is a serious global public health problem. The problem of HBV infection is well-recognised, but efforts to control the virus have not been satisfactory as a significant impact on disease incidence or prevalence has not been observed. The presence of HBeAg in the serum of patients with hepatitis B virus is a reflection of active viral replication in hepatocytes and is considered a surrogate marker for the presence of the DNA of hepatitis B virus.[18] Testing for the HBeAg can also identify individuals with a high risk of developing liver cancer.[8] In this study, 15.2% HBeAg prevalence was recorded among HBsAg-positive individuals. This reflects a pool of individuals who are highly infectious and serve in sustaining viral transmission and evolution suggesting that the future burden of liver cancer associated with HBV is likely to be high. Joseph et al conducted a study in North-Central Nigeria to estimate the prevalence of HBeAg-positive patient, which is 19.2% reflecting a high proportion of HBV infectivity and transmissibility rates.[19] In a recent study in Enugu,[20] low prevalence at 8.6% was found among asymptomatic adults. In Benin City,[21] the overall HBe-antigenaemia prevalence among adults was 7.3%.

HBeAg-negative individuals constitute 85.5% in the study. Effective prevention measures and ageing of existing carriers has probably led to increase in the number of HBeAg-negative CHB. In a study from Italy, HBeAg-negative CHB prevalence increased from 41% during the 1975-1985 period to 90% during the 1990s.[22,23,24,25] The predominance of HBeAg-negative CHB nowadays has been supported by a French study as well.[26]

Among the total HbsAg-positive individuals in the study, there are about 61% males and 39% females. The present demographic data shows a discrete predominance of men, which has been observed in other studies. Moosa et al and Awan et al reported a high (59.1%, 58.3%) prevalence in males than females (40.9%, 41.7%), respectively.[27,28] Most studies have shown a sex ratio of 1.3:1 to 2:1.[29]

Among the HBeAg-positive individuals, 83.7% were male. There is a high preponderance for males to have active HBV infections. Similar studies were conducted in Nigeria, Enugu and Benin City, which concluded that HBeAg-positive individuals were dominated by males.[30] HBeAg-negative individuals were also dominated by males, which accounts to 82.1%. Hadziyannis SJ et al conducted a study in Greece concluding that in HBeAg-negative CHB patients, male sex predominates with male-to-female ratio varying 4.6-17%.[31] Higher HBV infection in males as compared to female maybe

due their being employed outsides their homes, visiting barber shops and also their involvement in blood transfusion practices. While women are mostly involved in house-hold activities based on the social, cultural and religious preferences and influence.

Majority of the individuals (60%) are in between the age group 21 to 40 years. Prevalence of HBV is considerably less (4%) in individuals over 60 years. HBV infection being higher in young respondents maybe due to their greater exposures and interaction in society as compared to children and aged persons. Alam et al reported a significantly higher infection in persons with age between 21-40 years, followed by 41-60 years age.[32] Very young and old individuals were very less frequently infected by HBV. Study conducted in Asian-Pacific Islander Populations[33] in New York City, the highest prevalence was seen in young adults.

Family screening for hepatitis B infection plays an important role in epidemiological point of view. With the clear-cut aim to assess the spread of HBV infection in families with an infected member and to identify the family members with the risk of infection. All the members in the family were screened for markers of HBV by enzyme-linked immunosorbent assay. 20% of the study population had members in their family positive for HbsAg, which is quite high keeping in mind the recent advances in the vaccination era. Study done by Chakravarty R et al on hepatitis B infection in Eastern Indian families where the family members of hepatitis B patients were tested, it was found that 19.4% of them were HBsAg positive.[34] Horizontal transmission between family members is equally important for HBV transmission in our community. So, there is a need for screening of adult siblings and mothers of HBsAg carriers.

During evaluation of hepatitis B viral infection, level of alanine transaminase is essential because high ALT levels are thought to be associated with chronic HBV.[35,36] Host factors and viral factors also play an important part in HBV infected individuals. Many previous reports have attempted to define an HBV DNA threshold that corresponds to the presence of active liver disease. Owing to complex host-virus interactions, a precise viral load threshold cannot be identified that is accurate in all patients at all times.[37] HBV DNA and Alanine Aminotransferase (ALT) levels are more important to characterise the phase of infection and to decide further plan of action. HBV DNA load and liver damage appears to be different in HBeAg positive and negative patients. From the study, it is understood that there is a positive correlation between HBV DNA levels and ALT levels suggesting that inflammation increases in patients with elevated HBV DNA levels as HBeAg has immunomodulatory action.[38]

Various studies have shown that for HBeAg-negative individuals, low HBV DNA levels are associated with less liver damage.[39] The prediction of future active disease was associated with the corresponding HBV DNA titre, the baseline ALT and a history of disease activity. In HBeAg-negative individuals, 63.9% had HBV DNA less than 2000 copies/cu mm and ALT <30 U/L for male and 19 U/L for female. Such patients require regular follow-up every 6 months. There is a high chance of future ALT elevation. Otegbayo JA et al conducted a study in Nigeria concluded that HBeAg positivity among HbsAg-positive individuals was infrequent and whenever present was associated with active liver disease indicated by raised ALT value.[40] Serial testing of HBV DNA and alanine transaminase is essential to determine the appropriate followup and the need for antiviral therapy. The fluctuating levels suggest that decisions should not be made based on a single measurement.

CONCLUSION

The present study has thrown some light on virological profile of hepatitis B patients in Tripura. Most of the patients were young adult male in their third and fourth decade. Their virological profile was dominated by HBeAg-negative chronic hepatitis and inactive chronic HBV carriage. One sixth of HbsAg-positive individuals are positive for HBeAg, which suggests active HBV infection as it is a marker of active viral replication and transmission. There were quite a significant number of family members infected with hepatitis B virus in the study population. This is quite high and shows possibility of high horizontal transmission. There is need to screen pregnant women for hepatitis B and strengthen the childhood vaccination programmes and implement treatment programmes to reverse the harmful effect of HBV infection. Family members should be screened for HBsAg routinely. Full course of hepatitis B vaccine should be recommended to those negative for HBsAg. Proper measures should be taken to ensure regular screening of blood during transfusion process. Universal precaution should be taken, while treating these patients in the hospital. High hepatitis E antigen negativity is the pattern of infectivity, hence regular follow up and screening including imaging is required to avoid complications. Genotype evaluation and mutant study among this group of patients may provide important information. High prevalence of immunologically-active patients increases the burden of therapy and high probability of complications in the future. Necessary steps should be taken to strengthen awareness programs involving both the media and public sectors organisations to decrease the future burden of HBV.

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