Commentary

HCV genotyping in India - Quo vadis?

In India, the prevalence of hepatitis C virus (HCV) antibody (Ab) ranges from 0.12 per cent to 0.68 per cent in the general population. Since nearly 70 per cent of HCV Ab positive individuals are viraemic, the number of individuals harbouring circulating virus in our country could range from one to six million.

HCV is transmitted principally through parenteral routes. The parenteral modes of transmission include blood transfusions, blood product infusions and organ transplantation. Vertical transmission of HCV has also been described. Though reports on the sexual transmission have been controversial, a fact that clearly emerges is that transmission of HCV through this route occurs at a very low efficiency, lower than that for hepatitis B virus and human immunodeficiency virus. However, in a significant proportion of patients the route of HCV transmission is unknown. In developing countries, nonconventional routes such as acupuncture, tattooing, circumcision, commercial barbering and dental extractions are potential routes of transmission. In addition, the commonly held belief that an injection is more effective than oral medication can expose people to HCV through the use of unsterilised needles. Controlling such sources of infection is also difficult. A large pool of viraemic carriers coupled with the unrecognized routes of transmission indicate that HCV infection in the Indian subcontinent is poised to become a silent epidemic in the coming years posing major health problems.

RNA viruses are known for their genetic heterogeneity, HCV being no exception. HCV exhibits genetic variability with the frequency of nucleotide substitutions per nucleotide per year ranging from $0.41 \times 10^{-3}$ to $0.74 \times 10^{-3}$ in the non-structural 5B (NS5B) and the envelope (E2) regions of its genome respectively. Today, HCV is classifiable into 6 major genotypes (clades) and more than 80 subtypes. The term genotype corresponds to the broadest hierarchical classification of the HCV genome. HCV genotypes differ from one another by $\geq 34$ per cent over the length of the entire genome. Subtypes correspond to more closely related variants within the major genotypes. The six genotypes are numbered from 1 to 6 and subtypes denoted by alphabets a, b, c and so on in the order of discovery.

Knowledge about prevalent HCV genotypes in a particular geographical region has important implications. The infecting genotype often determines the course and severity of the disease process. Recent studies clearly point to the pivotal role HCV genotyping has in determining the duration and efficacy of antiviral therapy. The infecting HCV genotype determines prognosis after orthotopic liver transplantation and the regional distribution of HCV genotypes influences the configuration of diagnostic assays and vaccine design. Additionally, since the route of transmission of HCV infection remains unknown in 39 per cent of infected individuals, genotype detection may be a useful tool for tracing sources of infection.

HCV subtypes represent the next level of hierarchical stratification of the HCV genome. HCV subtypes may also have a bearing on disease progression, antiviral response and overall prognosis. Subtype 1b is associated with a high risk of progression to chronicity. Similarly, subtype 3h, is associated with poor response to the widely used antiviral drug alpha-interferon.

In India, HCV genotypes show differing distributions in different geographic regions. In north India, HCV genotypes 1, 2 and 3 have been detected with genotype 3 being most frequently detected. In eastern and western India, HCV genotype 3 is the predominant genotype. In south India, the commonly occurring genotypes are genotypes 1, 3 in decreasing order of frequency. In south India, the commonly occurring genotypes are genotypes 1, 3 in decreasing order of frequency. Studies carried out on patients attending our tertiary care hospital in south India reveal the presence of HCV genotype 6 exclusively in south Indian patients (unpublished observation), and the presence of HCV genotype 6 in two patients from east India (unpublished data). The article by Singh et al in this...
issue of the journal reports on HCV distribution in a small group of patients (n = 36) and essentially mirrors the information harnessed by previous studies. Moreover in this study, the geographical origin of the study subjects and other patient characteristics have not been well defined. In summation, HCV genotypes 3 and 1 seem to be the predominant genotypes in India with variations only in the proportions of patients infected with these genotypes in different geographic regions.

A wide variety of techniques with differing efficacies for HCV genotyping exist. Nucleotide sequencing of the viral genome is considered the ‘gold standard’ for genotyping. Representative regions that can be used for nucleotide sequencing include the core (C), envelope (E1), and the non-structural (NS5) regions. Most of the genotyping techniques are based on the amplification of a subgenomic fragment of HCV by the polymerase chain reaction (PCR). After amplification, genotypes can be determined by (i) differential amplification using type-specific primers; (ii) digestion of the amplified products from the 5′non coding region with restriction endonucleases to reveal digestion patterns specific to individual genotypes; and (iii) reverse hybridization of biotin-labelled PCR products to immobilized type-specific probes in a line probe assay format (Inno-LiPA HCV, Innogenetics, Belgium). Singh et al. have used the line probe assay for HCV genotyping. A commercial serotyping assay using serotype-specific peptides is also available (Murex Biotech, UK).

Though innumerable techniques exist for HCV genotyping, the choice of a genotyping system should be made keeping in mind the following:

(i) The category of patient population. Serotyping techniques may have limited utility for typing immunocompromised patient populations since this technique depends on the production of genotype-specific antibodies. On the other hand, a type-specific primer-based genotyping technique may have to be used in patients having high prevalence of mixed infection with more than one HCV genotype.

(ii) The heterogeneity of HCV in a geographical area. There is evidence that HCV has been endemic in India and other south east Asian countries for a long time. This prolonged endemicity provides an explanation for the increased number of subtypes seen in our country.

In this scenario, use of commercial assays such as the Inno-LiPA, geared for the detection of subtypes prevalent in industrialized countries, may have limited application in detecting subtypes indigenous to the Indian population.

Clearly, Indian data generated so far underscore the predominance of genotype 3 followed by genotype 1, and also alerts us to the entry of geographically restricted genotypes such as genotypes 4 and 6, probably as a consequence of increasing globalization and population migration.

What then are the thrust areas for HCV research in India in the coming years? Cohort studies will delineate the true pathological role of the existing HCV genotypes/subtypes in the Indian population. Research should also focus on the development of simple, cost-effective and relevant genotyping assays in the Indian setting. This information will empower clinicians to design treatment algorithms tailor-made for the Indian HCV carrier and will augment the global HCV vaccine initiative.

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References