INTRODUCTION

The liver is one of the most critical organs in the body and is responsible for over five thousand life-sustaining functions, produces most of the building blocks used by the rest of the body and removes harmful chemicals. Its main function is the secretion of bile and the production of enzymes, which is essential for digestion of fats and control of the chemical processes in the body, respectively. The liver metabolizes most of the nutrients that are absorbed by the intestine, stores them, produces proteins and also detoxifies blood by removing drugs, alcohol, and potentially harmful chemicals from the bloodstream that would otherwise be poisonous. It has a role in the processing of cholesterol, maintenance of blood sugar levels, and the processing of drugs. All the varied roles performed the liver makes it susceptible to onslaught of various toxic components and damage which may eventually lead to advanced liver disease and hepatocellular carcinoma (HCC). Liver diseases and liver cancer shows a marked worldwide geographic and ethnic distribution (Shields and Harris, 1991). Different etiological factors have been associated with liver diseases and cancer including hepatitis virus infection, liver flukes, aflatoxins, alcohol, smoking and dietary nitroso compounds etc. (Bortolotti, 1994; Bosch and Muñoz, 1988; Shank et al., 1972; Austin et al., 1986; Thamavit et al., 1978)

Hepatitis viruses are hepatotrophic viruses which causes progressive advance liver disease leading to cirrhosis and hepatocellular carcinoma. There are five major hepatitis viruses; viz, Hepatitis A, B, C, D and E. All hepatitis viruses are RNA viruses except Hepatitis B which is a partially double stranded DNA virus. Hepatitis A is the most common viral hepatitis caused due to infection with Hepatitis A virus (HAV). HAV infection is self-limiting and can produce effects that range from a lack of symptoms to death from fulminant hepatitis. HAV produces acute hepatitis, but never chronic disease, inducing life time immunity. Worldwide there are an estimated 1.5 million cases of acute hepatitis A annually (World Gastroenterology Organisation Practice Guidelines). The risk of fulminant hepatic failure (FHF) rate is very low (0.01–0.1%), but increases with age and in those with pre-existing liver disease. Poor hygiene and poor sanitation pose the greatest risk factors for HAV infection. Like Hepatitis A virus infection, hepatitis E virus infection is self limited. HEV is a small, non-enveloped,
icosahedral virus with a positive-sense RNA genome of 7.2 kb belonging to the Caliciviridae family. HEV is regarded as the sole member of the genus Hepevirus (Emerson et al., 2004). HEV is transmitted primarily by the fecal–oral route. Large epidemics with person to person spread have been known to occur. The overall mortality rate in HEV infection is 1–3% and in pregnant women it varies from 15–25%.

The persistent infection with hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide. Currently 210 million people are infected with HCV worldwide (Shepard et al., 2005) with the largest percentage reported from Egypt, 20% of the nation’s population (Frank et al., 2000) In India at least 20 million people are infected with HCV; more than twice as high as HIV infections. In fact, Acute HCV infection becomes persistent in about 85% of cases and may cause chronic hepatitis leading to cirrhosis and, eventually, hepatocellular carcinoma (HCC). Unfortunately, most acute and chronic infections are asymptomatic. Six major HCV genotypes have been distributed worldwide and more than 50 subtypes (Stuyver et al., 1996). The lack of a vigorous T-lymphocyte response and the high propensity of the virus to mutate appear to promote a high rate of chronic infection. The extensive genetic heterogeneity of HCV has important diagnostic and clinical implications, perhaps explaining difficulties in vaccine development and the lack of response to therapy.

Hepatitis B virus (HBV) infection is a major health burden globally and causes both acute and chronic hepatitis. HBV is a noncytopathic, enveloped, 42nm virion containing an incomplete, open circular DNA genome, which consists of a full length 3.2-kb minus strand and an incomplete plus strand. According to the World Health Organization (WHO) over 5 million cases of acute hepatitis B infection occur annually. More than 2 billion people alive today have been infected by HBV, with 350 million people chronically infected (Lok and McMahon, 2007), and HBV causes approximately 1 million deaths each year worldwide. Chronic HBV infection is associated with a wide range of clinical manifestations, from an asymptomatic carrier state with a normal liver histology to severe and chronic liver diseases, including cirrhosis and HCC (Previsani and Lavanchy, 2002). Prevalence and incidence of HBV varies widely in different areas of the world. It is mostly prevalent in China, south-east Asia, sub-Saharan Africa, most Pacific islands, and the Amazon basin. The incidence of HBV infection and patterns of transmission vary greatly throughout the world, depending on local
endemicity, with rates between 0.1 and 120 per 100,000. HBV is transmitted sexually, parenterally, and from mother to infant at birth. The age at which the infection occurs plays a very important role. In infants under 1 year of age, chronic infection develops in 80–90% of cases; in children between the ages of 1 and 5, 30–50% develops chronic infection. By comparison, 30–50% of adults, who become actively infected with HBV are symptomatic, but only 2–6% of these adults develop chronic infection. Eight HBV genotypes are known, and difference in HBV genotypes plays an important role in deciding the pathogenesis and severity of liver disease, and their geographic distributions are also different. Hepatitis D virus (HDV) infection occurs only in conjunction with hepatitis B where it seems to function as a parasite. Moreover, Hepatitis D virus (HDV) which is a defective single-stranded RNA virus of the Deltaviridae family needs the hepatitis B surface antigen to transmit its genome from cell to cell.

Differences in Hepatitis B and Hepatitis C genotypes are linked to various degree of liver disease severity and rate of disease progression towards hepatocarcinogenesis. Similarly, difference in HAV and HEV genotypes has been reported to be associated with various degrees of severity of liver disease globally. Unfortunately there is no data available on these important aspects in liver disease patients from Northeastern India (NEI) who are ethnically distinct from the other parts of India, and unfortunately has the highest number of cancer incidences of various etiologies in the country as per the survey done by the National Cancer Registry program of Indian council for Medical research.

Alcohol is the major cause of liver disease and cirrhosis in Northeast India, where drinking of alcohol and indigenous prepared alcoholic beverages is customary in many tribal and non-tribal communities. The safe limits for alcohol intake are controversial. Guidelines recommended by the Royal College of Physicians advise a weekly limit of 21 units (210g) of alcohol in men and 14 units in women. But excessive alcohol consumption is a familiar condition in NE India, resulting in a heavy burden of ALD patients in NE India. The three most widely recognised forms of alcoholic liver disease are alcoholic fatty liver (steatosis), acute alcoholic hepatitis, and alcoholic cirrhosis. Understanding of the mechanism and identification of key factors associated with ALD predisposition and severity is indispensable for correct and planned therapeutic
interventions for patient prognosis and treatment. In this line, the evaluation of genetic predisposition towards ALD will serve in identifying indigenous sub populations in NE India (especially the tribal population) with higher risk of liver damage in future.

The rate of development and progression of liver disease differs amongst different individuals, indicating the role of other factors in liver disease development. Along with the age, sex and the viral factors, alterations in host factors are also considered as one of the important event in the development of liver disease. According to epidemiological studies, 90% of cancers are associated with the impact of environmental factors, including nitrosamines, which are acquired through tobacco smoke, vehicle exhaustions and foodstuffs (Guslitser, 2001). Studies show that nitrite alone can cause cancer; however, an even more serious cause of concern is the well documented potential of nitrites/nitrates to cause cancer through the formation of nitrosamines. Cytochrome P450IIIE1 (CYP2E1) is an N-nitrosodimethyl-amine demethylase, which is constitutently expressed primarily in the liver. It takes part in the metabolism of drugs, but also activates a lot of precarcinogens and prepoison. Cyp2E1 activates N-nitrosamines, contained in tobacco smoke and foodstuffs and several industrial and endogenous carcinogens (Guengerich et al., 1991; Nakajima and Aoyama, 2000; Bartsch et al., 2000). Cyp2E1 activity is mediated by various determinants-obesity, fasting, liver dysfunctions-and also by a number of environmental factors (Camus et al., 1993). Cyp2E1 activity is accompanied by generation of significant amount of active oxygen form, which damage cell membranes and macromolecules and lead to formation of DNA abducts. Polymorphism in Cyp2E1 gene has been associated with malignancies of different cellular origins including liver. CYP2E1 polymorphism in the 5’regulatory region with C→T replacement at position -1019 and Rsal restriction site loss (CYP2E1*5B) is one of the most important polymorphisms identified (Watanabe et al., 1990; Hayashi et al., 1991). Homozygous c2/c2 genotype is associated with 10-times increase in enzymatic activity which leads to growth in carcinogens content in human body and to initiation of malignancies (Kang et al., 2007; Nomura et al., 2006).

Oxidative stress increases damage to cellular components, including DNA. 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 (Kiran et al., 2009). Genetic variants of OGG1 and XRCC1, important enzymes
participating in BER pathway, may confer inter-individual variations in susceptibility to liver diseases and cancer (Srivastava et al., 2009). The human OGG1 protein catalyzes the excision of 8-oxoG from DNA. The hOGG1 1245C→G polymorphism results in substitution from serine → cysteine at codon326, finally resulting in reduced DNA repair activity. Another enzyme of BER system is XRCC1, which encodes a scaffold protein implicated in both single-strand break repair and BER. It interacts with DNA polymerase β, and OGG1. Genetic polymorphisms of DNA repair genes have been reported to determine susceptibility to several cancers, including lung, esophageal, bladder, and non-melanoma skin cancers, liver cancer etc (Ratnasinghe et al., 2001; Xing et al., 2001; Stern et al., 2001; Cho et al., 2003; Cleaver and Crowley, 2002).

Reactive oxygen species (ROS) possess a high reactivity of a variety of biological linked molecules, among which, DNA is one of the most important targets (Halliwell, 1998). Oxidative DNA damage, caused by either endogenous or exogenous source of ROS, has been to aging, chronic degenerative diseases, inflammatory diseases and cancers. Among various types of DNA base modifications induced by ROS attack, 7,8-dihydro-8-oxoguanine (8-oxoG) has been the most widely studied and is considered as a key biomarker of oxidative DNA damage(Kasai, 1997). Leaving unrepaired, 8-oxoG is highly mutagenic because of its propensity to mispair with adenine during DNA replication, ultimately yielding GC to TA transversion. To minimize 8-oxoG accumulation within genomes, this lesion is subjected to DNA repair primarily through the base excision repair pathway. A key component of this pathway in eukaryotes is OGG1, a DNA glycosylase/α-lyase that recognizes 8-oxoG opposite cytosine (Bruner et al., 2000). Inactivation of the OGG1 gene generates a mutator phenotype characterized by GC-TA transversions in yeast. Analysis of the human OGG1 gene (hOGG1) and its transcripts in normal and tumoral tissues has revealed alternative splicing, polymorphisms and somatic mutations. The repair effectiveness of OGG1 may be modulated by gene polymorphisms. A Cys326Ser substitution in exon 7 has been the most extensively studied. The Cys326 isoform is postulated to exhibit reduced 8-oxoG-repair activity, increase susceptibility to cancers, nevertheless, controversy still remains (Janssen et al., 2001; Xu et al., 2002; Wikman et al. 2000; Hanaoka et al., 2001).
Application of molecular genetic techniques in human disease and cancer risk assessment has emerged as a method of identifying subpopulations with different sensitivities to carcinogen exposure. Liver diseases and cancer show a marked worldwide geographic and ethnic distribution. Northeastern part of India is a totally diverse population from rest of India with their unique and diversified cultural systems and food and living habit. As such there is limited or almost no study till date signifying the prevalence of different hepatitis viruses and their genotypes in liver disease cases in Northeast India. Similarly, study of host genetic factor alterations and gene-environment interaction (Cyp2E1, XRCC1 and hOGG1 gene) in hepatitis infection cases and risk of developing advanced liver diseases is of immense importance. We therefore have undertaken the present case-control study to explore the viral, environmental and genetic risk factors for liver diseases susceptibility and severity in Northeast India using molecular diagnostic tools. It is assumed that the scientific data generated through the experiments with respect to Northeast India will be immensely helpful in better liver disease patient management, development of prognostic markers and novel therapeutics in the future.