3.0 Exposition of the problem
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Hepatitis A is a major public health problem that causes significant morbidity and mortality all over the world. Approximately 1.5 million cases of disease with overt symptoms are reported annually worldwide. However, this figure underestimates the true incidence of the infections on account of asymptomatic infections (Martin and lemon 2006:s164-s172). Globally endemicity of HAV infections has been described in four major patterns that include very low, low, intermediate and high patterns, based on the age specific prevalence of antibodies to HAV. Transmission occurs primarily from person to person, however, outbreaks due to contaminated food or water are also reported (Hutin et.al 1999:595-602; Poonawagul et.al 1995:705-708). Hepatitis A is a classic example of an infectious disease in which the degree of endemicity is closely related to the level of economic development and accompanying living conditions. In recent times, improvements in sanitary and hygienic conditions in India have led to a reduction in the endemicity of the disease from hyperendemic status to intermediate status that is associated with epidemic form of the disease (Chitambar et.al 1996: 781-783; Chitambar et.al 1999:273-276; Arankalle et.al 2001:293-303, Arankalle et.al 2006:760-769). This transition can be expected to result in disease patterns characterized by increased morbidity, increased disease burden and large community outbreaks thereby increasing healthcare cost.

Hepatitis A is known to exist in a wide spectrum of disease presenting mild to severe forms. Severity of disease is manifested as persistent infection, fulminant hepatic failure, renal failure, late recovery, clinical relapse, cholestatic hepatitis and chronic hepatitis (Chio and Bakir 1992:413-416; Inoue et.al 1996:322-324; Bendre et.al 1999:1107-1112; Saunders et.al.1979:569-584; Glikson et.al.1992:14-23; Schiff,1992:s18-s20; McDonald et.al.1989:223-228). Several neurological syndromes such as myelitis, peripheral neuropathy, myeloradiculopathy, mononeuritis, exacerbation of multiple sclerosis and Guillain-Barre Syndrome (GBS) have been reported in association with hepatitis A (Adams and Asbury 1980:2097-3030; Owen et.al.1980:2307-2309; Bosch et.al.1983:685-687; Pelletier et.al. 1985:53-56; Chitambar et al 2006:1011-1014).

The causative agent of hepatitis A - HAV has been characterized at molecular level to determine the genotypes in different geographic regions. Using different HAV strains available globally seven genotypes were defined on the basis of molecular sequences in the VP1/2A junction region (Robertson et.al 1992:1365-1377). However, full length genome sequencing reclassified the two strains CF-53 and SLF88 as genotype IIA and IIB respectively which were previously classified as genotype II and
genotype VII using VP1/2A junction region (Ching et al. 2002:53-60; Lu et al. 2004:2943-2952). The new classification resulted into only three human (I-III) and three simian (IV-VI) genotypes of HAV. The discrepancies of the genotyping method based on VP1/2A junction region surfaced vividly when three antigenic variants, two strains from shellfish borne outbreak in Spain and one strain (Uru-3) from Uruguay of HAV could not be differentiated (Costa Mattioli et al., 2002:9516-9525; Sanchez et al., 2002:4148-4155). Necessity of nucleotide sequences of other or larger genomic fragments for phylogenetic analysis was also identified due to limitations of VP1/2A junction region for classification of the strain DL3 from China (Guo-Dong et al. 2003:499-504).

Although, the studies conducted for genotyping of HAV strains indicated predominance (80%) of genotype I all over the world, these studies over represented the strains from USA, Sweden, Japan and France-the countries with low endemicity of hepatitis A and lacked the data on characterization of HAV strains circulating in hyperendemic regions such as South America, India, North and Central Africa (Costa Mattioli et al., 2003: 3191-3201).

Limited studies carried out to characterize HAV strains from India have shown cocirculation of genotype IA, IB along with predominance of genotype IIIA (Khanna et al. 1992:118-124; Arankalle et al. 2006:760-769; Hussain et al. 2005:16-24; Chitambar et al. 2007 :85-93). The prototype strain of genotype IIIA, PA21 was originally isolated from Panamanian owl monkeys (Brown et al. 1989 :4932-4937) and was subsequently reported from Sri Lanka, Nepal, Malaysia and USA (Jansen et al. 1990:2867-2871; Robertson et al. 1992:1365-1377;). This genotype has caused outbreaks and re-emergence of the disease in Europe and is becoming more prevalent than formerly assumed (Stene-Johansen et al. 1999:3725-3729; O’Donovan et al. 2001:469-473; Costa-Mattioli et al. 2003:3191-3201, Tallo et al. 2003:187-193; Stene-Johansen et al. 2005:2739-2745; Spada et al. 2005:958-964; Tjon et al. 2005:360-366). Moreover, greater genetic variability within genotype IIIA than within genotype IA strains which are predominant globally has been suggested (Stene-Johansen et al. 2005:2739-2745).

Globally, large numbers of HAV isolates have been characterized by analyzing short genome fragments. This strategy has ignored the possibilities of recombination events that may occur in other genomic regions. Hence, complete genome sequencing was recommended in determining the frequency of intra and intertypic recombination of HAV and the newly emerging genetic and antigenic variants (Costa-Mattioli et al. 2003:51-59). Further, characterization of full-length genomes becomes indispensable in India due to cocirculation of more than one genotype (Hussain et al. 2005:16-24; Chitambar et al. 2007:85-93). To date entire or nearly entire nucleotide sequences of a total of 33 human and 1 simian HAV strains have been deposited in
These 33 human HAV isolates include only 5 strains of genotype IIIA, one each from Norway and Germany and three from Japan (made available in the GenBank in March 2007) the countries with low endemicity of hepatitis A. Thus at the time of inception of the present study full length genome characteristics of HAV genotype IIIA were underreported from the regions known to be endemic for hepatitis A. In view of this, present study was undertaken to characterize full-length genomes of HAV genotype IIIA that is circulating predominantly in India.

Another important reason to examine full-length genomes of HAV is the data from in vivo and in vitro studies on attenuated and cytopathic HAV strains (Karrcn et al 1988:338-345; Tedeschi et al, 1993:16-22; Brack et al 1998:3370-3376). The two biological properties of HAV, replication in cell culture and its virulence in primates and humans have been found to share at least some important genetic determinants (Mao et al 1989: 621-624; Rezende et al 2003:613-618). It has been emphasized that studies on genetic factors affecting the immune response are essential to analyze the mechanism underlying the disease severity (Sainokami et al 2005: 1165-1175). Few studies have been carried out to determine nucleotide substitutions in the HAV genome of FHF patients (Fujiwara et al 2001:112-119; Fujiwara et al 2007; 871-877; Fujiwara et al 2007;560-566). Genotyping has not been found beneficial as genotype-determining region has not always reflected antigenic variations and has shown no correlation with severity of the disease (Fujiwara et al 2003:124-134). Moreover, though the severity of hepatitis A could be attributed to the host factors (Stapleton et al 1995:s9-s14) the variant virus may need further examination for the possible potential to alter clinical status of disease that might originate in the mutations of HAV itself. Thus, even though HAV has been studied extensively, and variable spectrum of hepatitis A is known, correlation of the molecular characteristics of the virus and the clinical status has not been established.

The present study reports molecular characterization of full length genome sequences of three Indian strains of HAV genotype IIIA recovered from hepatitis A patients, two with acute infection and one associated with GBS subtype acute motor axonal neuropathy (AMAN).