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Risk factors associated with hepatitis B virus disease in different states of North Eastern India and their distribution

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ABSTRACT

To determine whether risk factors such as fever, anorexia, abdominal discomfort, haematemesis, weight loss, high coloured urine, blood transfusion, alcoholic intake and multiple sexual partners are highly associated and derive a novel risk score for the development of HCC. Different liver diseases were screened for the positivity of HBsAg were were followed up (mean Age and SD) for the occurrence of HCC. The risk factors were recorded and found the statistical significant with the disease. The distribution of the different categories of HBV disease in six different states of Northeastern India region were recorded .The number of Chronic cases are found as the highest followed by Acute viral hepatitis, cirrhosis, HCC and FHF. The mean Age±SD of HCC was was recorded as 53.3 ± 9.57 which was greater than other study groups of HBV. The risk factors such as fever, high coloured urine, blood transfusion and Multiple sexual partners were recorded as mostly significant($p<0.05$). These risk factors are closely associated with the progression of the liver diseases than other recorded in this study . The other risk factors were also recorded and the values were not found as a highly associated but may be increase up to a level . The risk score, based on the present and absent of the factors can estimate the chance of development of HCC in few years . It can be used to identify high-risk CHB patients for treatment and screening of HCC.

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INTRODUCTION

Hepatitis B is a public health problem and more than 350 millionpeople are said to be infected with the hepatitis B virus worldwids (Kim et. al., 2016) . Hepatitis B virus infection is mainly associated with an acute liver disease which includes liver failure and also chronicity which can lead to cirrhosis and liver cancer (Liang et. al., 2009). Hepatitis B virus (HBV) is a major bloodborne and sexually transmitted infectious agent that is a significant global public health issue Currently, eight HBV genotypes (A-H) have been described and diverge by at least more than eight per cent in their nucleotide sequences (Kramvis et. al., 2005). The occult hepatitis B virus infection is defined as "the presence of HBV DNA in the liver (with detectable or undetectable HBV DNA in serum) in individuals testing HBsAg negative by currently available assays" (Raimondo 2008; Hollinger 2010 and Metaferia et. al., 2016).

Earlier, naturally occurring deletions in the pre-S2/S promoter region were observed in several cases of occult HBV infection (Chaudhuri 2004; Mu 2009), chronic HBV infection (Fan et al., 2001), and patients with progressive liver diseases (Chen et. al.,2006) . In a subsequent study, it was demonstrated that these deletions can cause altered surface protein expression, and an increased large-HBsAg (L-HBsAg) to major/small-HBsAg (S-HBsAg) ratio leading to reduced HBsAg secretion (Sengupta et al., 2007).

HBV is spread predominantly by percutaneous or mucosal exposure to infected blood and other body fluids with numerous forms of human transmission. The sequelae of HBV infection include acute and chronic infection, cirrhosis of the liver and primary liver cancer. The likelihood of progression to chronic infection is inversely related to age at the time of infection. Around 90% of infants infected perinatally become chronic carriers, unless vaccinated at birth. The risk for chronic HBV infection decreases to 30% of children infected between ages 1 and 4 years and to less than 5% of persons infected as adults (McQuillan 1999; Wasley 2010).

Healthcare personnel are at increased risk of occupational acquisition of hepatitis B virus (HBV) infection. While effective vaccination for HBV is widely available, the prevalence of HBV and vaccine acceptance in hospital personnel have not been recently assessed.Some liver diseases are potentially preventable and are associated with lifestyle choices. Alcohol-related liver disease is due to excessive consumption and is the most common preventable cause of liver disease.Hepatitis B is a viral infection most often spread through the exchange of bodily fluids (for example, unprotected sexual intercourse, sharing unsterilized drug injecting equipment, using non-sterilized equipment for tattoos or body piercing).

The hereditary liver disease can be passed genetically from generation to generation. Examples include Wilson's disease (copper metabolism abnormalities) and hemochromatosis (iron overload). Chemical exposure may damage the liver by irritating the liver cells resulting in inflammation (hepatitis), reducing bile flow through the liver (cholestasis) and accumulation of triglycerides (steatosis). Obesity/overweight increases the risk for liver disease. Obesity often results in the accumulation of fat cells in the liver. Acids that are secreted by these fat cells (called fatty acids) can cause a reaction in the body that destroys healthy liver cells and results in scarring (sclerosis) and liver damage.From previous studies in Ethiopia have demonstrated that the important factors of HBV transmission include blood transfusion; tattooing; a history of surgery, unsafe injections, or abortions; multiple sexual partners; and traditional practices such as scarification, circumcision, and also ear piercing (Awole 2005; Walle 2008; Ramos 2011; Zenebe 2014; Tegegne 2014) .Although the association between HIV and HBV has become less prominent in Africans, evidence has been found indicating that HIV makes HBV-related liver disease develop more quickly (Metaferia et. al., 2007) and HIV/HBV co-infection has serious effects on both pregnant women and infants. A previous study among pregnant women in Bahir Dar city showed an HIV/HBV co-infection rate of 1.3% (Chen et. al., 2006) . The risk of developing liver disease varies, depending on the underlying cause and the particular condition. General risk factors for liver disease include alcoholism, exposure to industrial toxins, heredity (genetics), and long-term use of certain medications.

Age and gender also are risk factors for liver disease. These factors vary, depending on the particular type of disease. For example, women between the ages of 35 and 60 have the highest risk for primary biliary cirrhosis and men aged 30-40 are at higher risk for primary sclerosing cholangitis.

In our study we have included the following factors having unprotected sex with more than one partner or with an infected partner , Having a sexually transmitted disease (STD) ,Using IV (injected) drugs ,Living with an infected person , Having end-stage kidney disease and receiving hemodialysis treatments,Being exposed to human blood at work (e.g., health care workers) .

MATERIAL AND METHODS

Study area: The North-eastern region, where the where the study was conducted, is a less developed region of India in terms of economic, social, and health indices. Insufficient health services and lack of public awareness of health-related issues have increased the prevalence of

diseases, particularly communicable diseases. In order to achieve the objectives of the study, we enrolled patients with various types of liver diseases seen at various participating centre of Northeastern states which include Regional Institute of Medical Sciences, Imphal, Manipur; Agartala Govt. Medical College, Tripura; STNM Hospital, Gangtok, Sikkim; Naharlagun General Hospital, Arunachal Pradesh,Gauhati Medical College, Guwahati, Assam; NEIGRIHMS, Shillong, Meghalaya .Patients with HBV infection who had achieved a virological response were collected and recorded the data since Nov 2012 to May 2015.

CLINICAL DATA

The patients of AVH were evaluated on the basis of history and clinical examination. The liver function tests (LFT) was done at the first visit. The patients of Acute Viral Hepatitis (AVH), Fulminant Hepatic Failure (FHF) were evaluated on the basis of history, physical examination, liver function test and serological test for HBsAg. Samples which were positive for HBsAg were also screened for IgM anti-HBc and HBV-DNA. To rule out any co-infection with other hepatotropic viruses IgM anti-HAV, anti-HCV, IgM, anti-HEV infection was done. The patients of chronic liver disease viz. chronic hepatitis, cirrhosis of the liver and hepatocellular carcinoma was evaluated on the basis of history, physical examination, Liver function profile, Prothrombin time, serological markers for HBsAg. Samples which are positive for HBsAg were further tested for IgG anti-HBc and HBeAg followed by HBV DNA. Anti-HCV and HCV RNA was done in the cases which are positive for HBV DNA to rule out cases of co-infection. The serum of voluntary blood donor was collected from blood banks of all the hospitals .They were screened for HBsAg using commercially available ELISA kits (3rd generation) at each centre.Serum samples from each subject were stored at -80®C until use. The patients with HCC cases, the serum samples were collected at the time when liver biopsy was performed.

Inclusion and Exclusion Criteria for the study:

All those patients who were clinically diagnosed as Acute Viral Hepatitis (AVH), Fulminant Hepatic Failure (FHF), chronic active hepatitis (CAH), Cirrhosis, Hepatocellular Carcinoma (HCC) were included in the study. Voluntary blood donors and Healthcare workers were also included in this study. Professional blood donors, high-risk group like IV drug abusers will be excluded from the study.

EXTRACTION OF HBV DNA

HBV DNA was extracted using slightly modified Phenol-Chloroform method as described by Teresa Santantonio et al 1991. Briefly the method involves 100 µl of serum sample incubated with 0.5% SDS, 10mM Tris-HCl (pH-7.5), 10mm EDTA & 10mg / ml Proteinase K at 37°C overnight. Then the serum DNA would be extracted twice with Phenol / Chloroform and precipitated with ethanol in the presence of 30 µg of tRNA. The pallet was air dried and resuspended in 25 µl of distilled water.Part of the surface gene (nucleotide position 425 to position 840) was amplified by nested polymerase chain reaction (PCR) for the HBV DNA.

Study design

Before starting the study, approval from the local ethical committee was received, and those subjects over the age of 15 who had consented to participate in the study were included. Informed consent forms, as well as information about the aims of the study, were provided for each subject. Age, residence site (rural/urban), a level of education, and marital status of the subjects, and any family history of jaundice were recorded.

Statistical analysis

Categorical variables of HBV diseases were reported as a number of cases and were grouped into states. The statistical analysis was done by using the SPSS version 13.1 to confirm the association.For the descriptive analyses, we calculated the values and were presented as either a number (percent;%) or mean SD (standard deviation). Statistical significance levels were determined by two-tailed tests and considered the significant P value < 0.05 ($p < 0.05$). Statistical analysis data were plotted in excel.

RESULTS AND DISCUSSION

DEMOGRAPHICS AND OUTCOMES

The distribution of the different categories of HBV disease in six different states of Northeastern India region is shown in Table1. Different states have the distribution in different ranges and the numbers were showed in the first table. The number of Chronic cases is found as the highest followed by Acute viral hepatitis, cirrhosis, HCC, and FHF. All the values were recorded and a bar graph was plotted to express the distribution levels. The mean and S.D values of age were also recorded and it was distributed among all the groups of the disease infected cases of Hepatitis B virus. The table 2 showed all the mean and S.D values of the different stages of liver disease. The table 3 showed the risk factors of HBV-related liver diseases were recorded and which indicated the factors such as fever, anorexia, Abdominal discomfort,Haematemesis,weight loss, High coloured urine, blood transfusion, alcoholic intake, multiple sex-

Table 1: Distribution of HBV infection among the different parts of North Eastern India.

S. NO	STATES	AVH	FHF	CAH	CIRRHOSIS	HCC
1	ASSAM (n=427,34.4%)	157	0	180	62	28
2	SIKKIM (n=260,20.9%)	151	2	48	54	5
3	MEGHALAYA (n=125,10%)	19	0	67	29	10
4	TRIPURA (n=186 ,14.98%)	32	2	105	33	14
5	ARUNACHAL (n=117 ,9.4%)	48	0	47	6	16
6	IMPHAL (n=126,10.1%))	7	4	58	46	11
	TOAL (n=1241)	414(33.3%)	8(0.64%)	505(40.7%)	230 (18.5%)	84 (6.76%)

ual partners, and ascites.In Northeast India HBV is a predominant underlying disease (52%).

In this report we showed that the CAH was distributed the highest in number(n=505,40.7%)followedbyAVH(n=414,33.3%),Cirrhosis(n=230,18.5%),HCC(n=84,6.76%) and FHF(n=8,0.64%).So the number of FHF was the least 0.64% among all. The highest number of infections were recorded in Assam(n=427,34.3%) and the incident rate of CAH was more and no FHF cases were recorded during the study. In Sikkim 20.9% prevalence rate was recorded and the incident rate of AVH(n=151)was the highest followed by cirrhosis(n=54),CAH(n=48),HCC(n=5)andFHF(n=2).10% of incident rates were recorded in Meghalaya and the number of CAH(n=67) were more than another group of the liver disease. In Tripura, the HBV positive cases were recorded as 14.98%.Where CAH was recorded as the highest (n=105) and only a few(n=2) were recorded as FHF .9.4% of HBV were recorded in Arunachal Pradesh with AVH(n=48)as the highest incident rates and no FHF cases were recorded. In Imphal, only 10.1% cases of HBV were recorded and CAH was the highest incident .

In the Table2, the mean Age±SD of HCC was was recorded as 53.3 ± 9.57 which was greater than other study groups of HBV. The infection with AVH was recorded as 34.1 ± 4.33 .

Table2: The mean age of the study groups among various categories of the HBV infection.

s. no	Study group	Number (n=1241)	Mean Age±S.D
1	AVH	414	34.1 ± 4.33
2	CAH	505	40.1 ± 11.45
3	CIRRHOSIS	230	45.2 ±10.95
4	HCC	84	53.3 ± 9.57
5	FHF	8	35.3 ± 7.83

The risk factors such as fever ,high coloured urine, blood transfusion and Multiple sexual partners were recorded as mostly significant($p<0.05$).These risk factors are closely associated with the progression of the

Table 3: The independent risk factors and their significant role in the progression of the liver disease.

Risk Factors	HBV DNA PRESENT (n=712)	HBV DNA ABSENT (n=571)	P value
Fever: Present: Absent :	 206(29%) 506(71%)	 204(36%) 367(64%)	0.01*
Anorexia: Present Absent	 165(23%) 547(77%)	 144(25%) 427(75%)	0.40
Abdominal Discomfort Present Absent	 220(31%) 492(69%)	 196(34.%) 375(66%)	0.4
Haematemesis: Present Absent	 83(11%) 629(89%)	 66(11%) 505(89%)	0.68
Weight Loss: Present Absent	 216(30%) 488(70)	 183(32%) 382(68%)	0.51
High coloured urine: Present Absent	 76(11%) 636(89%)	 90(16%) 481(84%)	0.003*
Blood Transfusion Present Absent	 22(3%) 690(97%)	 30(4%) 541(96%)	0.004*
Alcohol intake Present Absent	 306(43%) 406(57%)	 263(46%) 308(54%)	0.27
Multiple sexual partners: Present Absent	 37(5%) 665(95%)	 25(4%) 546(96%)	0.049*
Ascites: Present Absent	 130 581	 95 476	0.44

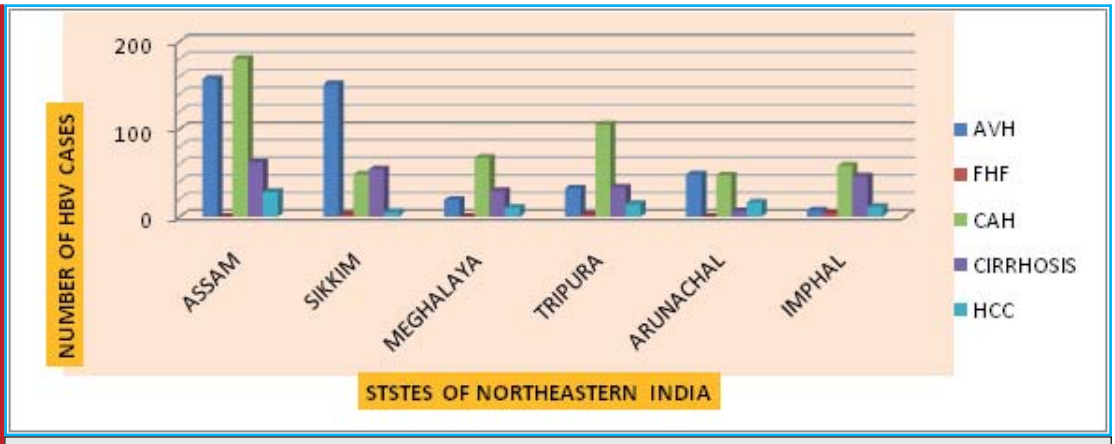

FIGURE 1. The distribution of the HBV-related liver disease among all the states of NorthEastern India.

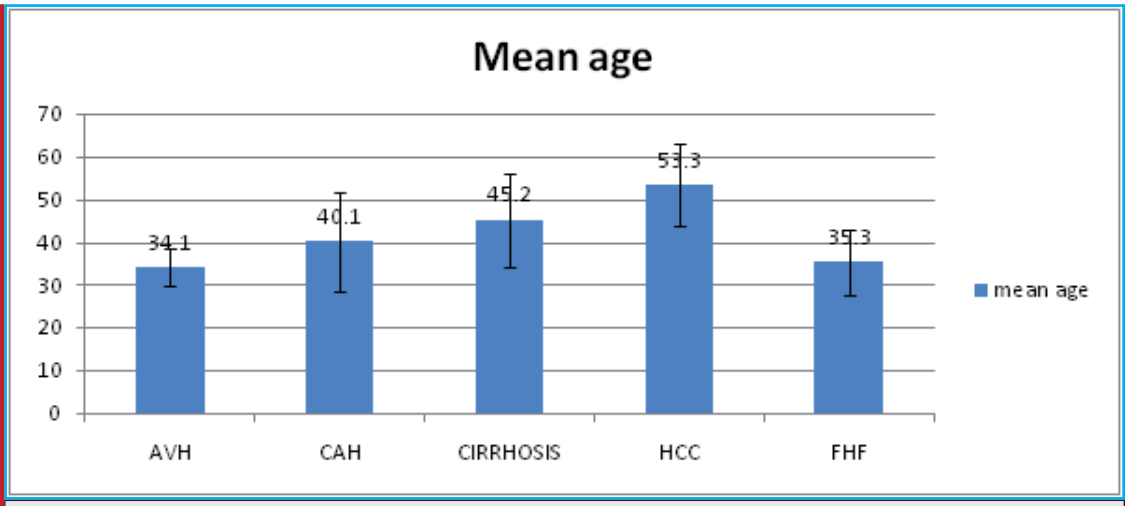

FIGURE 2. The distribution of different age groups as means±S.D in different cases of HBV.

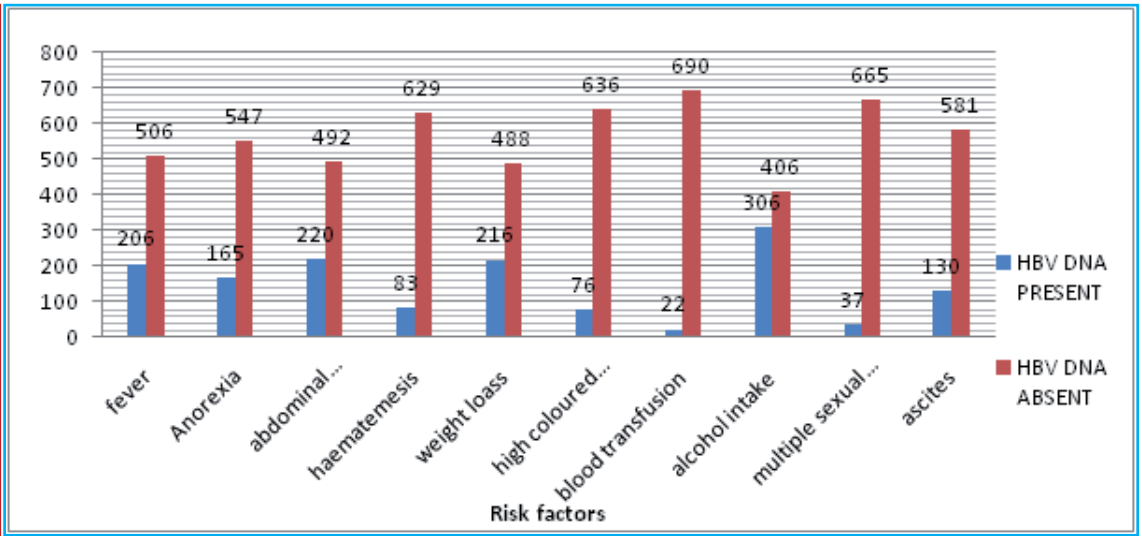

FIGURE 3. The independent risk factors of HBV-related liver diseases in all the states by differentiating the HBV DNA present or absent.

liver diseases than other recorded in this study. The other risk factors were also recorded and the values were not found as a highly associated but may be increased up to a level.

In conclusion, it has been demonstrated that the characteristic of Hepatitis B virus disease HBsAg surface marker identification is the most important to find the prevalence rate of the liver disease. Along with the identification of marker, the risk factors are also necessary to find so that it may help the physicians to give the treatment and also to reduce the high risk of this disease.So the awareness should be made among the incident areas. Finally, the significant distribution of major risk factors raises the possibility of association of this HBV disease.

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ABBREVIATIONS

- CHB, chronic hepatitis B
- HBsAg, hepatitis B surface antigen
- AVH,Acute viral Hepatitis
- HCC,Hepato cellular carcinoma

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