

Prevalence of Hepatitis C Virus Genotypes in Asymptomatic Blood Donors in NCT of Delhi

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Abstract

Hepatitis C virus shows high genomic diversity which poses a challenge for drug therapy and vaccine development. Here, we present a study on prevalence of HCV genotypes and subtypes in blood donors of Delhi. Forty two HCV samples were taken from blood bags found positive at various blood banks located in Delhi. From these samples 5'UTRs were amplified, sequenced and genotyped. Genotype 1, 3 and 4 were detected but genotype 1 of HCV was found prevalent. Based on sequence analysis the HCV isolates were further categorised to represent subtype 1a, 1b, 3a, 3b and 4a.

Keywords: Hepatitis, Hepatitis C virus, HCV

1. Introduction

Hepatitis C virus (HCV) belongs to family Flaviviridae and genus Hepacivirus. The molecular techniques were used for the first time in 1989 for its detection and later characterized to have single stranded RNA of positive polarity as genomic material. The HCV genome harbours single open reading frame flanked by untranslated regions (UTRs) at both ends[1]. After the discovery of hepatitis C virus, it became clear that HCV was the major cause of acute hepatitis after a blood transfusion. In most of the cases of acute Hepatitis-C, patients are asymptomatic and unaware of the underlying infection. It is also recognized as one of the common generic agent for liver cirrhosis. Large differences have been observed in the HCV genome between strains from different geographical regions allowing the virus to be classified into six major genotypes; and each genotype containing a number of more closely related subtypes[2]. The distribution and prevalence of the HCV genotypes seen to have a geographic influence and specific genotypes tend to be concentrated within certain geographic areas. On a global distribution analysis, HCV subtypes 1a and 1b of genotype 1, are most common in United States[3], while in Japan subtype 1b is responsible for about

73% of HCV infection[4]. HCV genotype-4 is predominant in North Africa and the Middle East[5]. In India, genotype-3 is predominant in all parts of India except south, while genotype-1 is shown to be the predominant genotype in southern parts of India[6]. Hissar *et al* reported the predominance of genotype-3 in central India in addition to northern parts[7]. The incidence of genotype-1 was also reported from North, West and Eastern regions of India. Besides genotype-1 & 3, genotype 4 has been reported from south India[8]. Very few studies have been done on the distribution of various hepatitis C virus genotypes in India and mostly from metropolitan cities[9]-[13]. Recently, a study also hinted towards geographic variation in the prevalence of various HCV genotypes in India[14]. Different genotypes of HCV responds differently to different therapies. The different responses to different therapies have been shown by HCV genotype-1, genotype-2 and genotype-3[15][16]. Therefore, determination of genotype of the infecting Hepatitis C virus is very important for disease management, disease treatment, epidemiological studies and development of vaccines. This strategy

may be helpful in controlling the Hepatitis C viral infection before causing diverse loss to the patient.

The present assignment was undertaken to find out the prevalence and dispersion of HCV genotypes and subtypes in the blood donors in NCT of Delhi.

2. Materials and Methods

Forty two blood bags found positive for HCV, during the screening at blood banks located in NCT of Delhi were selected. HCV RNA was extracted using QIAmpMiniElute virus spin kit (QIAGEN, Germany). The nested-PCR was performed to amplify conserved UTR. The 5'UTR from HCV RNA was reverse transcribed and amplified with primers 5'-ACT GTC TTC ACG CAG AAA GCG TCT AGC CAT -3' and 5'-CGA GAC CTC CCG GGG CAC TCG CAA GCA CCC -3'¹⁷ using one step RT-PCR kit (M/s QIAGEN GmbH, Germany) with its standard reaction conditions. The inner region of 5'UTR amplicon was amplified using *Taq* PCR kit (New England Biolabs) with 20 pmoles of each inner primers 5'-ACG CAG AAA GCG TCT AGC CAT GGC GTT AGT -3' and 5'-TCC CGG GGC ACT CGC AAG CAC CCT ATC AGG -3'¹⁷. The thermal cycling was performed in GeneAmp PCR system 9700 (Applied Biosystems) with: denaturation at 94 °C for 5 min followed by 40 cycles consisting of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min and final extension at 72 °C for 7 min. The final 256-bp amplicons were purified using QIAquick PCR purification kit (QIAGEN, Germany). The nucleotide sequences were determined in both directions by using BigDye Terminator v 3.1 Cycle Sequencing kit and Genetic Analyzer 3130xl (Applied Biosystems). The sequences were analysed using BLAST and clustal W alignment tools. Genotype and subtype identities were assigned to these sequences based on pair wise alignment to the HCV full length reference sequences, using the NCBI HCV genotyping tool. The percentages of genotype and subtype were calculated. The sequences were submitted to EMBL/GenBank under accession numbers LN681360 to LN681401.

3. Results

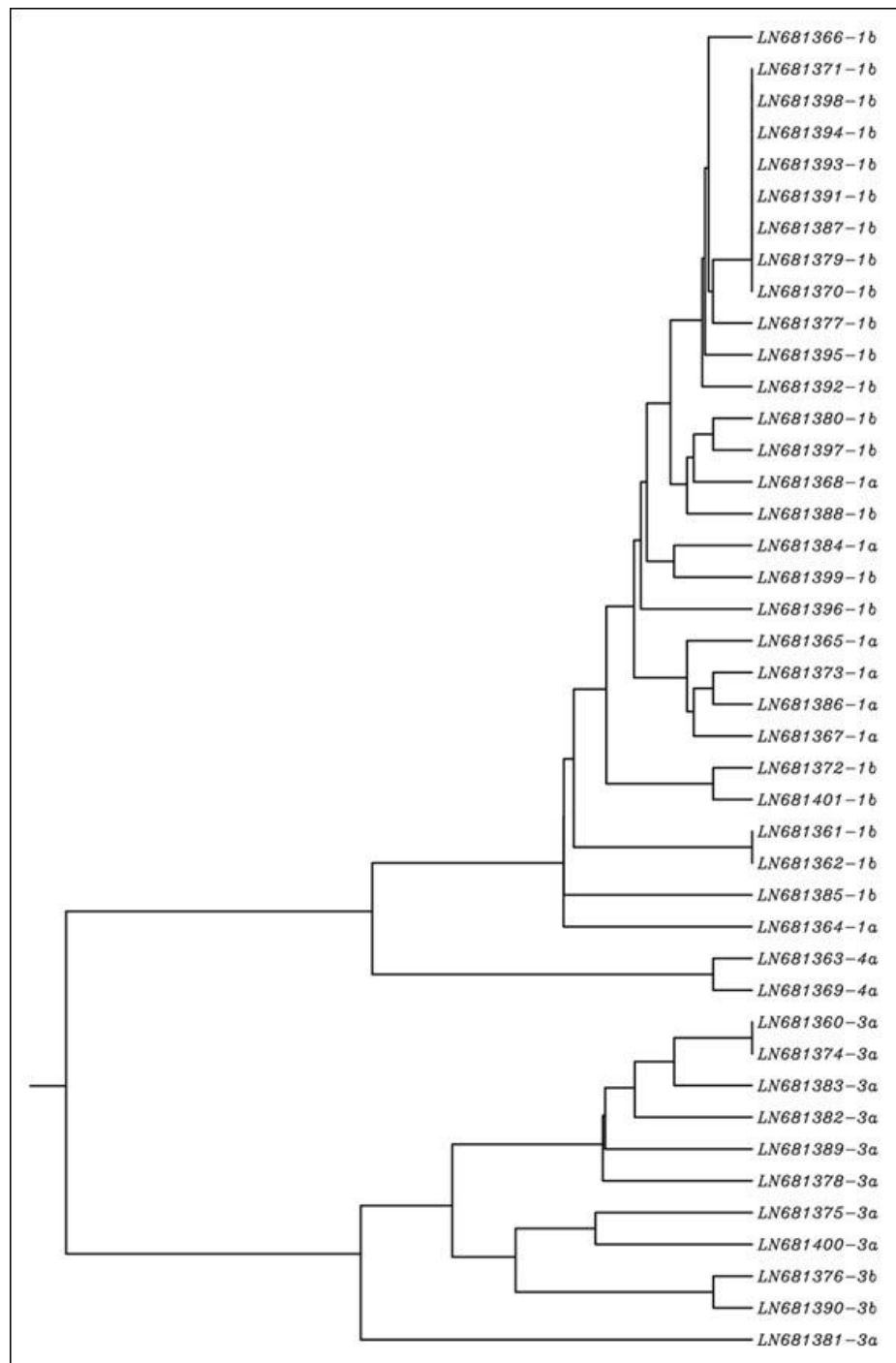
All 42 HCV RNA positive samples were subjected to genotype determination by sequencing. The analysis revealed the presence of genotype 1, 3 and 4. Genotype 1 was detected in 29 (69.04 %) samples. The genotype 3 was observed in 11 (26.19 %) samples. Genotype 4 was seen in only 2 samples. The subtype and other details are mentioned in table 1.

Table 1: Prevalence of Hepatitis C virus in blood donors in NCT of Delhi

Genotype	Subtype	No of cases	% age
1	1a	7	16.66
	1b	22	52.38
3	3a	9	21.42
	3b	2	4.76
4	4a	2	4.76

Figure 1 shows the rooted phylogenetic tree, generated using the neighbour-joining method for nucleotides. All the isolates were seen to form clusters to match their genotypes (Figure 1). This study shows that genotype 1 is more prevalent among blood donors in NCT of Delhi. The predominance of genotype 1 as per this report is in contrast to other reports because; the samples used in this study belonged only to asymptomatic blood donors. The overall predominance of a genotype can only be determined when samples are taken randomly and from all patients at various stages of infection. The heterogeneity and divergence have been observed among sequences of HCV isolates. During BLAST and multiple sequence alignment all the isolates showed very good phylogenetic relationship with corresponding subtypes. These isolates showed above 90% nucleotide identities to corresponding domains of sequences present in database. The information provided in the present study may prove to be helpful to physicians in clinical decision making and vaccination strategies. It must be noted, that the reported distribution of the various genotypes or subtypes in asymptomatic blood donors can change with increasing number of blood donors.

Figure 1: Phylogenetic relationship of 42 HCV isolates from Delhi, established using neighbour-joining tree. Each sequence is designated by its accession number and subtype



4. Discussion

The dispersion of Hepatitis C virus genotypes and subtypes vary depending on the geographical region. The Genotypes 1, 2 and 3 are widely distributed throughout the world. Subtype 'a' of genotype 1 is prevalent in Europe, Australia and America. Subtype 1b is common in Europe, North America and many parts of Asia. The different regions of Indian subcontinent show prevalence of different genotypes of HCV. Most of the studies

revealed predominance of genotype 3 in northern, eastern and western parts of India, whereas southern part is predominated by genotype-1[5]-[7],[9]-[13]. These findings are further supported by Das *et al*[14]. The present study showed genotype 1 to be the most common in blood donors in NCT of Delhi. The findings of this study differ from earlier studies because the samples included in this study belong only to asymptomatic blood donors. The predominance of genotype 3 had been reported for all patients at different stages of HCV infection in north

India. However, present study reports predominance of genotype 1 only in patients at asymptomatic stage. Delhi being capital and metro city, gets patients from various parts of country, which might have also affected the predominance in this case. Chakravarti *et al* reported that average viral load of the patients infected with HCV genotype 1 was significantly higher than average viral load of patients infected with genotype 2 and 3[18]. The higher degree of HCV genotype 1 replication in comparison to others, might be responsible for higher viral load and more severity of liver disease. The results of our study may also be used to explain the reason behind the high severity of liver disease in case of HCV genotype 1 infection. The predominance of genotype 1 in blood donors indicates that genotype 1 infected patients remain asymptomatic for longer period than that of other genotypes and by the time it is detected by symptoms, it becomes chronic. Therefore, future prevention and treatment strategy should be directed towards HCV genotype 1, without neglecting genotype 3 and others. In our study, HCV genotype 2, 5 and 6 were not detected in the asymptomatic blood donor samples. It also indicates that the predominant risk factors associated with the HCV infection could be blood transfusion if not screened properly. Therefore, there is a need of highly sensitive methods like NAT for blood donor screening especially in this era where we still await a suitable HCV vaccine.

With the detection of subtypes 1a, 1b, 3a, 3b, 4a and nucleotide heterogeneity between genotypes, this study gives an idea of HCV genetic variability. The evolution of viral genomes, like that of all other genomes, is a process that is ultimately dependent on mutations and changes in their genomes. HCV exhibits a significantly high variability at genomic level with six recognized genotypes. Each of these HCV genotypes have still more variant forms classified as subtypes. It is explicit that the identification of genotype and subtype of Hepatitis C virus infecting a patient is of high importance in the management of the disease and in the design and development of drugs. Such data is also useful in keeping track of the source and course of infection, both in the population and the patient as it is clear that the severity and prognosis of hepatitis C depend on the genotype and subtype of HCV infecting the patients[19]. This fact needs major attention as no single drug has so far been able to have the same effect in all the genotypes or even in subtypes of HCV[20]. Clinical prognosis and disease progression may also be affected by biological differences in the manifestation of infection with

different genotypes and subtypes. A viral genotype is important prognostic variable, knowledge of which might be useful in treatment decisions. The analyses of divergence and heterogeneity in the sequences of the isolates may be important in vaccine research and in understanding the host pathogen interaction. There is a need for public education in India about routes of transmission of HCV and it is important to create awareness to prevent the acquisition of HCV.

5. Conclusion

We have detected subtypes 1a, 1b, 3a, 3b and 4a of HCV in blood donors in NCT of Delhi. The genotype 1 is found predominant in these blood donors in contrast to genotype 3, which predominates all patients at different stages of HCV infection in north India. The blood donor units must be screened properly before transfusion.

Conflict of interest: None declared

References

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