

Prevalence of Hepatitis G Virus Infection in Patients with Liver Diseases in Singapore

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ABSTRACT

The prevalence of hepatitis G virus (HGV) infection in patients with liver diseases in Singapore and its pathogenic role in these patients was studied. One hundred and forty-eight patients who had chronic hepatitis or acute non A-E hepatitis were studied. Presence of HGV RNA was determined by nested polymerase chain reaction of the 5'non-coding region of the virus in all the patients. Hepatitis G IgG antibody to the envelope (E2) antigen was tested with an enzyme immunoassay (Boehringer Mannheim, Singapore) in 76 of them.

Most patients (93%) were ethnically Chinese, predominantly males (74%) and chronic hepatitis B (72%) patients. Others had chronic hepatitis C (19%) or cryptogenic cirrhosis (6%). Four patients had acute non A-E hepatitis. HGV RNA and anti-HGenv were present in 3.5% and 8.3% of those with chronic liver disease. HGV infection did not account for any of the acute non A-E hepatitis and most of the cryptogenic cirrhosis.

Keywords: Hepatitis G, Cryptogenic cirrhosis, Chronic hepatitis, Non A-E hepatitis, Co-infection

Singapore Med J 2002 Vol 43(2):067-069

INTRODUCTION

As we enter the new millennium, despite the marked improvement in our environmental hygiene and advancement in medical science, including the advent in molecular biology, we are still faced with hepatitis and liver cirrhosis of unknown etiology every now and then. After the description of a new virus, hepatitis G virus (HGV), towards the end of the 20th century, its prevalence and pathogenic roles in various liver diseases have been well explored in various populations worldwide^(1,2). The relatively higher prevalence of hepatitis G virus infection in the Western and Japanese populations is probably related to the similar route of transmission of the infection as, and co-infection with, hepatitis C virus that is known to be prevalent in these areas.

In contrast, perinatally transmitted hepatitis B is a commoner infection in the South East Asian population, particularly among the Chinese. So far, data on HGV infection in such patients are mainly derived from the native Chinese living in Taiwan and China^(3,4). We therefore went about determining the prevalence of HGV infection in patients with liver diseases in Singapore where there is a predominantly ethnic Chinese migrant population.

METHODS

This is a cross-sectional study involving one hundred and forty-eight consecutive patients who consulted in the Department of Gastroenterology in November 1997 and were diagnosed to have acute non A-E hepatitis or chronic liver diseases. Patients' sera were collected using standard sterile technique and stored at -40°C immediately until further study was performed.

RNA was extracted from 0.2 ml of serum using TRIzol reagent (Life Technologies, Gaithersburg, MD, USA) for all patients. The extracted RNA was reverse transcribed with the outer, antisense primer used for PCR. Previously published conserved nested primers to the 5'non-coding region were used for the PCR⁽⁵⁾. Standard PCR technique, with employment of Hepatitis G virus RNA control set, consisting of low (1 x 10⁽⁴⁾ GE (genome equivalent)/ml) and high positive (5 x 10⁽⁵⁾ - 1 x 10⁽⁶⁾ GE/ml) controls provided by Boehringer Mannheim, and blank controls in each PCR run, was carried out to ensure specificity and sensitivity of our in-house PCR technique.

Enzyme-linked immunoassay for the qualitative determination of IgG antibodies to the HGV E2-antigen was used according to the manufacturer's instructions (Boehringer Mannheim, Mannheim, Germany), in 76 of these patients. These include all patients, except one, who have cryptogenic cirrhosis or acute non A-E hepatitis and all who were tested positive for HGV RNA. The rest were randomly selected proportionally from all patient groups with chronic viral hepatitis.

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Table I. Hepatitis G virus infection in various patient groups with liver disease.

	HGV RNA positive n (%)	Anti - HGenV positive n (%)
Chronic hepatitis B	2/106 (1.9)	3/50 (6.0)
Chronic hepatitis C	3/29 (10.3)	2/14 (14.3)
Cryptogenic cirrhosis	0/9 (0)	1/8 (12.5)
Acute non A-E hepatitis	0/4 (0)	0/4 (0)
Total	5/148 (3.4)	6/76 (7.9)

DEFINITION

Patients who had liver cirrhosis and were seronegative for Hepatitis B and C, with absence of autoimmune markers or incriminating etiologic factors (e.g. alcohol) were diagnosed to have cryptogenic cirrhosis. Liver cirrhosis was diagnosed based on presence of clinical evidence of decompensating liver function, as well as clinical and radiological evidence of portal hypertension and shrunken liver.

Patients who had acute hepatitis but tested negative for all serological markers of all currently detectable hepatitis viruses (including HBsAg, anti-HBc IgM, anti-HAV IgM, Anti-HCV IgG, anti-HEV IgM and IgG), and who were seronegative for autoimmune markers for autoimmune hepatitis and had absence of other etiological factors, including alcohol and drugs, were diagnosed to have acute non A-E hepatitis.

RESULTS

There were 109 male and 39 female patients, majority (93%) ethnically Chinese, average aged 47 years old. Four of these patients presented with acute non A-E hepatitis. The rest have chronic liver disease, including 106 (71.6%) with chronic hepatitis B infection, 29 with chronic hepatitis C infection and nine with cryptogenic cirrhosis.

Of the 148 patients, five were HGV RNA positive: they included 2/106 (1.9%) and 3/29 (10.3%) of those with chronic hepatitis B and C co-infection, respectively ($p = 0.03$). None of the patients with cryptogenic cirrhosis or acute non A-E hepatitis were HGV RNA positive. Six of the 76 patients tested for anti-HGenV IgG were seropositive. All of them had chronic liver diseases: three and two of them were among those with chronic hepatitis B and C infection, respectively ($p = 0.31$), and one belonged to the group with cryptogenic cirrhosis. All four patients who had acute non A-E hepatitis were seronegative for anti-HGenV IgG (see Table I).

Among the chronic hepatitis B patients, mean s. alanine transferases (s. ALT) were 52 U/l and 79 U/l in the HGV RNA-positive and -negative patient groups, respectively ($p = 0.4$). Half (2/4) of those with positive marker(s) of hepatitis G infection (HGV

RNA and/or anti-HGenV IgG positive) were cirrhotic, whereas only 8.6% of those without positive marker of hepatitis G infection were cirrhotic (N.S.).

Similarly, among the patients with chronic hepatitis C, the mean s. ALT were 48 U/l and 94 U/l in the HGV RNA-positive and -negative patient groups, respectively ($p = 0.05$). Forty percent (2/5) of these patients with positive marker of hepatitis G infection were cirrhotic, in contrast to 11.1% of those without cirrhosis (N.S.).

There were a total of seven cases of hepatoma. Only one patient was weakly positive for HGV RNA and seropositive for anti-HGenV IgG at the same time. The rest were negative for HGV RNA and anti-HGenV IgG.

Ten of the chronic hepatitis patients had background of end-stage renal disease. Nine and one of them were infected with hepatitis C and B, respectively. Seven of these nine hepatitis C patients and the only hepatitis B patient had all undergone renal allograft transplantation. The remaining two were on regular haemodialysis. 2/9 of these hepatitis C patients had hepatitis G co-infection, with positive HGV RNA. The only renal patient infected with chronic hepatitis B was not co-infected with hepatitis G.

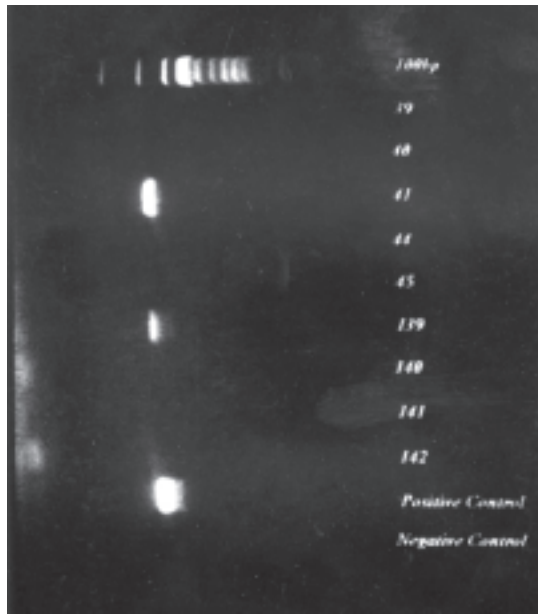
DISCUSSION

Our study showed that HGV RNA was present in 3.5% (5/144) of our patients who have chronic liver diseases but none among those with acute non A-E hepatitis. The latter is similar to the Taiwanese findings⁽⁶⁾.

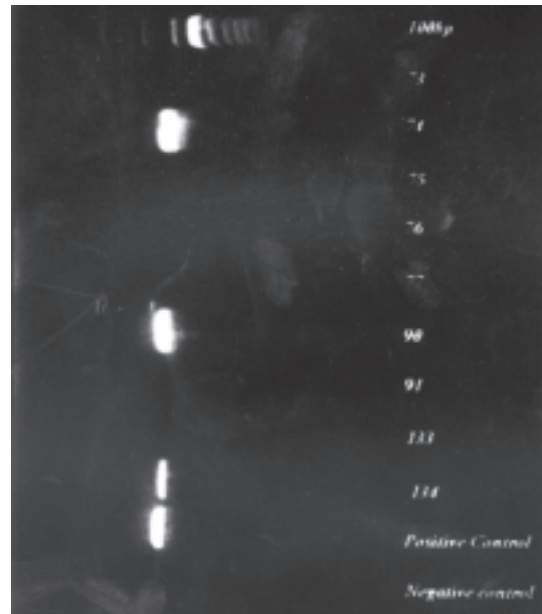
On determining if hepatitis G virus plays a role in the pathogenesis of chronic liver disease in our patient population, it is apparent that hepatitis G was not a significant etiologic agent in our patients with cryptogenic cirrhosis. On the other hand, it appears, numerically, that there was a higher frequency of HGV exposure among patients with chronic hepatitis B or C infection who developed cirrhosis. This was, however, not statistically significant. In fact, the probable lack of pathogenic role of HGV in these patients is consistent with the lack of augmentation of necroinflammation, in terms of s. ALT, in patients with co-infection. While our numbers are too small to be of any statistical significance, this clinical impression is consistent with the findings in larger series in the West⁽⁷⁻⁹⁾.

Of note too, is the relatively common co-infection of hepatitis G virus with hepatitis C virus despite the higher prevalence of hepatitis B infection in our population, suggesting that the high association between these two viruses in the Western population is more than a regional coincidence. Their frequent co-existence is probably, as suggested previously,

Fig 1 PCR product of HGV RNA in patients with chronic hepatitis B and C (a weakly positive HGV RNA, compared to positive control, is noted in patient 139).



Chronic hepatitis B patients.



Chronic hepatitis C patients

accounted by their common mode of transmission via blood products. Hence, the relatively high prevalence of hepatitis G and C co-infection among our patients with end-stage renal disease who required haemodialysis or renal transplantation in comparison with the other patients. This prevalence rate is similar to that reported by Wong et al in a local study⁽¹⁰⁾. However, it is interesting to note that Chen and his co-workers^(11,12) reported high propensity of persistent hepatitis G virus infection if it is acquired during infancy, especially among those who has high-titred maternal viraemia. Hence, knowing that most of the chronic hepatitis B patients in our population acquired their infection perinatally, the low prevalence of concomitant hepatitis B and G infection must be due to an indeed low hepatitis G viraemia, if present at all, in our maternal hepatitis B population.

In addition, our study also found mutually exclusive presence of HGV RNA and anti-HGenv antibodies in all, except one, patients. Thus confirming that, unlike hepatitis C infection, the acquisition of antibodies to the hepatitis G antigen (anti-HGenv) signifies past, rather than present, HGV infection. Our one exceptional case was probably caused by a patient who was at the verge of seroconversion, when viral elimination was still incomplete, as suggested by a relatively weak positive HGV RNA in this patient (see Fig. 1).

In summary, hepatitis G virus infection does not contribute significantly to the cause of cryptogenic cirrhosis and acute non A-E hepatitis in our limited number of patients. On the other hand, co-infection of hepatitis C and G was found in more than 10 % of our chronic hepatitis C patients, commoner than its association with our chronic hepatitis B patients.

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