Chapter 6

Summary
F. SUMMARY

Hepatitis C virus (HCV) is the leading cause of most cases of non-A-non-B hepatitis (NANBH) worldwide. World health organization (WHO Report, 1999) has estimated 170 million (3%) people of the world are infected with HCV, which highlights HCV as a major human pathogen. In India, approximately, 1.8% of the general population are infected with HCV till date. The recent reports from Western, Northern and Southern regions of India revealed increasing prevalence of HCV infection in different risk groups and this situation necessitated further molecular biological investigations to reveal the molecular characteristics of the HCV strains circulating in India. This study was initiated to carry out genotyping of HCV in different risk populations and also in general population and to perform a comparative genome analysis of selected isolates showing interesting features from Eastern India.

The summary of the outcome of the study are:

1. A very high prevalence of HCV infection was observed among liver disease patients with 11.09% of the acute and 25.32% of the chronic cases. This data clearly indicates that HCV is emerging as a major cause of liver diseases in this part of the country.

2. Among the cirrhosis and hepatocellular carcinoma patients, infection rate of HCV was quite high, with 16.6% and 58.6% among them respectively. On the other hand, a very high percentage (42.4%) of HCV positive cases were detected from intravenous drug users of Kolkata and its suburbs. Among
haemophiliacs 10.3% and among thalassemic 42.8% patients were positive for HCV.

3. A relatively high prevalence (4.1%) was observed among the voluntary blood donors in urban areas, whereas, among asymptomatic village population the incidence was comparatively low (0.87%). In both the groups, 3a was the predominant genotype.

4. Among the acute liver disease patients, the predominant genotype was 3b -38.06% followed by 3a -29.57%. A significant percentage (7.04%) of samples were 'untypeable' by RT-PCR. Among the chronic hepatitis population, the most prevalent genotypes were 3a -34.67% and 3b -47.67%. Moreover, 5.31% samples could not be typed by the present genotyping scheme and 1.23% were of mixed genotype (3a/3b). Genotype 1b was predominant among hepatocellular carcinoma and cirrhosis patients.

5. Phylogenetic analysis of the untypeable isolates from liver disease cases showed that 8 of them (NB67, NB82, NB125, NB128, NB131, NB135, NB187, NB192) had maximum homology with type 3a isolates and five (NB42, NB57, NB134, NB211, NB236) showed maximum homology with type 3b isolates. Another isolate (NB56) showed maximum homology with the strain Th580, which is believed to be of genotype 6b. Another one (NB74), showed 90.4% homology with an American strain H77, typed as 1a and 2 isolates (NB179 & NB193) showed maximum homology with an Indian strain, IND308, which was closely
related to a Nepalese strain NE137, typed as 3b. Three untypeable isolates from asymptomatic village population showed maximum homology to a Nepalese isolate NE145, which was designated as genotype 3e.

6. The analysis of all population parameters (Shannon entropy, Intrasample genetic distances & Mf) revealed a fluctuating behavior in the structure of the circulating quasispecies during the study period. Average genetic distance of the individual clones in the quasispecies population was greater in genotype 1b than in 3a or 3b, indicating the greater capability of 1b to evolve into chronicity.

7. Nucleotide and amino acid entropy, genetic distances, the proportion of synonymous and nonsynonymous substitutions, and $K_a/K_s$ ratios did not differ among the different genotypes. None of the parameters used to assess core-HVR-1 heterogeneity correlated with age.

8. Nonsynonymous substitutions were significantly more frequent than synonymous substitutions in the responders while there was no significant difference in the nonresponders.

9. Genetic divergence was outstanding in the envelope regions (E1 and NS1/E2, > 76.9% identity) in contrast to the sequence conservation of the 5' non-coding (> 95.8% identity) or core regions (> 82.0% identity). Significantly more nucleotide mutations were observed in the C-terminal one-third of HCC
isolates compared with the number of mutations in isolates from patients without HCC.

10. Although hypervariable regions were found in the NS1/E2 region, hydropathicity was maintained for all clones, reflecting the biological functions of these regions.