Hepatitis is a general term meaning inflammation of the liver and is mostly caused by a variety of different viruses such as hepatitis A, B, C, D and E; or by other non-viral agents like alcohol, aflatoxins etc. According to the World Health Organization (WHO), 2 billion people have been infected with the hepatitis B virus (HBV), and more than 350 million have a chronic HBV infection. In addition, it has been estimated that up to 3% of the world’s population has been infected with hepatitis C virus (HCV), of which 170 million people are chronically infected. Hepatitis A and E viruses are two representatives involved in the complexity and severity of liver disease which may cause acute and fulminant and results in acute liver failure in many cases globally. Approximately 1.4 million reported cases of hepatitis A virus (HAV) infection are documented annually. Hepatitis E virus (HEV) is found predominantly in regions with poor sanitation. In pregnant women, HEV has been associated with a substantially increased risk of maternal, fetal, and neonatal death. Apart from viral hepatitis, chronic alcoholism contributes in a major way in the total liver disease burden globally. The rate at which the liver disease develops varies from one individual to other. There is sufficient evidence to suggest that the disease progression is multi-factorial, including viral, host immunological, and genetic factors, environmental factors etc. Along with age, sex and viral factors, alterations in host genetic factors and environmental factors are also considered to be important in the development of liver disease, but unfortunately, there are very limited data available on these important aspects in liver disease patients from Northeast India, who are ethnically distinct from those in other parts of the country.

In this study, we determined the prevalence of systemic hepatotropic viruses and their genotypes, genetic alterations of key base excision repair (BER) pathway genes hOGG1 and XRCC1, polymorphism of key metabolic regulator gene Cyp2E1, and the presence of dietary toxic environmental carcinogens such as nitrites and nitrosamines in fresh and fermented food from Northeast India, as well as evaluated the levels of 8-oxo-dG as an oxidative stress biomarker; and evaluated the role that they might play in the severity of the liver disease or its predisposition.

**Molecular epidemiology of hepatitis viruses in liver disease cases**

The highest prevalence of fecal-oral infection occurs in regions where low standards of sanitation promote virus transmission (Ceyhan M et al., 2008). In most industrialized nations,
where hepatitis A is no longer considered a childhood disease, infections with HAV are increasingly contracted by adults (Mathur and Aora, 2008). Despite the high prevalence of antibody in highly endemic populations, the virus perpetuates in the region due to its high physical stability. In our study, HAV infection was found in 30.80% of the population and most of the infected individuals were children and young people. Our data also supports earlier global data that majority of the HAV related cases are from the pediatric age group (Hussain Z et al., 2006, Hollinger and Ticehurst., 1990). But a good fraction of patients are of young and adult age group as reflected by a mean age group of 23±16 years; and the prevalence in higher age groups is also supported by recent reports from India (Hussain Z et al., 2011). Seven HAV genotypes have been defined. Majority of the HAV isolates reported from India were found to be of genotype IIIA [Robertson BH et al, 1992, Hussain Z et al., 2005]. Similar to the majority of the studies from Indian population, in our study cohort the majority of the HAV related cases belong to genotype IIIA, and a very few belong to genotype IA, which is distinctly different from what has been reported from western India recently (Vaidya SR et al., 2002). Moreover, genotype IIIA was found to be associated with both acute and fulminant HAV infection.

The incidence of HCC in HBV-related cirrhosis in East Asian countries has been reported to be 2.7% (Michielsen PP et al., 2005). The annual risk of HCC is 0.5% for asymptomatic HBsAg carriers and 0.8% for patients with chronic hepatitis B (Michielsen PP et al., 2005, Liaw YF et al., 1986), while patients with HBV-cirrhosis have 1000 times higher risk of developing HCC, compared to HBsAg negative individuals (Michielsen PP et al., 2005, Beasley RP et al., 1981). In the present study, HBV infection was found in 25.89% of the study population, which is relatively on the higher side compared to other reports from different parts of India (Nayak NC et al., 1987; Roychoudhury A et al., 1989; Tandon BN et al., 1984; Verma J et al., 1989). This is a major concern because HBV infection is associated with a high rate of hepatocellular carcinoma (Sarin SK et al., 2001). HBV genotype D was the most predo-minant genotype in our cohort, which is contrary to a report from the sister state in Northeast India, Arunachal Pradesh (Borkakoty BJ et al., 2008). Analyses of genomic sequences of HBV isolates from India are mostly reported from Western India and Northern India, where genotypes D and A are found (Thakur V et al., 2002; Gandhe SS. et al., 2003). Reports from Eastern India illustrate that it is a geographical area where genotypes D and A of mainland India and genotypes B and C of China and Southeast Asia converge. Recently genotype C has been reported from Eastern India (Banerjee A et al., 2005; Vivekanandan P et al., 2004; Datta S et al., 2006; Banerjee A, 2006). Our data also shows the presence of genotype C in a high percentage of cases (18.33%), and is
thus comparable with the recent eastern and north-eastern data, but contrary to data from north
and other parts of India. Since HBV genotype C is associated with rapid progression and severity
of HBV related liver disease, as well as response to antiviral therapies; the presence of genotype
C in a large fraction is of clinical significance. One of the isolates from our study showed
phylogenetic relatedness with genotype G; which is similar to very recent studies from Idu
Mishmi primitive tribe of northeast India using detail phylogenetic analyses which showed
recombination between genotypes A, G and C, and has recomm-ended to be recognized as
genotype I (Arankalle et al., 2010).

Hepatitis A and B vaccination is less effective in patients with advanced liver disease. Our
observations lead to the recommendation that patients should undergo hepatitis A and B
vaccination early in the natural history of their chronic liver disease. Vaccination rates are low in
clinical practice in northeast India, and public health and educational programs are needed to
overcome barriers to facilitate timely implementation of these recommendations. In our studied
cohort, the percentage of cases with hepatitis A and B co-infection was found to be low (0.89%),
and were in the age group of 30-50. But this group of patients are clinically important and are
predisposed to have severe liver injury, as reported by pre-existing global data. Data available
from a large outbreak of acute hepatitis A in Shanghai in 1988 and from cases of hepatitis A
reported to the Centers for Disease Control and Prevention (CDC) between 1983 and 1988
demonstrated that HAV infection was more severe in patients with pre-existing CLD (Keeffe
B virus (HBV) infection was associated with a 5.6-fold and 29-fold increased risk of death,
respectively, in the Shanghai outbreak and the CDC analysis of reported cases (Yao G, 1991;

HEV and HCV infection was found in 7.14% and 3.13% of the cases, respectively. Viral
hepatitis is a major public health problem in India, which is hyperendemic for HAV and HEV.
HEV is also the major cause of sporadic adult acute viral hepatitis and acute liver failure, and
many epidemics of HEV have already been reported in India. HCV infections in India have a
population prevalence of around 1%, and occur predominantly through transfusion and the use of
unsterile glass syringes. HCV genotypes 2 and 3 are found in 60%-80% of the population
(Acharya SK et al., 2006). In a recent published data, Medhi et al., 2011 has reported the
presence of genotype 1,2,3,4 as well as 6 in a large cohort based study from northeast India. Our
results which constituted of only 7 HCV patients, showed the prevalence of different HCV
genotypes, namely, 2, 3, 4 and 6, which is similar to what has been reported by Medhi et al., 2011. This study highlights the fact that geographical variations occur with respect to HCV genotypes, which could influence the course and progress of different type of liver diseases seen in India and especially in northeast India. The present data warrants further study on HCV cases, including large cohort populations from all Northeast Indian states. There are 4 mammalian genotypes of HEV found to have unique geographic distributions. Genotype 1 includes Asian and African HEV strains, genotype 2 includes the single Mexican HEV strain and few variants identified from endemic cases in African countries, genotype 3 includes human and swine HEV strains from industrialized countries, and genotype 4 includes human and swine HEV strains from Asia, particularly China, Taiwan and Japan. Hepatitis E virus with genotype 1 is most frequently recovered from patients in developing countries (Asia, North Africa). This genotype and genotype 2 appear to be more virulent than genotypes 3 and 4 (Purcell RH, 1997). Here, to the best of our knowledge, we reported the presence of HEV genotype 1 in Northeast India for the first time, which is similar to reports published from other parts of India (Arankalle et al., 1998; Panda et al., 2007, Bose PD et al, 2011).

Alcohol consumption is well entrenched in the social fabric of many adult populations, virtually constituting a behavioural norm. The level of alcohol consumption in a population is an important determinant of health and social well-being (Rehm J et al., 2009). Widespread and increasing use of alcohol is drawing attention to the health consequences of alcohol consumption. Alcohol-related liver disease (ALD) is a significant burden on health, with alcohol consumption accounting for an estimated 3.8% of global mortality (Rehm J et al., 2009). Alcohol is a major cause of liver cirrhosis in the Western world. Alcoholic cirrhosis is increasingly seen in countries such as Japan and India which traditionally had a low prevalence of the disease (Walsh K et al., 2010). IN OUR STUDIED COHORT A MAJOR PROPORTION OF THE CASES HAD AN UNDERLYING ALCOHOLIC ETIOLOGY (21.43%). The burden of alcohol related liver disease is likely to be higher in the future as indicated by our results which showed that a alcohol related liver disease cases are predominant in the younger age group of 11-40 years; and since the consumption of alcohol is been taken up in the early phase of life in northeast India; and moreover it is customary to consume alcohol in several tribal groups of Assam and northeast India.

Since the rate of development and progression of liver disease with different underlying aetiologies differs amongst individuals, therefore additional factors may be instrumental in dictating the liver disease development and course. Amongst the critical factors reported from
various available literatures, genetic alterations of the host genetic factors as well as environmental factors are of major importance and were evaluated through the present study.

**Host genetic factor alterations and liver disease susceptibility**

Oxidative stress increases damage to cellular components, including DNA. The repairing of different types of DNA damages is important for safeguarding genomic integrity. Cells overcome the DNA damage by repair mechanisms. Base excision repair (BER) pathway constitutes the primary defense against lesions generated by DNA damaging agents like viruses (McKillop IH et al., 2006). Genetic variants of OGG1, an important enzyme participating in BER pathway, may confer inter-individual variations in susceptibility to liver diseases and cancer. The enzyme 8-oxoguanine DNA glycosylase 1 (OGG1) initiates the BER pathway, implicated in elimination of 8-oxoG. The human OGG1 protein catalyzes the excision of 8-oxoG from DNA (Ide H et al, 2004; Zhang H et al., 2007). The 1245C→G polymorphism (Ser326Cys) is a well-known OGG1 gene polymorphism which results in substitution from serine to cysteine at codon 326 (Sakamoto T et al., 2006; Park HW et al., 2007), finally resulting in reduced DNA repair activity (Kohno T et al., 1998). Our results show that the presence of variant genotype significantly increased the risk of liver disease by more than two folds {OR=2.322 (1.555-3.467) at 95% CI, p<0.001}.

Moreover, the presence of hOGG1 variant genotype was found to significantly increase the risk of liver disease liver disease with underlying HAV [OR=1.989, p=0.024], HBV [OR=2.897, p=0.002] and alcoholic [OR=2.872, p=0.005] aetiology; and non-significantly increased the risk of HCV [OR=1.889], HEV [OR=1.663], and cryptogenic [OR=2.267] related liver disease; which signifies the role of hOGG1 variant genetic background in predisposing the patients to liver disease and severity with different underlying etiology in northeast India. Our data is contrary to recent reports from Srivastava et al., (2009), which showed that the frequency distribution of variants in controls was comparable in case of gall bladder cancer cases. It needs to be noted that high percentage of control cases (56.95%) from the northeast India enrolled and studied in our present study also harboured the hOGG1 variant genotype; which indicates the genetic predisposition of northeast Indian population towards different diseases susceptibility and severity, including liver diseases with different underlying etiologies. Our hOGG1 genotypic data is also clinically important since recent reports suggest that hOGG1 326cys polymorphism is associated with HCC risk (Yuan T et al., 2012; Sakamoto T et al., 2006). This fact is proved
by the fact that our data shows that hOGG1 variant genotype increases the risk of cirrhosis by more than three folds [OR=3.275], and most importantly in alcoholic cirrhosis cases [OR=3.023].

Another enzyme of BER system is X-ray repair cross complementing group 1 (XRCC1), which encodes a scaffold protein implicated in both single-strand break repair and BER (Han J et al, 2003). It interacts with DNA polymerase β (Caldecott KW et al., 1996; Kubota Y et al., 1996) and OGG1 (Marsin S et al., 2003). The presence of variant XRCC1 genotype was found to be associated with increased risk of liver disease in our studied cohort [OR=1.545 (1.059-2.255) at 95% CI, p=0.028]; and most importantly in alcoholic [OR=2.776, p=0.006] and HBV related liver disease cases [OR=1.991, p=0.037]. The frequency of homozygous variant genotype of codon 399 was observed in the present study (26.79%) was higher than the earlier reports from Indian population involving different diseases and carcinomas (Kiran M et al., 2009; Syamala VS et al., 2009; Sreeja L et al., 2008; Mittal RD et al., 2008; Majumder M et al., 2007; Ramach-andran S, 2006); and also higher than variant genotype frequencies in Caucasians which was 14 per cent for homozygous variant genotypes (Duell EJ et al., 2002). Our data also shows the alarming presence of variant XRCC1 genotype in control population (17.39%), which is comparable to recent reports from New Hampshire based study on white population which showed existence of variant genotype in 16.5% of the normal subjects (Nelson HH et al., 2002); but The frequencies were different from that reported in the Asians where 10% and below of the controls had variant genotypes for codon 399 (Ratnasinghe D et al., 2001; Zhang X et al., 2005; Park JY et al., 2002; Kim SU et al., 2002).

We found a significant increase in the risk of alcohol related cirrhosis compared to chronic [OR=4.127, p=0.003] and acute alcoholic cases [OR=3.00] because of the presence of variant XRCC1 genotype; which also signifies the prognostic importance of the XRCC1 genotype especially in alcoholic liver disease cases and is concurrent with recent reports from other groups (Li QW et al., 2012). Our novel data on XRCC1 protein expression analysis using immunohistochemistry method showed that the down-regulation of XRCC1 protein expression as a result of mutated XRCC1 genotype correlated with the staging and severity of alcoholic cirrhosis cases; which proves that deregulation in XRCC1 expression is associated with liver disease severity. This was also supported by the fact that XRCC1 variant allele was also found to be associated with higher ALT in all stages of ALD; and higher HAI score in Chronic hepatitis cases (p<0.029) and cirrhosis cases (p<0.001) for cases where details of biopsy based staging was available. Our data also showed that variant XRCC1 genotype is associated with increased
risk of FHF compared to controls [OR=2.063] and AVH [OR=1.375]; and risk of AVH compared to controls [OR=1.501]; which is similar to the very recent data from the Mexican American populations (Zhang L et al., 2012).

Cytochrome P450IIIE1 (CYP2E1) is an N-nitrosodimethyl-amine demethylase, which is constitutently expressed primarily in the liver (Ingelman-Sundberg M et al., 1993). Cyp2E1 takes part in the metabolism of drugs, but also activates a lot of precarcinogens and prepoison (Ramaiah SK et al., 2001). Cyp2E1 activity is mediated by various determinants-obesity, fasting, liver dysfunctions-and also by a number of environmental factors (Camus AM et al., 1993; Lucas D et al., 1998). Cyp2E1 activity is accompanied by generation of significant amount of reactive oxygen form (ROS), which damage cell membranes and macromolecules and leads to the formation of DNA abducts. Polymorphism in Cyp2E1 gene has been associated with malignancies of different cellular origins including liver (Sheng Y et al., 2009, Kang JS et al., 2007). CYP2E1*5B polymorphism (c2 genotype) is accompanied by a significant (10-fold) increase in enzymatic activity which leads to growth in carcinogens content in human body and to initiation of malignancies. (Watanabe J et al., 1994; Nomura F et al, 2003). The functional significance of CYP2E1*5B polymorphism might be due to its localization in presumed binding sites for hepatic transcription factor, hepatocyte nuclear factor-1 [Nomura F et al., 2003]. Rare c2 allele frequency constitutes 24%-30% for Asian populations [Kato S, 1992], 2%-3% for Caucasians [Hayashi S et al., 1991], 0.3%-7% for Afro-Americans [Hayashi S et al, 1991; London SJ et al, 1996], 15% for Mexican Americans [Wu X et al, 1997], and 18% for Taiwanese [Hildesheim A et al., 1995]. Our study showed that the prevalence of mutant c1/c2 Cyp2E1 RSA I allele was significantly higher in liver disease patients (p=0.002); and the presence of variant genotype increased the risk of liver disease by almost five folds {OR=4.937}. The prevalence of the c1/c2 genotypes was lower than that reported in other Asian countries, but amongst the highest reported in the Indian population [Soya SS et al, 2005].

Induction of cytochrome P450 2E1 by ethanol is believed to be one of the central pathways by which ethanol generates a state of oxidative stress and causes hepatotoxicity. Hepatic CYP2E1 enzyme activity is significantly higher in alcoholic patients with liver disease than in those without signs of liver disease [Dupont I et al., 1998]. It has been clearly shown through mice experiments that CYP2E1 plays an important role in binge ethanol-induced fatty liver, through inhibition of autophagy which promotes binge ethanol induced hepatotoxicity, steatosis and oxidant stress via CYP2E1 (Defeng Wu et al., 2012). In our study, Cyp2E1 c1/c2 (OR=11.30,
p<0.001) was significantly associated with alcoholic liver disease. In alcoholic liver disease cases, presence of Cyp2E1 variant allele significantly increased the risk of acute hepatitis [OR=8.071, (p=0.037)], chronic hepatitis [OR=11.30, (p<0.001)], and cirrhosis [OR=14.125, (p<0.001)] by multiple folds compared to controls. Mutant C1/C2 allele was also found to be significantly associated with cryptogenic hepatitis in liver disease patients from Northeast India [OR=8.071, p=0.020]. Our results on polymorphism of CYP2E1 influencing the risk for ALD is simi-lar to some reports [Ingelman-Sundberg M et al., 1993; Pirmohamed M et al., 1995], but contrary to others who have failed to find such an association [Savolainen VT et al., 1997].

ASSOCIATION OF ENVIRONMENTAL FACTORS IN LIVER DISEASE SUSCEPTIBILITY

Apart from the viral and host genetic factors, many ecological risk factors, especially dietary habits including Alcohol consumption, smoking, tobacco chewing and consumption of food containing higher concentration of nitrites and n-Nitroso compounds have been identified to play important role in the severity of liver disease and its further progression. Kirkali et al (2000) had previously demonstrated increased serum levels of nitrite and nitrate, which are metabolites of nitric oxide, in patients with liver cirrhosis. Our results which shows that the nitrite levels in plasma of liver disease cases were significantly higher (0.0346 ± 0.0237) compared to controls (0.0175 ± 0.0103) (p=0.011), which confirms the previous report from Kirkali et al. Compared to controls, the difference in plasma levels were found to be significantly elevated in HEV related liver disease cases (p=0.019), and chronic hepatitis B (p=0.002). Significantly higher nitrite levels in chronic cases compared to AVH cases (p=0.042) also. In alcoholic liver disease cases, significantly higher nitrite levels were present in ALD cases (p<0.001) and cirrhosis cases (p=0.007) compared to controls. Hence present data is indicative of the association of higher nitrite levels with susceptibility and severity of liver disease. The present data of higher nitrite concentration is clinically more important because of the higher prevalence of variant Cyp2E1 genotype in northeast India.

There is a concern to maintain the levels of nitrite as low as possible because of suspected adverse effects on oxygenation of the blood, and/or indirect carcinogenic effects, through formation of nitrosamines. Nitrites have been known to cause cancer directly. Although there is little correlation between nitrate/nitrite and nitrosamine content of food, nitrates and nitrites are agents in endogenous nitrosamine formation in the gastrointestinal tract [Spiegelhalder B et al., 1976]. Therefore, the presence of high nitrite concentration in raw and fermented mustard, radish
and betel leaf and nut as found in the present study, is also a high risk factor for adverse health effects, along with genetic and viral factors. Addition of nitrite-containing salts for storage of some dried fish products and fermented pork also adversely affects the quality of the food. The European Commission Scientific Committee for Food (document 111/5611/95) has recommended that nitrate and nitrite should be limited to an acceptable daily intake of 0.06 mg/kg, while the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2002) based on effects on the heart and lung in a 2-year rat study, recommended a acceptable Daily Intake (ADI) of 0-0.07 mg/kg bw/day, (as nitrite ion). Therefore, ingestion of the above food products that contain high nitrite concentrations is a high risk factor, especially for children.

Case-control studies have suggested that exposure to exogenous and possibly endogenous nitrosamines in food or tobacco in betel nut and cigarettes plays a role in the development of liver disease and cancer. There is evidence that endogenous nitrosation of areca nut alkaloids can occur in animals and humans, and areca-nut-derived nitrosamines, including 3-(methylNitrosamino) propionitrile, have been detected in the saliva of betel quid chewers which is a common practice in Guwahati and throughout Northeast India. Epidemiological data have linked the use of areca nut with other cancers such as liver cancer [Tsai JF et al., 2004]. The presence of volatile nitrosamines (N-diethylnitrosamine and N-dimethylnitrosamine) in raw fish was detected using the protocol followed by Mitacek et al (1999). The presence of volatile nitrosamines could be an indication of increasing pollution of the River Brahmaputra, which is one of the life lines of Northeast India, and its tributaries, from where the fish PUNTIUS SARANA is caught and fermented and dried. Our results is of grave importance as case-control studies conducted in Thailand have implicated traditional lifestyle and especially consumption of fermented-style fish and fermented vegetables [Srivatanakul P et al., 1991]. Fortunately, the nitrosamine levels were undetectable in fermented and dried fish, contrary to what has been reported in other countries [Mitacek EJ et al., 1999]. The non-detection of nitrosamines in fermented fish, irrespective of its presence in raw fish, could be attributed to the activities of lactic acid bacteria during fermentation.

It is suggested that generation of an oxidative stress through generation of ROS in the cell plays a central role in the damage of lipids, proteins, lipids and DNA (Davies, KJ, 1987; de Zwart et al., 1999; Marnett, LJ, 2000). A major product of ROS attack in genomic DNA is the pre-mutagenic lesion 7,8-dihydro-8-oxoguanine (8-oxoG), which causes G-to-T transversions (Grollman, AP et al., 1993; Michaels, ML et al., 1992). Analysis of 8-OHdG has been
established as an important biomarker to evaluate oxidative stress and to assess risk to cancer after exposure to various carcinogenic substances, environmental pollutants, and lifestyle factors (Kasai H et al., 2001; Cooke MS et al., 2002). the levels of 8-OH-dG was higher in the liver disease cases with different underlying aetiologies compared to controls, apart from HBV related liver disease cases which was comparative, the ALD cases showing the highest levels. The presence of 8-OH-dG was significantly higher in alcoholic liver disease patients compared to controls (p=0.03). The 8-oxoG levels were significantly elevated in HAV-FHF cases compared to controls (p=0.002) and HAV-AVH (p=0.035); and in alcoholic-cirrhosis cases compared to controls (p=0.001); and liver disease cases compared to controls (p=0.010). Higher 8-oxoG levels correlated significantly with mutant hOGG1 genotype (p<0.001). The main defense against the mutagenic effect of 8-oxoG is the base excision repair pathway, which in eukaryotes is initiated by the OGG1 protein, a DNA glycosylase that catalyzes the excision of 8-oxoG from DNA (Boiteux S et al., 2000). With the prevalence of higher hOGG1 variant genotype in liver disease patients as well as control population in northeast India as revealed from our study, it doubles the predisposition towards disease and carcinomas of different tissue origins as well to different grades of severity of liver disease in association with the hepatitis virus aetiology or alcoholic and cryptogenic liver disease.
Since limited information is available on the underlying molecular aetiology of liver disease development and progression in Assam and other parts of northeast India, which incidentally has a high load of liver disease patients of different grades of severity; the present case-control prospective study to explore the viral, environmental and genetic risk factors for liver diseases in Assam using molecular diagnostic tools. The clinically proven liver disease patients were enrolled from the Central hospital, NF Railway, Guwahati, with informed consent and all the clinical details; and blood samples were collected following the standard protocols following the ICMR regulations and guidelines. The following are the highlights from the present study:

I. The molecular epidemiology of hepatitis viruses involved in the liver disease development was evaluated and revealed the following:

- Hepatitis A virus is associated with maximum number of cases (30.80%) followed by HBV (25.89%) and Alcohol related liver disease cases (21.43%); while HCV infection was the least prevalent in our studied cohort (3.13%).
- Elevated SGPT levels (a marker for liver injury) was found to be elevated highest in case of HEV and HAV infection, while the SGOT levels was highly elevated in alcoholic liver disease cases.
- HbeAg status was found to be higher in chronic HBV cases compared to HBV related cirrhosis.
- Screening of two chronic HBV cases with HbeAg –ve, Anti-Hbe –ve cases is of clinical significance, as these cases as per available literature, are more resistant to antiviral therapies and are harder to treat.
- HBV Genotype D (51.66%) was the most prevalent in the HBV positive cases of our cohort, which also showed higher prevalence of Genotype A (20%) and Genotype C (18.33%) and was found to be associated with the severity of liver disease compared to genotype D.
- In HAV cases where genotyping was possible, HAV genotype IIIA was the major genotype in both the AVH and FHF group, while HAV genotype IA was the only other genotype found in our studied cohort.
• The existence of multiple HCV genotypes in a small number of isolates is indicative of existence of heterogeneity in HCV infecting strains as well as resulting diversity in the severity of HCV related liver disease in northeast India which may in turn relate to differences in treatment outcome.

• HEV genotype 1 was the only genotype found in our studied cohort. This has clinical significance, since genotype 1 and 2 have been reported to be associated with more severity of liver disease compared to other genotypes.

II. Along with viral factors the alterations in host genetic factors have been shown to play an important associative role in liver disease susceptibility, and were thus studied in the present study. Since DNA repair genes and metabolic pathway genes are instrumental in neutralizing the genotoxic stress because by assaults by different endogenous and exogenous agents, genotypes or polymorphism in key genes of BER pathway (hOGG1 and XRCC1) as well as Cyp2E1 gene was evaluated for their association with the predisposition of liver disease with different underlying etiologies. It was found that:

• The variant hOGG1 genotype was significantly associated with liver disease risk (p<0.001) and increased the risk of liver disease by more than two folds compared to controls [OR=2.322].

• The variant hOGG1 genotype was significantly associated with HAV (p=0.021), HBV (p=0.002) and alcohol related hepatitis (p=0.004), and significantly increased the risk of HAV [OR=1.989, p=0.024], HBV [OR=2.897, p=0.002] and alcohol [OR=2.872, p=0.005] related liver disease.

• The presence of the variant hOGG1 genotype increased the risk of cirrhosis by more than three folds [OR=3.275, p=0.068].

• The presence of variant hOGG1 was found to be associated with the severity of HBV related liver disease as it was found that the presence of hOGG1 polymorphisms increased the risk of chronic hepatitis compared to controls and AVH-B cases.

• In alcoholic liver disease cases, presence of hogg1 variant allele significantly associated with higher risk of alcohol related chronic hepatitis and cirrhosis compared to alcohol related acute hepatitis cases and healthy controls.
• Compared to controls the presence of *hogg1* variant allele significantly increased the risk of AVH (p=0.026) [OR=2.00, p=0.028]; and non-significantly increased the risk of fulminant hepatitis (p=0.447) [OR=1.889, p=0.702].

• The distribution of XRCC1 codon399 mutation was higher in liver disease cases (p=0.024), and it also significantly increased the risk of liver disease {OR=1.545, p=0.028} compared to controls.

• The variant XRCC1 genotype was associated significantly with HBV (p=0.029) and alcohol related hepatitis (p=0.004).

• The presence of XRCC1 variant genotype non-significantly increased the risk of cirrhosis by almost two folds [OR=1.816, p=0.310].

• In alcoholic liver disease cases, presence of *XRCC1* variant allele significantly increased the risk of chronic hepatitis compared to controls [OR=4.127, p=0.003] and non-significantly compared to acute hepatitis [OR=3.00, p=0.327].

• The presence of XRCC1 homozygote genotype increased the risk of alcohol related chronic hepatitis [OR=3.167, p=0.007], and cirrhosis [OR=3.167, p=0.089], by more than three folds compared to controls.

• *XRCC1* variant allele was also found to be associated with higher ALT in all stages of ALD; and higher HAI score in chronic hepatitis (p=0.029) and cirrhosis cases (p<0.001).

• The XRCC1 protein expression was down-regulated in cases of alcoholic-cirrhosis compared to controls, and interestingly, the cases showing down-regulated XRCC1 expression had XRCC1 mutated a genotype case; which underlines the importance of XRCC1 genotype and expression in the staging, severity and advancement of alcoholic liver disease.

• XRCC1 polymorphism was also found to be associated with severity of HAV related liver disease.

• The distribution of variant Cyp2E1 genotype c1/c2 was found to be significantly higher in liver disease cases compared to controls (p=0.002), and increased the risk of liver disease by almost five folds [OR=4.937].

• Presence of the variant Cyp2E1 genotype significantly increased the risk of liver disease in alcoholic cases [OR=11.30, p<0.001] and cryptogenic cases [OR=8.071, p=0.020]; and
non-significantly increased the liver disease risk in HAV \([\text{OR}=3.477]\) and HBV cases \([\text{OR}=3.082]\).

- Presence of Cyp2E1 variant allele also non-significantly increased the risk of cirrhosis \([\text{OR}=1.750]\), and chronic hepatitis \([\text{OR}=1.4]\), compared to acute hepatitis; and significantly cirrhosis \([\text{OR}=14.125, \ p=0.021]\) and chronic hepatitis \([\text{OR}=11.30, \ p<0.001]\) risk compared to controls.

III. Gene environment interaction plays an important role in deciding the rate and fate of disease progression, and hence, certain key and critical environmental factors were evaluated for their association with liver disease predisposition in northeastern population. These included the screening for the presence of nitrite in cases and controls as well as analysis of food samples which are routinely consumed in northeast India (some of which are indigenously prepared) for presence of nitrites and volatile nitrosamines. Presence of 8-oxo-dG, a DNA damaging agent and a marker of oxidative stress was also analyzed in liver disease cases and compared with control status. The experimental data revealed the following details:

- The nitrite levels in plasma of liver disease cases were found to be higher \((0.0346 \pm 0.0237)\) compared to controls \((0.0175 \pm 0.0103)\), the difference being statistically significant \((p=0.011)\).

- Compared to controls, the difference in plasma levels were found to be significantly elevated in only HEV related liver disease cases \((p=0.019)\).

- In cases with HBV aetiology, significantly higher nitrite levels was found in chronic HBV cases compared to controls \((p=0.002)\), and AVH-B cases \((p=0.042)\).

- Significantly higher nitrite levels were present in ALD cases \((p<0.001)\) and cirrhosis cases \((p=0.007)\) compared to controls.

- Majority of the fermented food products showed presence of very high value of nitrite which is detrimental to health.

- The highest amount of nitrite in raw material was found in mustard seed \((42\text{mg/kg})\), and the highest amount of nitrite in fermented food was found in fermented mustard i.e. kharoli \((43.2\text{mg/kg})\) which is consumed in large amounts in upper Assam areas; followed by beetle leaf \((pan, \ 9.6\text{mg/kg})\) and gundruk \((fermented \ radish \ leaf, \ 8.6\text{mg/kg})\).
• Detectable amounts of N-nitrosamines were found to be present in raw fish. Fortunately the presence of nitrosamines was undetectable in the fermented product.

• Normal range of 8-OH-dG levels in plasma ranges between 4-21pg/ml. 8-OH-dG levels was found to be much higher in both controls as well as liver disease cases.

• The presence of 8-OH-dG was significantly higher in alcoholic liver disease patients compared to controls (p=0.03).

• The 8-oxoG levels were significantly elevated in HAV-FHF cases compared to controls (p=0.002) and HAV-AVH (p=0.035); and in alcoholic-cirrhosis cases compared to controls (p=0.001); and liver disease cases compared to controls (p=0.010).

• Higher 8-oxoG levels correlated significantly with mutant hOGG1 genotype (p<0.001) which is the key enzyme for the repair of 8-oxoG related DNA damage.

Limitations of the study:

• The study had to be conducted with a limited number of liver disease samples, since it was single hospital/centre based, but since the Central hospital, NF Railway, Guwahati is a referral center for the entire Assam and many parts of NE India, non-biased representation of cases belonging to both tribal and non-tribal background could be enrolled and studied.

• Follow-up based study couldn’t be complete for many of the patients suffering from HAV or HEV, which limited the co-relation/associative studies to co-relate the genetic alteration(s) data with disease susceptibility and time course required to completely recover from the disease.

• Family screening based analysis for determining the path of infection could not be performed because of various limitations.

• The liver tissue based analysis for the protein expression of the genes evaluated in the present study was limited by the non-availability of liver tissue biopsies.

• The gold standard for diagnosis of cirrhosis is a liver biopsy, through a percutan-eous, transjugular, laparoscopic, or fine-needle approach. A biopsy is not necessary if the clinical, laboratory, and radiologic data suggests cirrhosis (Grant A et al, 1999). Hence it was not possible for us to collect the liver tissue sections for either all cirrhosis cases or for chronic hepatitis cases; which limited our protein expression analysis study.
Despite of these limitations, a planned and conscious effort has been made to elucidate the critical factors associated with the liver disease susceptibility, and the study results are novel to northeast India which incidentally has a high load of liver disease patients.

**CONCLUSIONS**

**To conclude,** the diversity of etiological factors associated with liver disease burden in Northeast India is enormous with respect to the high prevalence of certain hepatitis viruses, such as HBV, as well as the various HBV and HCV genotypes found in our study cohort. Moreover, strict vigilance and upgrading of overall hygiene standards is mandatory to investigate epidemics of HAV or HEV, which are also prevalent in Northeast India. Chronic alcoholism is critically associated with liver disease susceptibility and severity. High prevalence of genetic alterations of the critical hOGG1 and XRCC1 genes predisposes patients to susceptibility towards liver disease severity by restricting or malfunctioning the BER pathway activity. Higher oxidative stress due to higher 8-OH-dG in plasma of different liver disease sub groups as well as controls, with a faulty BER mechanism is a critical factor to liver disease risk in NE Indian population and is associated with the severity of liver disease in patients through gene-environment interaction. XRCC1 genotype and expression has prognostic significance with respect to liver disease risk and severity respectively. CYP2E1 polymorphism is supposedly associated with the risk of liver disease, especially in non-viral hepatitis patients, and the presence of higher nitrite concentration in fermented dietary products in Northeast India, and nitrosamines in ARECA CATECHU (betel nut) and raw fish, have clinical significance, because these environmental factors can act as additional risk factors in liver disease susceptibility, by virtue of the gene-environment interaction.
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Kits, Buffers and Reagents:

All the chemicals, media and reagents used were of Molecular Biology grade.

- **1M Tris**: 12.11 g of Tris-base was dissolved in 80 ml water. Desired pH was adjusted with the help of concentrated HCL. The final volume was adjusted to 100 ml with the help of MQ water; pH was adjusted as per requirement and was sterilized by autoclaving.

- **0.5 M EDTA (pH 8.0)**: 18.61 g of Disodium salt of Ethylene diamine tetra acetic acid 2 H2O was added to 80 ml water. Stirred vigorously on a magnetic stirrer. NaOH was used to adjust the pH to 8.0. It was sterilized by autoclaving after making the volume to 100 ml.

- **5M NaCl**: 29.22 g of sodium chloride was dissolved in 80 ml of water, volume raised to 100 ml with water and sterilized by autoclaving.

- **Proteinase K**: 0.15 g of powdered proteinase K was dissolved in 10 ml of autoclaved MQ water. It was filter sterilized.

- **10X Sera Lysis Buffer (50 ml)**:
  - 1M Tris pH 8.0 = 0.5 ml
  - 0.5 M EDTA = 1.0 ml
  - 5.0 NaCl = 1.5 ml
  - MQ H2O = 47.0 ml

- **TE buffer**:
  - 10mM Tris (pH 8.0)
  - 1mM EDTA (pH 8.0)

- **10X TBE (per litre)**:
  - Tris = 121g
  - Boric acid = 61.8 g
  - EDTA = 9.3 g
- **Gel Loading buffer:**
  - 0.25% bromophenol blue
  - 40% (w/v) Sucrose in water

- **Proteinase K buffer**
  - 1M Tris HCl (pH 7.4) 10 uL
  - 1M CaCl2 2 uL
  - MQ H2O 988 uL

- **Equilibrated Phenol:** Phenol was melted at 68°C. 8-Hydroxyquinoline was added to make the final concentration at 0.1%. Then several times extraction was carried out with equal volumes of 0.1 M Tris (pH 8.0) till pH 7.5-7.8 was reached. Finally two changes in 10mM Tris and 1mM EDTA (pH 8.0) was given and stored at 4°C after the last change.

- **Ethidium Bromide:** 5 mg/ml stock solution was made in water.

- **20% Sodium Dodecyl sulphate (20% SDS):** 20 gm of SDS was dissolved in 90 ml of water. Heated at 68°C to assist dissolution. pH was adjusted to 7.2 by adding 1-2 drops of concentrated HCl. Final volume was adjusted to 100 ml.

**Kits:**
- enzyme immuno assay kits for HAV IgM, Anti-HCV, anti HEV IgM was procured from the Immuno vision US, General Biologicals Corp. Taiwan, Wantai, China respectively

- Gel extraction, and PCR purification kit, DNA extraction kit was procured from Qiagen.

- ELIZA Kit for 8-hydroxy-2-deoxy Guanosine estimation procured from Cayman, US.

**Molecular biology reagents:**
- Taq DNA polymerase-PCR amplification and Sequencing kits were used from Perkin Elmer, Promega and MBI Fermentas, Germany.
- PCR purification and Gel extraction kits were obtained from Qiagen, Germany.
DNA ladders and PCR markers were bought from Promega and MBI Fermentas, Germany.

Restriction enzymes were procured from MBI Fermentas, Germany.

dNTP, 10X PCR buffer, 25mM MgCl₂ were procured from MBI Fermentas.
Annexure

PAPER PUBLISHED:


Paper presented at International Conferences:

- **Moumita Bose**, Bharati Baruah, Rajkumari Deblakshmi, Purabi Deka Bose, Subhash Medhi, Sujoy Bose, Manab Deka. Study the associative role of genetic alterations of BER pathway genes in liver disease susceptibility in Northeast India. Asia Pacific Association For The Study Of The Liver, Bangkok, Thailand, 2011 (Young Investigator Award by Liver Care Foundation)


- **Moumita Bose**, Sujoy Bose, Anjan Saikia, Bharti Baruah, Subhash Medhi, Anupam Sarma, Manab Deka. Risk Factors associated with hepatitis A virus (HAV) and alcohol related liver disease severity- a molecular diagnosis based study from Northeast India. EASL Annual conference, Bercelona, Spain, 2012. (Young Investigator Bursary award)


Paper presented at National Conferences
