SUMMARY AND CONCLUSIONS

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 polyno-orphism increased the risk of chronic hepatitis compared to controls and AVH-B cases.

• In alcoholic liver disease cases, presence of hog1 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 XRCC1codon399 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 [OR=3.477] and HBV cases [OR=3.082].

- Presence of Cyp2E1 variant allele also non-significantly increased the risk of cirrhosis [OR=1.750], and chronic hepatitis [OR=1.4], compared to acute hepatitis; and significantly cirrhosis [OR=14.125, p=0.021] and chronic hepatitis [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 ± 0.0237) compared to controls (0.0175 ± 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 (42mg/kg), and the highest amount of nitrite in fermented food was found in fermented mustard i.e. kharoli (43.2mg/kg) which is consumed in large amounts in upper Assam areas; followed by beetle leaf (pan, 9.6mg/kg) and gundruk (fermented radish leaf, 8.6mg/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 subgroups 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.