CHAPTER 1
INTRODUCTION
Hepatitis E virus (HEV), the causative agent of human hepatitis E, is a non enveloped, single-stranded, positive sense RNA virus in the genus Hepevirus of the family Hepeviridae (Ahmad et al., 2011; Emerson and Purcell, 2004). HEV is a key public health disease in many developing countries and is also-endemic in some industrialized countries (Ahmad et al., 2010; Purcell and Emerson, 2010). HEV transmission occurs primarily by the fecal–oral route through contaminated drinking water or water supplies in areas with poor sanitation (Ahmad et al., 2011; Kamel et al., 2011). The disease mainly affects young adults, and although the mortality rate is generally less than 1%, it can reach up to 28% among infected pregnant women causing acute liver failure (Bose et al., 2010; Hamid et al., 1996).

Acute liver failure in pregnant women is an explosive disease with short pre-encephalopathy period than acute liver failure in non-pregnant women (Khuroo and Kamili, 2003). These patients had rapid development of severe encephalopathy and more often presenting with characteristics of cerebral edema and cerebellar coning.

Nucleotide substitution, insertion and deletion (indel) events are the key driving forces that have formed genomes. Single point mutations in the capsids of poliovirus (Westrop et al., 1989), infectious bursal disease virus (Loon et al., 2002), and adeno-associated viruses (Wu et al., 2006) have all been recognized to have considerable effects on both in vitro and in vivo growth uniqueness of the viruses. DNA mutation patterns in the human genome have been thoroughly verified with the identified human ribosomal protein (RP) pseudo gene sequences, (Zhang and Gerstein, 2003). Amino acid substitutions in viral proteins that are associated with changed pathogenesis have been well acknowledged (Brack et al., 1998; Raychaudhuri et al., 1998). The viral RNA 50- and 30-untranslated region sequences can also influence the expression of disease symptoms (Slobodskaya et
In a recent study, 25 specific nucleotide positions were identified to possibly being involved specifying the host range of HEV or determining the severity of hepatitis E disease (Fu et al., 2011). In an another study, one substitution common to almost all human HEV strains in a separate cluster was located in the helicase domain (V239A) and which might be associated with increased virulence suspecting a zoonotic origin of the cluster related viruses due to the presence of the signature substitution (Takahashi et al., 2009). Similarly, genotype 3 isolates revealed 15 unique amino acid substitutions and one deletion in open reading frame (ORF) 1, and three substitutions in ORF 2 (Si et al., 2009). Another study (Liang et al., 2010) reported that the amino acid occupying position 606 plays a critical role in maintaining the antigenicity of the HEV p166 proteins. In a previous study done in pigs it was observed that three amino acid mutations (F51L, T59A, and S390L) in the capsid protein of the HEV collectively contribute to virus attenuation (Cordoba et al., 2011). Further, a silent substitution of U3148 in HEV was observed which might be associated with the development of acute liver failure (Inoue et al., 2006).

In an Indian study, two mutants were reported demonstrating NTPase/RNA helicase activity of the helicase domain of HEV ORF1 which completely lost the ability to unwind RNA duplexes with 5' overhangs (Karpe and Lole, 2010). Inoue et al. in 2006 suggested that a silent substitution of U at nt 3148 in genotype 4 of HEV is associated closely with the occurrence of fulminant hepatitis and in the year 2009 he reported that the silent substitutions at U 3148 and C 5907 in genotype 3 and genotype 4 are closely associated with the occurrence of fulminant hepatitis and severity of hepatitis and also substitution at C 5907 is associated with high viral load of HEV. Other studies on Hepatitis A and B virus genomes have been reported to affect the disease severity and the final outcome (Fujiwara et al., 2001 and Koh et al., 1995). The full-genome-based comparisons of genotype-1 viruses from acute viral hepatitis and fulminant hepatic failure cases revealed that they are distinctly different from the majority of the genotype-1 viruses derived from acute viral hepatitis cases from different countries which suggests the probable role of virus in causing severe disease (Mishra et al., 2013).
HEV is a significant but extremely understudied human pathogen, and the mechanisms of HEV replication and pathogenesis are largely unknown. It has been well recognized that one or more amino acid changes in capsid or envelope proteins can contribute to the attenuation phenotype of viruses (Polo and Johnston, 1990). Though whole genome studies on HEV from India including North India have been reported, very few studies have reported about the nucleotide substitutions in HEV and its association with acute liver failure. In a study from Western India, in comparison to type 4 HEV isolates, 26 unique amino acid substitutions were recorded, 16 in ORF-1, 8 in ORF-2 and 2 in ORF-3 in an Indian swine isolate which showed insertion of 'C' at 5159 position while all other type 4 isolates had insertion of 'U' at the same position but whether those changes contributed towards observed absence of type 4 HEV infections in Indian patients needed to be determined (Chobe et al., 2006). Only a single study from western India has reported about the significance of amino acid mutations and nucleotide substitutions in the helicase domain which may play a vital role in determining outcome of HEV infection (Mishra et al., 2013). Such study on HEV in Northern region of India has not been carried out so far.

The present study was designed to study the nucleotide substitutions in the HEV isolates from North Indian population with the following objectives:

1. To study the substitution profile in the HEV genome consisting of the:
   i) non-structural ORF 1 region.
   ii) region encoding the ORF 2 protein which encodes the viral capsid protein.
   iii) region encoding the ORF 3 protein which is the regulatory protein.

2. To determine the viral load and genotypes of HEV in HEV positive patients.

3. To correlate the mutational profile of the genome with the viral load, disease severity and final outcome of the disease.

4. In-silico prediction of effective si-RNA targets against HEV.
Introduction

It is expected that the results obtained from the study will help in understanding the pathogenesis of HEV, the evolution of the virus and in the identification of new regions which may be the drug targets for the development of new vaccines of HEV. Besides, the association of the severity of the disease with the genotypes can be determined which are now confined to certain geographical regions of the world. The identification of new mutations will lead to new insights in the evolutionary origin of the virus by phylogenetic analysis. The study may prove to be useful in the development of strategies for identifying and screening approaches for the early detection of HEV infected patients who are in more risk of developing acute liver failure. Nucleotide substitutions can also help to study the effect of drugs like ribavirin which have been used in patients with acute on chronic liver failure who have ongoing HEV viremia and it is in this category of patients, Ribavirin therapy has been found to be useful.