

Hepatitis C Virus- Epidemiology and Genotyping

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Abstract: Hepatitis C virus (HCV) is one of the major causes of parenterally acquired hepatitis. It is a leading cause of chronic hepatitis and primary hepatocellular carcinoma (HCC). The common modalities of spread of hepatitis C infection are blood transfusion, injection drug use, unsafe therapeutic injections, health care related procedures, sexual and vertical transmission. HCV is known to have marked genetic heterogeneity. Presently HCV can be classified into at least 6 major types (1, 2, 3, 4, 5, 6) and series of subtypes. Genotype 1a is common worldwide. In India the most prevalent genotype is 3. HCV genotypes exhibit different profiles of pathogenicity, infectivity and response to antiviral therapy.

Keywords: Hepatitis C virus (HCV), chronic hepatitis, HCV transmission, HCV genotyping.

I. Introduction

In the 1970s, several researchers produced evidence for an infectious agent, distinct from the hepatitis A virus (HAV) and hepatitis B virus (HBV), that caused chronic hepatitis and was frequently transmitted by blood and blood products¹⁻³ called non- A non- B Hepatitis (NANBH) initially. In 1989, HCV was discovered by Harvey J. Alter. Hepatitis C virus is one of the major causes of parenterally acquired hepatitis. It is a leading cause of chronic hepatitis and primary hepatocellular carcinoma in most parts of the world. In the developing countries of Asia and Africa, though hepatitis B virus (HBV) infection is the commonest cause of chronic liver disease; HCV is fast evolving as an equally important infection among these populations.⁴ Moreover, with successful immunization programme against HBV infection in most countries of Asia, there is a decrease in the rate of HBV infection. On the other hand, chronic liver disease burden due to HCV infection has increased significantly.⁵

HCV is a RNA virus belonging to Flaviviridae family and genus Hepacivirus. The genome of HCV is single stranded RNA of positive polarity. HCV shows extraordinary genetic diversity. This is true both in terms of extent of quasispecies variation within single infected individuals, as well as in the genetic distances between viruses infecting different persons.

II. Prevalence

Worldwide HCV is the commonest cause of post-transfusion hepatitis. Globally, HCV is estimated to infect 170 million people, 3% of the world's population, creating a huge disease burden. In India about 20 million people are known to have HCV infection and a quarter of them expected to develop chronic liver disease in the next 10-15 years. The impact of this infection has started to emerge in India.⁶

The prevalence of HCV varies greatly in different countries. For example, in the Scandinavian countries <0.5% of the population is infected, whereas in Egypt >20% of the population is infected with HCV because of the use of parenteral anti-schistosomal therapy. The highest prevalence has been reported in Ukraine and in the central African countries of Gabon and Cameroon. The carrier rate among the general population in India is nearly the same as in developed countries. High prevalence of the infection ranging from 20 - 80% is reported in patients of chronic renal failure on maintenance haemodialysis, multi-transfused patients including patients with haematological disease, professional plasma donors and renal transplant recipients.⁷

The seroprevalence among the blood donor population in India is 1.8% - 2.5 % and the community seroprevalence has been reported to be 0.87%. HCV is the etiological agent in about 20% of patients with chronic hepatitis in northern India.⁷

HCV seroprevalence reported in South India is 0.22%⁶, 0.3% in Western India⁸, 1.8% in Central India⁹ and 1.9% in North India.¹⁰ In the developing world, unsafe therapeutic injections and transfusions are likely to be the major modes of transmission.¹¹

III. Transmission of HCV

The risk factors most frequently cited as accounting for the bulk of HCV transmission worldwide are blood transfusions from unscreened donors, injection drug use, unsafe therapeutic injections, and other health-care related procedures.

Blood transfusion is a major mode of transmission of HCV, as a large quantity of virion enters the blood. In most of the developed world risk of transfusion-transmitted HCV infection is less common due to mandatory screening of donors. Most blood donations in the developing world do not come from voluntary, non-remunerated donors, transfusion is probably a major source of HCV transmission throughout the developing world. In India, some observers have suggested that problems with regulatory oversight of the nation's blood transfusion service led to insufficient use of volunteer-donated blood. Most countries in the developing world do not screen blood donations for the presence of HCV. WHO's Global Database on Blood Safety estimates that 43% of donated blood in the developing world is not screened adequately for transfusion-transmitted infections, including HCV. In India, HCV screening of blood products is mandated by law but not usually done due to financial constraints.¹²

In New Delhi, among 182 anti-HCV-negative hospitalised patients studied prospectively following a blood transfusion, HCV infection developed in 5.4%.¹³ 6.9% of HCV patients received blood transfusion in a study conducted by Rehan et al.¹⁴

The prevalence of hepatitis C in healthy blood donors was reported to be 1.09% in Punjab¹⁵, 1.57% in Delhi¹⁶, 0.75% in Madurai¹⁷ and 87.3% in commercial blood donors in Maharashtra, India.¹⁸ Studies have shown prevalence of hepatitis C to be below 2% in voluntary donors^{8,10}. In India mandatory screening of blood and blood products for HCV was introduced in 2002.¹⁴

As is the case with other transfusion transmissible viruses, few cases of blood donors infected with HCV may be missed when antibody detection tests are adopted, instead of nucleic acid amplification test (NAAT) for screening.

Unsafe therapeutic injections: In many developing countries, supplies of sterile syringes may be inadequate or non-existent; non-professionals often give injections outside the medical setting.¹⁹ In this environment people may receive multiple contaminated injections over the course of a lifetime, incurring a substantial cumulative risk of HCV infection. Contaminated injection equipment appears to be the major risk factor for HCV infection in several countries, including several of the most populous nations in the world. In Egypt, the country with the highest reported seroprevalence in the world, transmission has been attributed to contaminated glass syringes used in nationwide schistosomiasis treatment campaigns from 1960 to 1987.²⁰ In India, seroprevalence of HCV infection among patients receiving multiple injections to treat kala-azar was 31.1%—well above the seroprevalence among hospitalised and community controls.²¹ Two 2003 studies among populations in different regions of India found substantial associations between prevalent HCV infection and frequent visits to “freelance” or unlicensed practitioners of medicine, as well as a history of therapeutic injections using reusable syringes.^{22,23}

Injection drug use (IDU) is the primary mode of transmission for HCV infection in the developed world. In countries such as the USA and Australia, where the highest seroprevalence is among middle-aged people, IDU has been the dominant mode of transmission for more than 30 years and accounts for 68% and 80% of current infections, respectively. The prevalence of HCV infection among long-term injection drug users is high—64–94% among those with a duration of injecting of 6 years or more.^{24,25} HCV infection is thought to occur rapidly after initiating injecting behaviour, based on a seroprevalence of 65% observed in the late 1980s among injection drug users with less than 1 year of injecting.²⁶ More recent studies among young injection drug users with 5 years or fewer of injecting have reported HCV seroprevalence rates of 20–46%.^{27,28} Fewer sharing partners are necessary to sustain HCV transmission than are necessary for other blood borne viruses,²⁹ and indirect drug sharing and preparation practices have been associated with HCV transmission.³⁰ Very little data exist regarding the prevalence of injection drug use and its contribution to HCV transmission in the developing world.¹²

In India, very few studies are available on IV drug abuse induced HCV infection. In a study conducted at YGRCARE Chennai 1158 Intravenous drug users were screened for HCV infection. The study reported a prevalence of 55%.³¹

Occupational transmission of HCV infection is largely confined to health-care workers who have sustained a contaminated needle stick injury and observed attack rates under these circumstances are as low as 0.3%.¹²

Patients undergoing **haemodialysis (HD)** are at high risk of acquiring blood-borne pathogens, since HCV is efficiently transmitted by the parenteral route. In addition, infected patients have an increased tendency to develop chronic hepatitis and to be also a potential reservoir for its transmission, possibly contributing to the

nosocomial spread of HCV in dialysis centres and hence contributing to the high prevalence of HCV infection among haemodialysis patients.³²

HCV prevalence in HD patients was found to be 4.3% in Delhi³³ and 1.11% in Mangalore³⁴. Prevalence of HCV RNA in the Haemodialysis population is 27.7%.³⁵ The incidence directly related to the prevalence of HCV in the dialysis unit. Units with a prevalence of <19% had an annual incidence of 2.5% compared to a 35.3% incidence in units with a prevalence >60%.³⁵

Case control studies of acute hepatitis C in the USA failed to find a significant association with tattooing, ear piercing, or acupuncture. A community-based cross-sectional seroprevalence study in Taiwan found a significant association with acupuncture ($p=0.05$), but not with tattooing¹². In a study by Khaja et al a significantly higher rate of transmission of HCV was noted in people exposed to tattooing (2.8%).³⁶

In epidemiological reviews relating to disease accelerating co-factors among HCV infected people male sex has been quoted as one such factor.¹²

Sexual transmission- Sex with an infected partner and with multiple partners have been identified as risk factors for HCV transmission, but sexual transmission of HCV is far less efficient than that of other sexually transmitted viruses. Among people in long-term monogamous relationships in particular, the risk of sexual transmission of HCV is extremely low.¹² Sexual transmission of HCV is not as common as it is with hepatitis B virus.⁷ The prevalence of HCV infection in individuals with STDs in a tertiary care hospital in South India was 6%.³⁷

Maternal-infant transmission is not common. In most studies, only 5% of infants born to infected women become infected. Information on hepatitis C virus (HCV) infection in pregnant women in India is scanty. The disease in new-borns is usually mild and free of symptoms. The risk of maternal-infant spread rises with the amount of virus in the mother's blood. Breast feeding has not been linked to HCV's spread.⁷ None of the known risk factors were found to be significantly associated with the HCV infection. Hence case identification and consequent management pose a particular problem and routine screening is not a viable option in our resource-poor setting.³⁸

Acquisition of HCV infection through perinatal transmission is estimated to occur in 2.7–8.4% of infants born to HCV infected mothers, and a higher proportion of infants born to HIV/HCV co-infected mothers.¹² Kumar et al,³⁸ in a study from Delhi reported a prevalence of 1.03% in antenatal population while the results of other studies at Shimla³⁹ and Vellore⁶ reported 0% prevalence.

IV. Factors accelerating disease progression

Several cofactors have been associated with accelerated progression of hepatic fibrosis among those infected with HCV, or with increased incidence of HCV-related complications of chronic liver disease and hepatocellular carcinoma (HCC). These cofactors are male sex, older age at acquisition of HCV infection, obesity, HIV co-infection, hepatitis B virus (HBV) co-infection, and alcohol consumption. Because the future burden of HCV-related complications may be altered substantially by the relative presence or absence of these cofactors among HCV-infected people, those cofactors that are modifiable through public-health prevention programmes—i.e., HIV, HBV are of particular interest.¹²

HCV and HIV co-infection is emerging as an important and frequent finding in patients seeking therapy for one or the other disease. The fact that both viruses share a similar route of transmission and mechanisms of epidemic spread appears to be the most important reason for the growing nature of the co-infection. The highest prevalence of co-infection is occurring in patients with a current or previous history of injecting drug use.⁴⁰ Studies on HCV-HIV co-infection from India have reported a prevalence of 3.02% in Andhra Pradesh⁴¹, 2.2% in Tamil Nadu⁴², 1.6% in Lucknow⁴³ and 1.06% in Vellore.⁶

HCV and HBV co-infection The proportion of HCV-infected people who also have chronic HBV infection will have an impact on the overall burden of chronic liver disease. HCV and HBV co-infection in chronic hepatitis patients has been associated with clinically and histologically more severe liver disease than that of chronic hepatitis patients with HCV infection alone. A meta-analysis found HBV/HCV co-infection to be more strongly associated with HCC than either infection alone, suggesting a synergistic effect between the two viruses in the carcinogenic process of HCC¹². In studies conducted by Reddy et al⁴⁴ and Kosaraju et al³⁴ prevalence of dual infection in haemodialysis patients was observed to be 3.7% and 4.4% respectively.

V. Genotyping of HCV

HCV is known to have marked genetic heterogeneity. Presently HCV can be classified into at least 6 major types (1, 2, 3, 4, 5, 6) and series of subtypes. Genotype 1a is common worldwide. In India the most prevalent genotype is 3.

Geographical distribution of HCV genotypes⁴⁵

The relative prevalence of the six major genotypes of HCV varies by geographic region. Genotype 1 comprises two major subtypes: 1a, which predominates worldwide, and 1b, which is widely distributed in Europe and North America, where it is typically found in older persons and those infected by transfusion. Genotype 2 is found predominantly in older persons from the Mediterranean region and in Asia; genotype 3 is particularly prevalent in Europe, where it is associated with injection drug use; genotype 4 is widely distributed in the Middle East; genotype 5 is common only in South Africa; and genotype 6 in Southeast Asia.

HCV types 1a, 2a, 2b show broad worldwide distribution, type 5a and 6a found in specific geographic regions. HCV infected blood donors and patients with chronic hepatitis from countries in Western Europe and USA is frequent with genotypes 1a, 1b, 2a, 2b and 3a, although relative frequencies vary. In many European countries, genotype distributions vary with age of patients, reflecting rapid changes in genotype distribution with time within a single geographic area.

A striking geographical change in genotype distribution is apparent between south east Europe and Turkey (both mainly type 1b) and several countries in Middle East and parts of North and Central Africa where type 4 predominates. HCV genotype 5a is frequently found amongst NANBH patients and blood donors in South Africa, but is found only rarely in other parts of Africa or elsewhere.

In Japan and Taiwan and probably parts of China genotypes 1b, 2a, and 2b are the most frequently found. Infection with type 1a in Japan appears to be confined to haemophiliacs who have received commercial blood products, such as factor VIII and IX clotting concentrates, provided in the USA. A genotype with a highly restricted geographical range is type 6a which was originally found in Hong Kong, where approximately one third of anti HCV positive blood donors are infected with this genotype, as are an equivalent proportion in neighbouring Macau and Vietnam.

Genotyping

Genotyping and subtyping of HCV is relevant to the epidemiology of HCV, vaccine development, clinical management and assessment of the risk benefit ratio of therapeutic measures against chronic HCV infection. It has been postulated that differences in nucleotide sequence could result in differential activity of HCV proteins that could alter the rate of HCV replication, sensitivity to the antiviral activity of interferon, or pathogenicity of the virus.

In recent years, substantial evidence has emerged indicating that typing and subtyping for HCV is important clinically; genotype 1 in particular cannot be treated efficiently with IFN- α , while genotypes 2 and 3 respond favourably. The causes of variation in treatment response are not well understood. Studies of Japanese patients infected with subtype 1b indicated that the outcome of interferon therapy was correlated with genetic variability in a portion of the NS5A gene (the interferon sensitivity determining region, ISDR), although subsequent studies of European patients did not confirm this result. Moreover, genotype 1 infection may proceed more rapidly to severe forms of chronic hepatitis, cirrhosis and hepatocellular carcinoma, when compared with genotype 2 and 3.⁴⁶

Several methods of genotyping HCV have been proposed. Because therapeutic decisions for chronic HCV-related hepatitis are made on the basis of genotype, it is important that genotype be accurately determined by clinical laboratories. The nucleic acid from the clinical sample is amplified by Reverse transcription-PCR (RT-PCR). Once the material is amplified (by RT-PCR), a number of approaches can be used such as sequencing of each amplicon, restriction fragment length polymorphism (RFLP), hybridization assays like line probe assay, DNA enzyme immunoassay, and finally type-specific primer nested PCR.⁴⁷ In addition to these assays, Real time reverse transcription PCR have recently become commercially available.⁴⁸

Direct sequencing is the most accurate method for HCV genotyping. However, many other methods have been used because of the expense and technical difficulties of direct sequencing. The most frequently used methods in clinical laboratories are LIPA (line probe assay) and sequencing of the 5' UTR (both from Bayer Diagnostics). Both assays require two steps: generation of the PCR amplicon and then evaluation of the amplicon by either hybridization or sequencing. An additional disadvantage is the use of the 5' UTR, which is less informative for genotyping than are other, more variable regions of HCV.

In contrast, type-specific PCR assays require only a single amplification step and thus are technically simpler. The first type-specific genotyping assay was described by Okamoto et al.⁴⁶ The assay utilized a first-round amplification of a large section of the core region, followed by a set of second nested amplification reactions. Several studies compared these type-specific assays to a variety of other genotyping methods. In each

of these studies, results from the type-specific assays had good agreement with other methods but had somewhat high rates of mixed samples and samples that did not amplify.

Recently, several methods have been described that utilize single-step real-time reverse transcription-PCR (RT-PCR) with target-specific TaqMan or LightCycler probes or SYBR green detection, further streamlining the method. Some of these real-time methods were designed only to distinguish between genotype 1 and non-1 types, and none have been evaluated with large numbers of samples.⁴⁶

Existing methods are often subjective, inaccurate, manual, time-consuming, and contamination prone. Therefore, real-time reverse transcription-PCR (RT-PCR) reagents that have recently become commercially available (Abbott HCV Genotype ASR) starts with purified RNA and can be performed in 4 to 5 hours. This assay detects genotype 1a (NS5b), 1b (NS5b), 1, 2, 3, 4, 5, 6 (5'UTR).⁴⁷

In the United States, HCV genotype 1 accounted for the majority (74%) of infections followed by genotype 2 (15%), genotype 3 (6%) and genotype 4 (1%).⁴⁸

In Northern and Western India, genotype 3 was found to be predominant.^{5, 51, 52, 53} Genotype 1 was predominant in Southern India.⁵⁴

VI. Conclusion

Blood transfusion is a major risk factor for HCV infection. Stringent blood banking laws need to be introduced. The latest specific measure is the introduction of viral nucleic acid amplification test (NAAT). NAAT screening is currently in use in most of the developed countries but not yet mandatory in India. It detects viral genes rather than antibodies or antigens. NAAT will enable earlier detection of HCV.

The complexity and uncertainty related to the geographic distribution of HCV infection and chronic hepatitis C, determination of its associated risk factors, and evaluation of cofactors that accelerate its progression, underscore the difficulties in global prevention and control of HCV. Because there is no vaccine and no post-exposure prophylaxis for HCV, the focus of primary prevention efforts should be safer blood supply in the developing world, safe injection practices in health care settings and decreasing the number of people who initiate injection drug use.

HCV genotypes exhibit different profiles of pathogenicity, infectivity and response to antiviral therapy. Therefore, genotyping is crucial in patient management.

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