The molecular epidemiology and Biochemical profiling of HBV infection in the North-eastern part of India

Namrata Kumari[1], Priyanka Kashyap[1], Subhash Medhi[1], Snigdha Saikia[1,2], Bhavadev Goswami[2], Premashis Kar[3], Th. Bhimo Singh[4], K.G Lynrah[5], M. R. Kotowal[6], Pradip Bhaumik[7], Moji. Jini[8] and Manab Deka[1]

1Bioengineering and Technology Department, Gauhati University Institute, Guwahati, Assam
2Department of Gastroenterology, Gauhati Medical College, Guwahati, Assam
3Department of Medicine, Maulana Azad Medical College, New Delhi
4Department of Medicine, Regional Institute of Medical Sciences Regional Medical College,Imphal
5Deptartment of Medicine, NEIGRIHMS, Shillong, Meghalaya
6Medical Adviser to the Hon'ble Chief Minister of Sikkim, STNM Hospital, Gangtok, Sikkim
7Dept of Medicine, Agartala Govt. Medical College, Agartala, Tripura.
8Directorate of Medical Education, General Hospital, Naharlagun, Arunachal Pradesh.
namrata388@gmail.com

Abstract: In India also HBV is considered a major public health problem. Among the general population of India the prevalence of HBV was recorded as a range from 2%-8% and found that India is in the intermediate HBV endemic zone. The population in the North east India is one of the most indigenous tribes with different ethnicity and has a higher incidence of drug abusers as well as HIV infection. The present case control prospective study has been undertaken to explore the viral and host genetic risk factors for the hepatitis B infection in Assam using different diagnostic tools. The present study was designed to express on many important aspects of molecular epidemiology of HBV which are very much important for identifying the population, at the risk of acquiring HBV and the developing of severity of liver disease and also propose about the transmission through different modes. From the overall samples collected 5.12% were found as the HBV positive cases and were further studied for the above objective. The male population (74%) are more prone to HBV related liver disease than women (26%). The biochemical parameters and risk factors were studied to find the severity. Also to study the seroprevalence of HBV infection among the healthy blood donors and Healthcare workers among the North eastern population, samples were first done with HBsAg ELISA and then confirmed with PCR. Out of the 751 HBV positive samples (5.12%) it was found that 4.5% were positive for blood donor and 0.6% were positive for health care workers. The blood donors with mean age 28.12±4.30 with Male: Female ratio of 31:3 and found as significantly associated with the disease ($p<0.05$).

[Namrata Kumari, Priyanka Kashyap, Subhash Medhi, Snigdha Saikia, Bhavadev Goswami, Premashis Kar, Th. Bhimo Singh, K.G Lynrah, M. R. Kotowal, Pradip Bhaumik, Moji. Jini and Manab Dek. **The molecular epidemiology and Biochemical profiling of HBV infection in the North-eastern part of India.** *Life Sci J* 2019;16(2):90-96]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 12. doi:10.7537/marslsj160219.12.

Keywords: molecular; epidemiology; Biochemical; profiling; HBV; infection; North-eastern; India

Introduction

Hepatitis B virus (HBV) is consider as a serious public health problem globally and the major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). It was calculable that close to two billion individuals have medical science proof of past or gift HBV infection. More than 350 million square measure chronic carriers of HBV (1). Because of the high HBV-related morbidity and mortality, the worldwide illness burden of hemoprotein is substantial. The prevalence of chronic HBV infection varies greatly in several a part of the planet. The prevalence of chronic HBV infection worldwide could be categorized as high, intermediate and low endimicity (2). The prevalence of HBV infection varies markedly throughout regions of the world (3). Hepatitis B is extremely endemic in developing regions with giant population like South East Asia, China, geographic region and therefore the Amazon Basin, wherever a minimum of 8 May 1945 of the population are HBV chronic carrier. In these areas, 70–95% of the population shows past or gift medical science proof of HBV infection. Most infections occur during infancy or childhood. Since most infections in kids are symptomless, there's very little proof of acute illness associated with HBV, however the rates of chronic disease and carcinoma in adults are high (4). Hepatitis B is moderately endemic partly of japanese and Southern Europe, the center East, Japan, and a part of South America. Between 10–60% of the population have proof of infection, and 2-7% ar chronic carriers. Acute illness associated with HBV is common in these areas as a result of several infections occur in adolescents and adults; but, the high rates of

chronic infection ar maintained largely by infections occurring in infants and children (5). In these areas, mixed patterns of transmission exist, together with baby, babyhood and adult transmission. The endemicity of HBV is low in most developed areas, like North America, Northern and Western Europe and Australia. In these regions, HBV infects 5–7% of the population, and only 0.5–2% of the population are chronic carriers (6). In these areas, most HBV infections occur in adolescents and young adults in comparatively well-defined risky teams, as well as injection user, homosexual males, health care staff, patients UN agency need regular blood transfusion or haemodialysis.

Transmission of Hepatitis:

HBV is unfold through contact with infected body fluids and also the solely natural host is human. Blood is the most important vehicle for transmission, but other body fluids have also been implicated, including semen and saliva (7). Currently, 3 modes of HBV transmission are recognized: perinatal, sexual and parenteral/percutaneous transmission. There is no reliable evidence that airborne infections occur and faces are not a source of infection. HBV isn't transmitted by contaminated food or water, insects or other vectors. Transmission of HBV from carrier mothers to their babies will occur throughout the perinatal amount, and appears to be the most important factor in determining the prevalence of the infection in high endemicity areas, notably in China and geographic region. Sexual transmission of hepatitis B may be a major supply of infection altogether areas of the globe, especially in the low endemic areas, such as North America. Hepatitis B is taken into account to be a sexually transmitted sickness (STD). The risk of chronicity is low (less than 5%) for transmission through sexual contact, intravenous drug use, acupuncture, and transfusion (8). Individuals in danger for these transmission modes sometimes acquire HBV infection throughout adolescence or adulthood while not immune tolerance. Instead, the sickness progresses on to the immune clearance section and is of short length, that most likely accounts for prime spontaneous recovery.

Material and methods:

The North-eastern region, 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 rate of diseases, particularly communicable diseases. This population based case-control study was carried out by Bioengineering and technology Department, Gauhati University, Department of Gastroenterology, Gauhati Medical College, Guwahati, Assam; Department of Medicine, Regional Institute of Medical Sciences Regional Medical College, Imphal; Department of Medicine, NEIGRIHMS, Shillong, Meghalaya; General Hospital, Naharlagun, Medical Adviser to the Hon'ble Chief Minister of Sikkim, STNM Hospital, Gangtok, Sikkim; Dept of Medicine, Agartala Govt. Medical College, Agartala, Tripura; Directorate of Medical Education, General Hospital, Naharlagun, Arunachal Pradesh. Arunachal Pradesh. The study was carried out from Nov 2012 to May 2015. A total of 7130(48.40%) liver disease cases were collected included HBV, HCV, HAV, HEV, Aloholic liver disease, Nonalcoholic liver disease and Blood donors (48.05%) and health care worker (03.53%).

This cases were based on clinical and laboratory parameters of both Male and Female with same number of control samples. Cases were pathologically and/or cytologically confirmed HBV subjects of both sexes (male and female) and for HCC cases with serum alpha-fetoprotein level greater than 400 ng/ml combined with at least one positive image on angiography, sonography, liver scan, and/or computed tomography scan were included. After obtaining written informed consent, trained social investigators collected the data from the eligible subjects by face-to-face interview using a pre-coded, closed ended questionnaire. The sociodemographic characteristics included participants' age, sex, educational status, occupational status and location of residence etc. The questionnaire included ranges of information including sociodemographic characteristics of participants, history of alcohol drinking habits, type of alcoholic beverages, dietary habits. Alcohol drinking habits were categorized into three categories, viz., non-drinker, former drinker, and current drinker. Subjects who were reported that they were regularly drink alcoholic beverage during index year were defined as current drinker, those who reported that they had stopped drinking the year before the index year or in the former were defined as former drinker, and those who reported that they never drank before were defined as non-drinker or abstainers. Diets and the dietary history of patients and controls were recorded based on 6 months of recall. Food frequency questions that contain details of dietary practices were included in the questionnaire. The frequency of consumption was classified as never eaten, occasionally, once a week, 2–4 times a week, and more than 4 times a week.

Clinical data analysis by Virological methods:

All those patients who were clinically diagnosed as Acute Viral Hepatitis (AVH), Fulminant Hepatic Failure (FHF), chronic active hepatitis (CAH), Cirrhosis, Hepatocellular Carcinoma (HCC) with HBsAg positive will be included in the study. Voluntary blood donors and the Health care workers (HCW) will be included in this study. Professional

blood donors, high risk group like IV drug abusers was excluded from the study. The HBV suspected patient‟s blood sample were collected in both EDTA and Clot activator vials. Serum/Plasma was separated by centrifugation method. Hepatitis B surface Antigen, Anti Hepatitis B core Antigen, Hepatitis B core IgM, Anti HCV, Hepatitis A core IgM, Hepatitis E core IgM were done using specific kit. HBsAg positive cases were subjected to HBV DNA extraction and further Polymerase Chain Reaction with specific Primers. All the liver disease patients were examined by qualified Gastroenterologists to determine the different symptoms of liver disease which includes nausea, vomiting, loss of appetite, jaundice, dark urine and pale stool.

Based on HBsAg positivity at presentation and after 6 months follow-up, anti-HBc-total positive, HBV DNA positive and PCR positive status were categorized as chronic carriers and were sub-categorized as chronic hepatitis B or cirrhosis after co-relating with the clinical details; else they were categorized as acute HBV cases. The HBeAg status was further screened by ELISA using commercially available kits.

Statistical analysis:

Descriptive statistics was performed to describe different characteristics of the HBV mediated liver disease. Univariate and multiple logistic regression was performed to assess the factors associated with HBV. The multiple logistic regression analysis was performed to calculate adjusted odds ratio (Adj. OR) and corresponding 95% CI for determining independent associations between the factors and HBV status after adjusting for potential factors. Only variables that were found to be significantly associated with HBV status in univariate analysis at 5% level of significance were included in multiple regression model. This study was approved by institutional ethical committee of Gauuhati University and Indian Council of Medical Research, New Delhi.

Results:

The burden of the viral hepatitis B infection in North-eastern states of India is not well characterised till date. To fulfil the objectives of the study a surveillance was conducted across different states of Northeastern part of India. To study the epidemic-prone diseases, information on the outbreaks of all forms of viral hepatitis, including A, B, C and E were collected. The data were collected from the Department of Gastroenterology, Gauhati Medical College, Guwahati, Assam; Department of Medicine, Regional Institute of Medical Sciences Regional Medical College, Imphal; Department of Medicine, NEIGRIHMS, Shillong, Meghalaya; STNM Hospital, Gangtok, Sikkim; Dept of Medicine, Agartala Govt. Medical College, Agartala, Tripura; Directorate of Medical Education, General Hospital., Naharlagun, Arunachal Pradesh. A total number of 14,729 samples with different types of liver diseases were screened for hepatitis B virus infection from all the centres, these screened data include 7130 (48.40%) different types of liver disease cases (HAV, HCV, HBV, HEV),7077 (48.05%) prospective blood donors and 522 (3.55%) health care workers and are depicted in table 1 and figure 1. To study the objectives of polymorphism and expression a total number of 294 control samples were included. From different parts of North-eastern states different liver disease cases were screened and further they were studied for HBV DNA positivity.

Table 1: **Different categories of sample screened**

Study Category	Number Of Sample Screened
Liver Disease	7130(48.40%)
Blood Donor	7077(48.05%)
Healthcare Worker	522(3.53%)

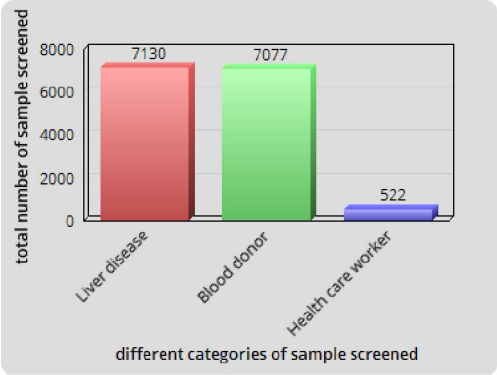

Figure 1: **Showing different categories of samples screened from all the States.**

Amplification of HBV viral samples:

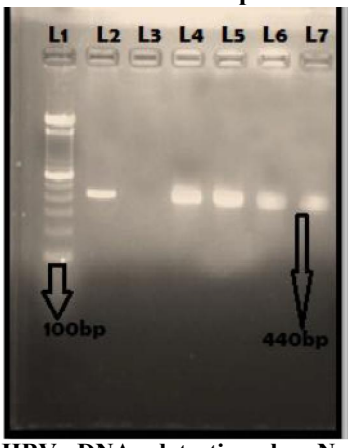

Figure 2: **HBV DNA detection by Nested PCR Method; Lane 1: 100 bp ladder; Lane 2,4,5,6,7: HBV Positive samples; Lane 3: HBV DNA Negative sample.**

All the screened samples were further amplified by nested PCR primers and found around 5.12% were HBV DNA positive and amplification of PCR result has been shown in figure 2. In figure 3 graphically the comparison was done among the overall liver disease cases and HBV DNA positive cases. All the screened samples were further amplified by nested PCR primers and found around 5.12% were HBV DNA positive (Figure 3; Table 2).

Table 2: **Distribution of HBV DNA positivity from the overall study samples.**

STUDY GROUPS	**Overall Cases (n=14,729)**	**HBV DNA positive (n=751) (5.12%)**
	781(5.3%)	221 (29.4%)
FHF	25(0.16%)	03 (0.3%)
CAH	3682(24.74%)	308 (41%)
CIRRHOSIS	2543(17.26%)	142 (19%)
HCC	162(1%)	162(1%)
BLOOD DONOR	7077(48%)	34 (4.5%)
HEALTH CARE WORKER	522(3.54%)	05 (0.6%)

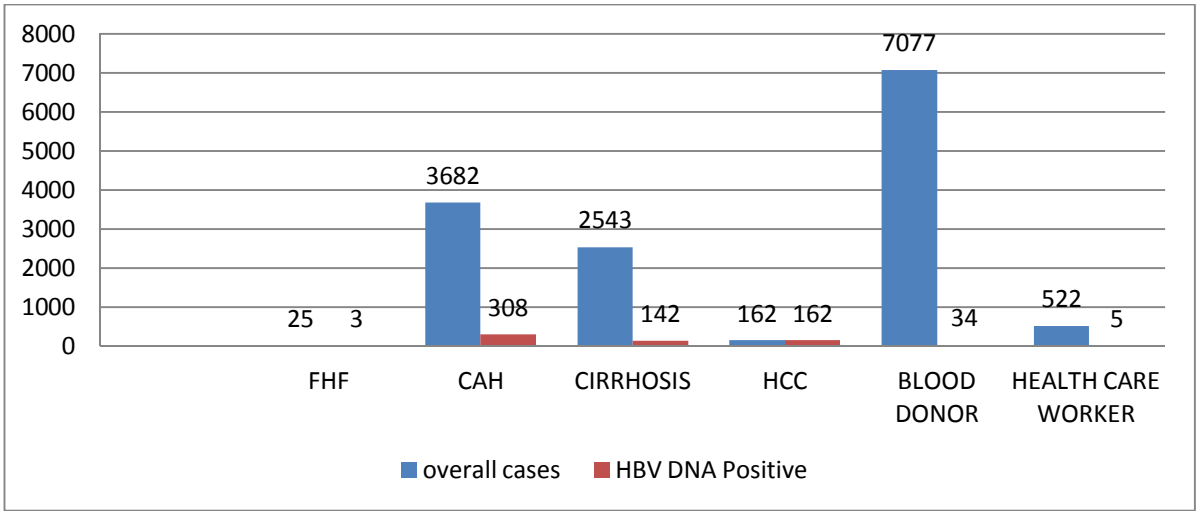

Figure 3: **Comparison of overall liver disease cases and HBV positive cases**

All the HBsAg positive cases were studied with demographical, biochemical, clinical and serological profile and compared with HBV DNA positive cases expressed in tabl3 and figure 4. The overall distribution of different HBV cases has been shown state wise. The biochemical parameters like ALT, AST, Total Bilirubin, Direct Bilirubin, Haemoglobin, Prothrombin time, serum creating, viral load, Na+, K+ were recorded. The p value was calculated by using unpaired t test. The study groups mean±S.D values were compared with the control samples and were represented in table 4. All the biochemical parameters were found as statistically significant and all are highly associated with the disease ($p<0.05$).

Table 3**: Overall collected samples and the HBV positive samples in all the States.**

States of NE	AVH (n=221)	FHF (n=3)	CAH (n=308)	CIRRHOSIS (n=142)	HCC (n=38)	BLOOD DONOR (n=34)	HEALTH CARE WORKER (n=5)
ASSAM	71(32.1%)	0	79(25.6%)	52(37%)	12(31.6%)	8(23.5%)	0
MEGHALAYA	2(0.9%)	1(33.3%)	68(22%)	34(24%)	3(7.9%)	10(29.4%)	0
TRIPURA	40(18%)	0	45(14.6%)	20(14%)	8(21%)	14(41.1%)	5(100%)
ARUNACHAL	26(11.8%)	0	34(11%)	21(14.8%)	5(13.1%)	1(2.9%)	0
SIKKIM	47(21.3%)	1(33.3%)	39(12.6%)	6(4.2%)	2(5.2%)	1(2.9%)	0
MANIPUR	35(15.9%)	1(33.3%)	43(14%)	9(6.4%)	8(21%)	0	0

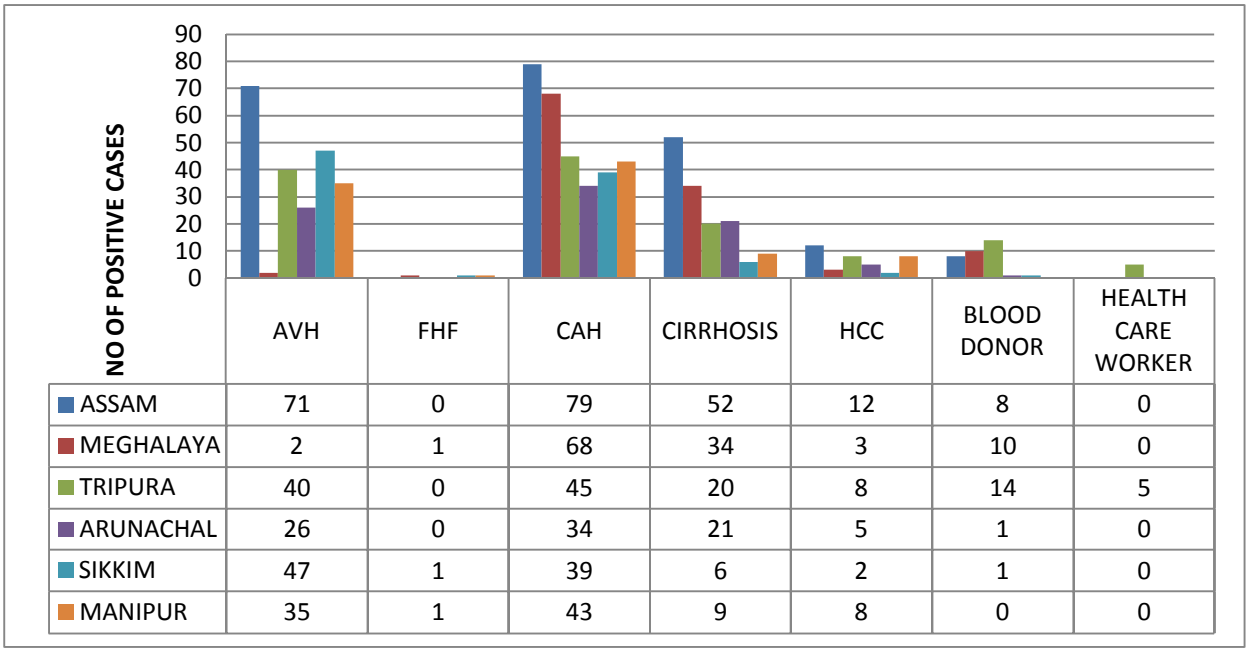

Figure 4: **Distribution of different stages of HBV in different study centres**

Table 4: **Unpaired t test results for comparing the different study groups with their biochemical profiles.**

	Male (n=556); 74%	**Female (n=195), 26%**	**P value**
Age:<20yr **21-30** **31-40** **41-50** **51-60** **>60**	23 162 274 68 21 8	2 119 45 21 7 1	0.2
ALT **<40 U/L** **>40 U/L**	238 318	89 106	**0.04**
AST **<30U/L** **>30U/L**	291 265	78 117	**0.016**
Viral load (IU/Ml) **<1000 IU/Ml** **1000-10000 IU/Ml** **10000-100000 IU/Ml** **100000-1000000 IU/Ml** **>1000000 IU/Ml**	31 114 189 142 80	6 30 49 86 24	**0.04**
Hb (%) **>12** **<12**	386 170	115 80	0.2
TotalBilirubin (mg/dl) **0.03-1.9** **>1.9**	245 311	78 117	**0.04**
DirectBilirubin (mg/dl) **<0.3** **>0.3**	257 299	68 127	**0.03**

Prothrombintime (INR) **<0.5** **0.5-1.5** **>1.5**	95 310 151	48 79 68	0.13
Serum creatinine (mg%) **<1.1-1.3** **>1.1-1.3**	286 270	87 108	**<0.05**
Na (mEq/L) **<135** **135-145** **>145**	74 169 313	39 69 87	0.16
K+(mEq/L) **<3.7** **3.7-5.2** **>5.2**	55 219 282	38 96 61	0.15
HBeAg status **Present (n=397)** **Absent (n=357)**	231 198	166 159	0.09

Additionally HBe antigen is used as an indicator of virological response when treating patients with chronic hepatitis B. The HBeAg positive/negative was studied among all the study groups and it was found that HBeAg is not highly associated with a specific phase of HBV DNA positive liver disease ($p>0.05$) presented in the table 5.

Table 5**: HBeAg profile among the different stages of HBV DNA positive liver disease.**

	HBeAg positive (N=397); 52.87%	**HBeAg negative (N=354); 47.1%**	**P value**
AVH (n=221)	103(25.96%)	118(33.33%)	0.46
CLD (N=308)	210(52.90%)	98(27.70%)	0.07
CIRRHOSI (N=142)	52(13%)	90(25.42%)	0.77
HCC (N=38)	20(5.04%)	18(5.08%)	0.37
FHF (N=3)	1(0.25%)	18(5.08%)	0.67
BLOOD DONOR (N=34)	10(2.6%)	24(6.78%)	0.92
HEALTH CARE (N=5)	1(0.25%)	4(1.13%)	Ref.…

Discussion:

To the best of our knowledge, this was the first study to assess the molecular and epidemiology study of HBV disease in the Northeast India. Different factors associated with HBV in different part of the Northeast India. We examined the relationship of different biochemical factors in different types of liver diseases associated with HBV. The present study found the incident rate of HBV among found illiterate and ever drinkers (current and former drinkers) are independently associated with increased risk of HCC. Illiteracy limits a person's ability to comprehend verbal and written health care information which adversely affect the awareness about healthy life style and precaution measure of various health issues. In the Northeastern region of India, preparation and consumption of traditional homemade alcohol is a social tradition of most of the tribe. Possibly people with low literacy level not able to identify the risk of excess consumption of any alcohol beverage, which make them vulnerable to liver disease. It has also been documented that one of the main cause of chronic liver disease and HCC is alcohol consumption chronic liver disease and HCC is alcohol consumption (9,10). Better approaches of large scale of HBV vaccination, identification and treatment of chronic HBV, alcohol reducing campaigns in high risk area are needed to prevent this important form of cancer.

Conclusion:

In conclusion, the findings of this study is first ever biochemical factors among HBV and Blood donor and health care workers patients in this increasing liver cancer incidence states of India. This

study also demonstrates a protective relationship of HBV with consumption of different food habbits. Prevention of primary infection by vaccination is an important strategy to decrease the risk of chronic HBV infection and its subsequent complications. The burden of adverse outcomes related to hepatitis B on individuals and communities, particularly in high-prevalence populations, is increasingly recognized. Although ensuring high coverage of infant vaccination will have a profound impact on this burden in coming decades, attention must be given to comprehensive policy responses now. Understanding the epidemiology of HBV infection will enable evidence-based and cost-effective public health and clinical interventions within countries and at the global level.

2/25/2019