

Hepatitis C, E and G Virus—Three New Viruses Identified by Molecular Biology Technique in the Last Decade

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Abstract

The development of molecular biology techniques has enabled us to clone the genetic sequence of three new hepatitis viruses, namely, hepatitis C virus, hepatitis E virus and hepatitis G, or GBV-C virus in the last decade.

Hepatitis C virus (HCV) is now known to account for the majority of the parenterally transmitted non-A, non-B hepatitis and hepatitis E virus (HEV) is the major cause of the epidemics of enterally transmitted non-A, non-B hepatitis in the less developed countries, mainly through contaminated water supplies. While 80% of those who were infected with HCV will become chronic carriers, there is no known chronic carrier state for HEV. To date, 6 major genotypes of HCV were identified, with various variants of genotype 6 described in Southeast Asia.

In Singapore, the majority of our chronic hepatitis C patients are infected with genotype 1. The seroprevalence rate of HEV is 10% to 14% in Singapore. This probably reflects our suboptimal sanitary condition in the past rather than a reflection of a currently high HEV infection rate in our population. However, local cases of acute hepatitis E have been reported.

As for the most recently cloned hepatitis G virus, its aetiological role in the remaining cases of non-A, non-B hepatitis is still unclear. Some preliminary evidence suggests that it may be just an innocent bystander with limited pathogenicity and it certainly cannot account for all the remaining cases of non A-E hepatitis. Hence, the search for yet another hepatitis virus continues.

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Introduction

For more than 10 years after the development of diagnostic tests for hepatitis A and B viruses, we were still left with a considerable number of patients who were suffering from hepatitis which were serologically negative for both the hepatitis viruses and were therefore “labeled” as suffering from non-A, non-B (NANB) hepatitis. The advent of molecular biology techniques has enabled us to identify the full genetic sequence of a virus, in some cases, before its identification microscopically. Three viruses, namely hepatitis C virus, hepatitis E virus and the latest addition, hepatitis G virus (also known as GBV-C virus), were identified by this technique in the last decade in patients who were known to have NANB hepatitis.

While hepatitis C virus would account for a significant proportion of the parenterally transmitted NANB hepatitis, and hepatitis E virus for the enterally-transmitted NANB hepatitis, the role of hepatitis G virus is still uncertain. In this article, we will try to summarise the characteristics and clinicopathological roles of each of these three viruses in patients who have NANB hepatitis, with particular reference to some of the specific

features found in our local setting.

Hepatitis C Virus (HCV)

HCV is a single-strand, positive sense, RNA virus (Fig. 1), belonging to the family of flaviviridae. The nucleotide sequence of HCV was first identified in 1989.¹ Since then, it has been found that there are major nucleotide variations of the virus which are classified into at least six major genotypes (identified by arabic numerals in Figure 2), with 31% to 34% difference in the nucleotide positions between each genotype within the entire sequence of the virus,² as described by Simmonds et al.³ Such genotypic differentiation is important as we now know that the various genotypes have different biological properties, different response to interferon therapy (the only established therapeutic agent for hepatitis C), and result in differing clinical outcome in terms of disease progression. In addition, there are also different subtypes (identified by small alphabetical letters) within each genotype with approximately 20% difference in their nucleotide sequences which are of no known biological consequences.⁴

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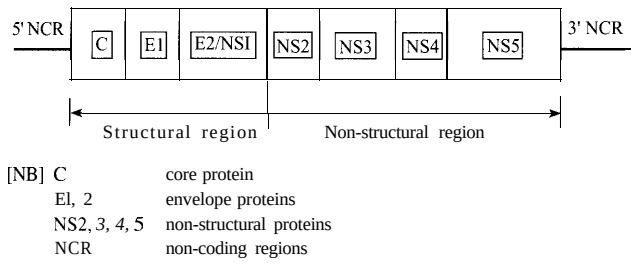


Fig. 1. Organisation of the Hepatitis C virus genome.

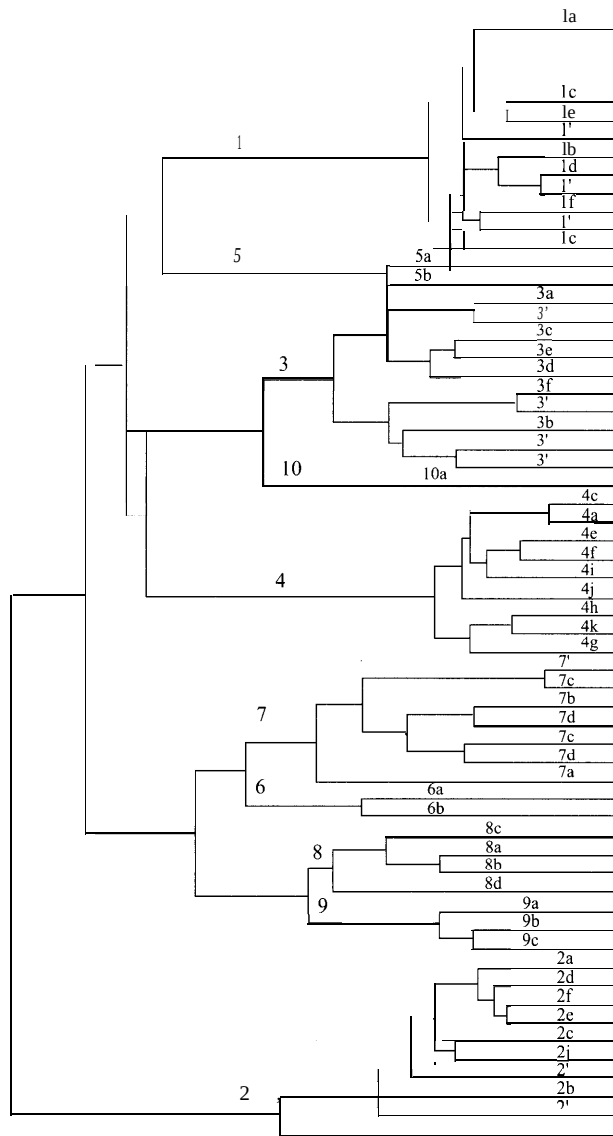


Fig. 2. Hepatitis C genotypic classification as illustrated by the phylogenetic tree of the NS5B region.

HCV has been reported to account for more than 80% of the acute and chronic NANB hepatitis both in the eastern and western population^{5,6} and is considered to be the predominant cause of parenterally transmitted NANB hepatitis. Unfortunately, 80% of the patients who acquire HCV infection will become chronic HCV carriers, of which 20% will develop liver cirrhosis and 1% of

these cirrhotic patients will develop liver cancer each year.⁷ However, this process is slow, evolving over a period of approximately 20 years, with the onset of symptoms after an average of 13 years post-exposure to the virus.⁸ Studies suggest that HCV genotype 1 is associated with more necroinflammatory activities in the liver,^{9,10} with higher rate of progression to fibrosis and cirrhosis.^{11,12} Genotype 1b, in particular, is associated with poorer response to interferon therapy^{13,14} and the likelihood to develop hepatoma in the absence of cirrhosis.

Initially, it was believed that the mechanism of hepatic damage caused by HCV is a cytopathic effect of the virus. It has become more controversial now, with increasingly more evidence suggesting participation of an immunopathological process.

There is a geographical difference in the distribution of the HCV genotypes. In the West, patients who acquire their HCV infection via blood transfusion tend to have infection of genotype 1, whereas the intravenous drug abusers tend to have infection of genotype 3a;¹⁴ while genotypes 1b, 2a and 2b predominate in Japan and China. In recent years, newer variants of HCV related to genotype 6 were discovered in patients in Southeast Asia.¹⁵

In Singapore, more than 80% of our chronic hepatitis C patients were infected with genotype 1.¹⁶ This may be related to the predominantly Chinese population here. In addition, more than 50% of the studied patient population were patients who were either undergoing haemodialysis or have received renal transplantation, thus probably acquired their infection via blood products. A few of our patients were found to have HCV genotype 6 infection. All of them had renal transplantation carried out in Guangzhou, China, from where they probably acquired their infection (Lee A S G et al, personal communication). It is unfortunate that the majority of our patients were infected with HCV genotype 1 as this will pose a relatively dismal outlook for our patients, considering the unfavourable prognosis associated with this particular genotype.

Hepatitis C is also associated with autoimmune manifestation. It is associated with the production of autoantibodies, such as anti-nuclear antibody (ANA),¹⁷ anti-liver, kidney microsomal antibody (Anti-LKM)¹⁸ and anti-GOR antibody.¹⁹ Autoimmune diseases, such as cryoglobulinaemia with or without vasculitis, autoimmune thyroiditis, glomerulonephritis and lichen planus are also associated with HCV infection.²⁰ A cross-sectional study done in our department has shown that 59% of our chronic hepatitis C patients were positive for ANA and/or smooth muscle antibody (SMA).²¹ Caution should therefore be exercised when we administer treatment to these patients. None of these patients have other

systemic autoimmune manifestation. The hepatic necroinflammatory activities of those patients who were positive for autoantibodies were not different from those without.

Diagnosis of hepatitis C is simplified by the development of immunoassays using the various epitopes in the structural and non-structural regions of the virus. Serological diagnosis is made by the detection of anti-HCV IgG antibodies to these epitopes. The third generation immunoassays (ELISA or RIBA) are highly sensitive and specific in detecting these antibodies. In the case of acute hepatitis C, seroconversion usually occurs with IgG antibodies detected at about 2 weeks after exposure to HCV.²² Diagnostic kit for detection of anti-HCV IgM using enzyme-linked immunoassay (EIA) has been developed, but its sensitivity is uncertain.²³ In addition, IgM antibodies may be detected in cases of chronic hepatitis,^{24,25} thus its lack of specificity in diagnosing acute hepatitis C. However, it may serve as a role in monitoring the patients' response to interferon treatment by its disappearance with successful treatment.²⁶

Alpha-interferon has been established as an accepted therapeutic agent for the treatment of chronic hepatitis C. Unfortunately, long-term response to previously established 6-month treatment regimen of interferon is no more than 20% to 30%. Longer treatment duration and combination treatment has been tried with more promising clinical outcome.²⁷⁻²⁹ The issue of treatment for acute hepatitis C remains controversial.

Hepatitis E Virus (HEV)

While HEV was first reported to be identified as an icosahedral non-enveloped viral particle by immune electron microscope in 1982³⁰ after the first documented epidemic outbreak of enterically-transmitted NANB hepatitis in Kashmir Valley in 1980,³¹ the cloning and sequencing of HEV was only reported in 1990.³² It is a single-strand, positive sense RNA molecule, measuring 7.5 kilobase in length, belonging to the family of Caliciviridae. Since then, detection of HEV RNA from patients' faeces and blood using polymerase chain reaction (PCR) technique has been documented, thus confirming faecal shedding of HEV and a transient viraemic phase during early infection.

Epidemics of hepatitis E related to consumption of contaminated water have been initially reported in various less developed countries,^{33,34} however evidence of exposure of hepatitis E by seroprevalence study were also reported in developed countries such as Netherlands³⁵ and France.³⁶ In Singapore, the seroprevalence of hepatitis E is between 10% and 14%.^{37,38} This seemingly high seroprevalence rate probably reflects the suboptimal sanitary situation and high infection rate in the past as the peak seroprevalence is found in the older population.³⁷

Hepatitis E is an infection which is transmitted by the orofaecal route. The average incubation period is approximately 6 weeks. The alanine transaminase (ALT) usually peaks at the onset of the symptomatic illness and normalises by the first month of illness. Cholestasis may be a predominant feature in a significant proportion of the patients^{39,40} and the course may be protracted.³⁹ There is no known chronic carrier state for this virus.

In contrast to the patients infected with acute hepatitis E in endemic areas, a local prospective study⁴¹ found that the patients presented here tend to have higher ALT (up to 72 times normal) and absence of faecal viral shedding at presentation. This may be related to an augmented host immunological response. The mean peak serum bilirubin was nearly 10 times normal and jaundice persisted for as long as 2 months. Some of our patients were found to have hepatitis A co-infection, and super-infection of hepatitis E was found in some chronic hepatitis B carriers. The course of illness in those with hepatitis A and E co-infection was similar to the rest. However, one of the two hepatitis B carriers who had superimposed acute hepatitis E had prolonged prothrombin time during his acute illness; he eventually recovered.

Diagnosis of acute hepatitis E can be made by the detection of the anti-HEV IgG and anti-HEV IgM antibodies by the ELISA technique. Both of these antibodies are highly specific and each is detectable in about 89% to 95% and 85% to 89% of patients, respectively, by about day 10 of illness.^{42,43} The problem of anti-HEV IgM is its unpredictability. It may be negative in some cases of acute infection, its appearance may be transient, or it may appear biphasically with periods of negativity.^{42,44} While protracted period of viraemia and faecal shedding of HEV were reported in endemic areas,⁴⁴ detection of HEV RNA by PCR technique may not be a useful diagnostic tool for patients in non-endemic areas based on our experience. Our patients' viraemic and faecal shedding phases seem to precede their symptomatic presentation.⁴¹

Like most other acute viral hepatitis, there is no specific treatment for acute hepatitis E. Special attention, however, should be paid to pregnant women who are infected with acute hepatitis E as they tend to develop fulminant hepatitis and are associated with a fatality rate of 5% to 25%. Unlike hepatitis B, there is suggestion that HEV-related fulminant hepatic failure is mediated by a cytopathic process.⁴⁵

Hepatitis G Virus (HGV)

As some of the NANB hepatitis are still not accounted for by hepatitis C and hepatitis E viruses, the search for new hepatitis virus continues. Two independent teams separately described a new viral agent, derived from patients with non-A-E hepatitis, which they cloned in

recent years and were named hepatitis G virus (HGV) and GB virus C (GBV-C), respectively. Both of these agents were later found to be almost identical in molecular structure with more than 95% molecular homology, hence, they are considered as two independent isolates of the same virus, belonging to a new genus in the family of flaviviridae; the same family as HCV. Below we will refer to both of these agents interchangeably as HGV.

HGV is a single-strand RNA virus, 9.4 kilobase in length. While belonging to the same family, HGV is considered as a different genus from HCV because they have less than 25% molecular homology. In addition, unlike HCV, HGV does not have a hypervariable region, nor does it have any systematic genetic variation which subdivides itself into various major genotypes.

At present, the mainstay of diagnosis of HGV infection is by reverse transcription-PCR (RT-PCR) technique to identify the specific sequences at the 5' non-coding region of the viral genome. Recently, a semi-quantitative RT-PCR hybridisation assay amplifying 2 independent regions, the 5' non-coding and the NS5a regions, has been described.⁴⁶ This is to make simple the technique involved in the diagnosis of HGV infection and hence, facilitate mass screening.

HGV is parenterally transmitted, hence, it is not surprising that there are cases of co-infection of HGV with hepatitis B or C.⁴⁷⁻⁴⁹ In addition, HGV have a much higher genomic concentration than HCV, thus making it more likely than HCV to be transmitted via sexual contact and between mother and child. Based on studies done on non-remunerated donors, the prevalence of HGV is much higher than that of HCV in the general population in the West.^{50,51} At present, no data is available with regards to the prevalence of HGV in Singapore. In Japan, Germany and the USA, the viraemic rate of HGV among blood donors is 0.9%,⁵² 4.7%⁵³ and 2%,⁵⁴ respectively. This is even higher among the patients on haemodialysis, with 3.1%⁵² and 20%⁵⁴ of them infected with HGV in Japan and the USA, respectively. However, the pathogenic role of HGV is yet to be proven definitively.

First, HGV seems to have relatively low transfusion infectivity despite its high prevalence in the blood products.⁴⁴ In addition, infected patients may be asymptomatic with absence of elevation of transaminases.^{52,54} Hence, in cases of acute non-A-E hepatitis where HGV was isolated, it is possible that the isolated HGV was but an innocent bystander and not necessarily the cause of the acute hepatitis which is yet to be identified.

The reports on the role of HGV on fulminant hepatitis are contradictory too.^{53,55-58} In some of these reports, HGV is at most a co-factor in causing fulminant hepatitis as these patients were co-infected with hepatitis B. In any case, at least 50% of the non-A-E fulminant hepatitis

in these reports were negative for HGV. Similarly, HGV was identified only in a small proportion of patients with chronic cryptogenic liver disease.⁵⁹ Hence, it remains for us to search for yet another agent to account for the aetiology of hepatitis in these cases.

In conclusion, molecular biology technique has assisted us in accelerating the identification and understanding of at least two new hepatitis viruses, namely HCV and HEV, which have a well-defined pathogenic role known to us now. However, whether the cloning of HGV is just adding to the confusion to an ever-growing list of hepatitis viruses is yet to be certain. In any case, it is expected to have yet another virus added to the list, as there are still cases of non-A-G hepatitis of which the aetiology needs to be elucidated.

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