# HEPATITIS G INFECTION AND THERAPEUTIC RESPONSE TO INTERFERON IN HCV-RELATED CHRONIC LIVER DISEASE

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**Abstract.** Circulating HGV-RNA was determined in 117 patients with HCV-related chronic liver disease and in 200 healthy blood donors. The patients, aged 50.8±13.8 years, were classified as chronic hepatitis (CH; n = 82), liver cirrhosis (n = 25) and hepatocellular carcinoma (HCC; n = 10). HGV-RNA was detected in 5 (4.3%) patients, all with CH and in 10 (5%) of blood donors. The majority of all groups (52% to70%) were infected with HCV genotype II/1b, including 4/5 patients with HGV co-infection. Of 5 patients with HGV co-infection, 4 were positive for anti-HBs and anti-HBc and none exhibited jaundice. A 24-week course of interferon treatment with 12-month follow-up was achieved in 27 patients with chronic active hepatitis, including 3 with HGV co-infection. Of these, 55.6% responded to the therapy, but only 6/27 (22.2%) patients were sustained responders. The majority of sustained responders were HCV genotype III/2a (4/6) while genotype II/1b was found in the majority of patients with relapse (7/9) and non-responders (9/12). At the 48- month follow up, 2/6 sustained responders (one with HGV co-infection) became HCV RNA positive. These results show that the prevalence of HGV infection in HCV-related chronic liver disease is low, as in the general population, and is found in younger patients with chronic hepatitis. HGV co-infection does not interfere with clinical severity, disease progression or response to interferon in patients with HCV-related chronic liver disease. The favorable factors of interferon treatment for HCV infection are young age, low HCV-RNA levels and HCV genotype III/2a.

## INTRODUCTION

Hepatitis G virus (HGV) is a recently identified flavivirus which is principally transmitted through parenteral routes (Simons *et al,* 1995; Linnen *et al,* 1996; Alter *et al,* 1997b). HGV viremia is found worldwide and is a major cause of hepatitis of unknown etiology (non-A-E) accounting for 9-35% of acute and at least 30% of chronic hepatitis (Simons *et al,* 1995; Linnen *et al,* 1996; Alter *et al,* 1997a,b; a; Jeffers *et al,* 1996; Pramoolsinsap, 1998). The prevalence of HGV viremia among the healthy population in different regions of the world varies considerably but in many countries it is 5-10 fold higher than HCV viremia (Alter *et al,* 1997b; Feucht *et al,* 1997; Loiseau *et al,* 1997). The pathogenic role of HGV in chronic liver disease appears to be mild and is less important compared to that of HCV, but this is still controversial. The significance of HGV infection in chronic liver disease is aggravated by concurrent infection with HBV or HCV. According to a few studies HGV may have an impact on the course of coexisting chronic HCV infection (Sugai *et al,* 1997; Suarez *et al,* 1997) but this has not been confirmed by many studies (Alter *et al,* 1997a; Tanaka *et al,* 1996; Pawlotsky *et al,* 1996; Bralet *et al,* 1997; Martinot *et al,* 1997). HGV appears to be more sensitive to interferon compared to HCV (Pawlotsky *et al,* 1996; Bralet *et al,* 1997; Martinot *et al,* 1997; Francesconi *et al,* 1997; Saiz *et al,* 1997; Nagayama *et al,* 1997) but in a few patients, the therapeutic response of HGV is only transient (Tanaka *et al,* 1996). The present study has determined the prevalence of HGV infection in patients with HCV-related chronic liver diseases. Their clinical course, biochemical, virology genotype and therapeutic response to interferon have been evaluated.

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## MATERIALS AND METHODS

### Patients

Patients with chronic liver disease related to HCV infection were recruited to the study at

Ramathibodhi and Phaya Thai hospitals, Bangkok, Thailand. HCV infection was defined by the presence of HCV-RNA or antibodies to HCV in serum. All patients had had persistent elevation of ALT levels for more than 6 months. Exclusion criteria were patients known to either have any systemic disease or other causes of chronic liver disease *eg.* alcohol abuse, metabolic or autoimmune disorders. Patients with hepatitis B virus (HBV) surface antigen or human immunodeficiency virus antibody were excluded. Informed consent was obtained from each subject and the study was approved by the research committee of Mahidol University, Thailand.

Blood samples were collected for complete blood count, biochemical and liver function tests, HCV-RNA concentrations and HCV genotypes were studied at Ramathibodi Hospital. Liver biopsy was performed in all patients with normal coagulation tests and liver histology was evaluated as described by DeGroote *et al* (1977) and Desmet *et al* (1994). The patients were classified according to clinical, biochemical, radiological findings and liver histology into 3 groups; chronic hepatitis (CH), liver cirrhosis and hepatocellular carcinoma (HCC).

Alpha interferon therapy was offered to patients with chronic active hepatitis who had detectable HCV-RNA with persistent ALT elevations above twice the normal level for at least 6 months. Only patients who gave informed consent to the therapy were recruited and these patients were followed up for at least 12 months after completion of interferon treatment.

### Viral serology

Presence of anti-HCV in serum was detected by the third-generation enzyme-linked immunosorbent assay (ELISA) kit (Abbot Laboratories, North Chicago Ill). All the sera were tested for hepatitis B infection by ELISA, Auszyme II (Abbot Laboratories, North Chicago, Ill), and anti-HIV was detected by the second generation ELISA (Abbot Laboratories, North Chicago, Ill).

### HCV-RNA concentration

Quantitative measurement of HCV-RNA was performed by the second generation test branched-DNA signal amplification assay version 2.0 (Chiron corporation) (Lau *et al,* 1995).

### HCV genotyping

The assay for HCV genotype was based on variations of the 5'-UTR region of the HCV genome, and on analyzing amplicons from the conserved 5'-UTR generated by nested reverse transcriptase PCR using genotype-specific primers of the core region categorized according to the method of Okamoto *et al* (1992). RNA extraction was performed by the guanidium thiocyanate method (Cha *et al,* 1991). Infections with double genotypes were confirmed by the nested PCR and the PCR products were sequenced. Reverse transcription and first PCR was carried out in a single reaction mix with primers from 5'-UTR. The first PCR used 35 cycles (94°C for 1.5 minutes, 55°C for 2 minutes, and 72°C for 3 minntes), the second PCR was performed with 37 cycles at 94°C for 1 minnte, 56°C for 1 minnte and 72°C for 3 minutes. The second PCR using each of four type-specific primers was performed in case of double genotype infections.

### HGV-RNA detection

The sera were separated from whole blood within 2-3 hours of collection and immediately stored at-70°C until testing for HGV-RNA at Chulalongkorn Hospital. Serum HGV-RNA was detected by reverse-transcription nested PCR. RNA extraction was performed by the guanidine method (Cha *et al,* 1991). Subsequently, denaturation was performed at 65°C for 5 minutes. The RNA samples were reverse-transcribed into cDNA in a total volume of 20 µl using 50 U of MuLV reverse transcriptase (Perkin Elmer, NJ, USA), 50 mmol/l KCl, 10 mmol/l Tris-HCl pH 8, 400 µmol/l dNTP, 10 U RNAse inhibitor, and subsequently incubated at 37°C for one hour. HGV-RNA was detected by nested polymerase chain reaction (PCR) (thermocycler Perkin Elmer 9600; CA, USA) using four primers created from the 5 untranslated region (UTR) of GBV- C. First amplification step: 5µl of cDNA samples were amplified in a 50 µl reaction volume containing 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 10 mmol/l Tris-HCl pH 8, 200 mol/LM dNTP, 1 U Ampli Taq DNA polymerase (Perkin Elmer Cetus, NJ, USA), 0.8 µmol/l each of outer sense primer, located at position 108, having the sequence 5' AGG TGG TGG ATG GGT GAT 3' and the outer

anti-sense primer, located at position 531, having the sequence 5' TGC CAC CCG CCC TCA CCC GAA 3', 1.5 mmol/l $MgCl_2$. The reaction was then performed for 30 cycles at 94°C for 0.6 minutes, at 55°C for 0.7 minutes and at 72°C for 1.5 minutes. Second amplification step: 20 µl reaction was performed as described above for the first amplification step, using 1 µl of the first step PCR product as template. The inner sense primer, located at position 134, had the sequence 5' TGG TAG GTC GTA AAT CCC GGT 3' and the anti-sense primer, located at position 476, had the sequence 5' GGR GCT GGG TGG CCY CAT GCWT 3' (R = A or G, W = A or T, Y = C or T) (Jarvis *et al,* 1996).

The above primers were employed for the second step, consisting of 30 cycles of amplification. The 10 µl of amplified product were fractionated by 2% Nusieve agarose gel electrophoresis in 1 x Tris borate buffer pH 8 at 120 volts for 50 minutes. Ethidium bromide was added to the gel on preparation and the PCR products could thus be visualized by UV fluorescence. The product band showed at 421 base pairs for the first amplification step and at 343 base pairs for the second step.

**Blood donors**

As a control, a series of 200 specimens taken from voluntary blood donors were collected at random from the National Blood Center, Thai Red Cross, Bangkok. All specimens were screened for HGV-RNA. Of these, 150 were males and 50 females aged 19 to 64 years.

**Biochemical test**

Blood chemistry including liver function tests was determined by an automated chemical analyzer, Beckman Synchron CX5 (BREA, California) at the Central Laboratory, Ramathibodi Hospital.

**Anti-viral therapy**

Recombinant interferon alfa-2b (Intron-A, Schering-Plough) was given to selected patients with biopsy-documented chronic active hepatitis and was administered subcutaneously at a dosage of 3 million units thrice weekly for 24 weeks. Patients were followed up for clinical assessments in the second and fourth week after the initiation of therapy and then every 4 weeks during treatment and at 2-3 month intervals for at least 1 year after completion of therapy. Serum ALT levels were measured at least once every 4 weeks during treatment, and then every 2-3 months.

Therapeutic responses to interferon were assessed by serum levels of ALT and HCV-RNA as sustained responders, relapse or non-responders. Sustained responders were patients with normalization of ALT levels and disappearance of serum HCV-RNA for at least 12 months after completion of therapy. Patients with relapse showed abnormal ALT levels with reappearance of HCV-RNA after temporary response and cessation of interferon therapy. Non-responders were patients with persistent ALT elevation and detectable serum HCV-RNA during and after the course of interferon treatment. In all patients with HGV co-infection, HGV-RNA was reassessed after completion of interferon treatment.

**Statistical analysis :**

The clinical and biochemical data are presented as mean ± SD or median as appropriate. Comparisons between groups of liver disease or between patients with and without HGV co-infection were made by Student's *t* -test or Mann-Whitney U test for quantitative variables and by chi-square test or Fisher's exact method for the categorical variables. A p-value of 0.05 or less indicated statistical significance.

## RESULTS

**Clinical features and biochemical profile**

The study enrolled 117 patients aged 19-84 years (mean±SD = 50.8±13.8 years) and the male: female ratio was 1.3:1 (Table 1). Of these, 82 were CH, 25 liver cirrhosis and 10 HCC. Among the 3 groups, patients with CH were the youngest (mean±SD = 45.9 ± 11.1 years, $p < 0.001$) and patients with HCC constituted the most common exposures to blood transfusion (90%, $p = 0.005$) but there were no significant differences in their HCV-RNA levels (Table1). Duration of blood transfusion was longest in patients with HCC (15.6 ± 6.2 years) and this was significant compared to patients with CH (p =

0.001) but not to patients with liver cirrhosis. Of all 5 intravenous drug users, 4 patients were in the CH group. An unknown risk of HCV infection was most common in patients with CH (46.3%) but was not significantly different compared to the other 2 groups (10-20%). History of previous major surgery was found in 50-90% of all groups.

The majority of the patients (62.4%) were positive for anti-HBs and anti-HBc and there were no significant differences regarding the prevalence of HBV among the three groups. Between patients with and without antibodies to HBV, there were no significant differences in ALT levels (148.7±111.5 *vs* 158.4±95.5 U/l, p = 0.65) or HCV-RNA levels (15.5±14.1 *vs* 20.5±11.0 MEq/ml; p = 0.18). The HCV genotype distributions of all patients were type II/1b or/and III/2a (Table 1). The majority of patients in all groups were infected with HCV genotype II/1b (52-70%) and infections with both genotypes II/1b and III/2a were found in 7 patients (6%). There were no significant differences in baseline laboratory results among the 3 groups of patients with chronic liver disease (Table 2).

**HGV infection**

Of all patients, HGV-RNA was detected in 5 patients, all with CH (Table 3). These were 2 males and 3 females aged 19-48 years. The prevalence of HGV was 4.3% of all patients, or 6.1% of patients with CH. Patients with concurrent HGV infections were significantly younger (36.8 ±10.0 years.) compared to the remaining patients with CH (46.5 ±10.9 years; p = 0.007). The majority of both groups (60.0% and 53.2%) had a history of previous exposure to blood transfusion. Intravenous drug abuse was found in 1 and 3 patients with and without HGV co-infection, respectively. All but one patient with concurrent HGV infection were infected with HCV genotype II/1b but the genotype distributions between patients with and without HGV co-infection were not significantly different. Circulating anti-HBs and anti-HBc was found in 4 (80%) patients with concurrent HGV infection and in 53.3 % of patients with HCV infection alone ( p = 0.24). Of 200 voluntary blood donors aged 19-64 years, HGV-RNA was detected in 10 subjects (5%).

All five patients with concurrent HGV infection had mild chronic hepatitis with mean ALT levels of 116.4 ± 36.0 U/l, and none was jaundice. The baseline HCV-RNA levels and ALT levels in patients with HGV co-infected were lower compared to patients with HCV infection alone, although these differences were not significant (p = 0.30 and 0.85). Other laboratory results including serum levels of gamma-glutamyl-transpeptidase, alkaline phosphatase and γ-globulin were not significantly different between patients with and without HGV co-infection (Table 3).

**Therapeutic response to interferon-α**

Of 30 patients with CH who received interferon therapy, 27 patients completed the 24-week therapy and were subsequently followed up for at least 12 months. The treatment was discontinued in three patients (including one HGV-positive) during the first 2-3 months of therapy due to interferon side effects. Each of these patients developed thrombocytopenia with neutropenia, thrombocytopenia and hyperthyroidism, respectively.

Of 27 patients who received the full course of treatment, 3 had HGV co-infections. The majority of all patients (55.6%) responded to the therapy, but only 6/27 (22.2%) patients were sustained responders or remained negative for HCV-RNA with normal ALT levels till the 6-month follow-up (Table 4). Compared to patients with relapse or non-responders, sustained responders were younger and had lower levels of serum HCV-RNA although these differences were not significant. Among the three groups of responders there were no significant differences in their baseline levels of ALT, AST or GGT. The majority of patients with sustained response were HCV genotype III/2a (66.7%) while genotype II/1b was found in the majority of patients with relapse (77.8%) as well as among non-responders (75.0%) (Table 4).

Of the 3 patients with HGV co-infection who received treatment, 2 were infected with HCV genotype II/1b and one with genotype III/2a (Table 5). The mean age of these 3 patients (34±10.7 years), was significantly lower compared to patients without HGV co-infection (p <0.001). Their baseline levels of ALT and HCV-RNA were also lower, although there were not significant differences compared to the latter group (Table 5). After completion of interferon treatment, 2 patients showed sustained disappearance of both HCV-RNA and HGV-RNA and one had a relapse of both infections during 24 weeks of follow-up. This relapse patient was infected with HCV genotype III/2a.

Table 1

Demographic data and other baseline data in patients with HCV-related chronic liver disease.

| Characteristic data | Groups of patients | | | |
|---|---|---|---|---|
| | Chronic hepatitis | Cirrhosis | HCC | Total |
| No. of patients (%) | 82 (70.1%) | 25 (21.4%) | 10 (8.5%) | 117 |
| Age (mean±SD, year) | 45.9 ± 11.1 | 60.6 ± 13.9 | 66.0 ± 9.6 | 50.8 ± 13.8 |
| Male: Female (N ) | 45: 37 | 15: 10 | 6: 4 | 66: 51 |
| HCV genotypes; N (%) | | | | |
| - Type II | 57/82 (69.5%) | 13/25 (52.0%) | 7/10 (70.0%) | 77/117 (65.8%) |
| - Type III | 21/82 (25.6%) | 10/25 (40.0%) | 2/10 (20.0%) | 33/117 (28.2%) |
| - Mixtypes II and III | 4/82 (4.9%) | 2/25 (8.0%) | 1/10 (10%) | 7/117 (6.0%) |
| Anti-HBs positive; N (%) | 45/82 (54.9%) | 21/25 (84.0%) | 7/10 (70.0%) | 73/117 (62.4%) |
| Source of infection; N (%) | | | | |
| - Post transfusion | 40 (48.8%) | 19 (76.0%) | 9 (90.0%) | 68/117 (58.1%) |
| - IV drug use | 4 (4.9%) | 1 (4.0%) | 0 | 5/117 (4.3%) |
| - Sporadic | 38 (46.3%) | 5 (20.0%) | 1 (10.0%) | 44/117 (37.6%) |
| History of transfusion (year) | 8.90 ± 5.50 | 11.74 ± 4.23 | 15.60 ± 6.24 | 10.41 ± 5.75 |
| History of surgery; N (%) | 41/82 (50.0%) | 15/25 (60.0%) | 9/10 (90.0%) | 65/117 (55.6%) |
| HCV-RNA (MEq/ml) | 19.1558 ± 5.8243 | 11.4192 ± 6.7915 | 12.1923 ± 8.4817 | 14.2545 ± 7.0334 |

Table 2

Baseline biochemical results (mean+SD) in 117 patients with HCV- related chronic hepatitis.

| Parameters | Groups of patients | | |
|---|---|---|---|
| (normal values) | Chronic hepatitis | Cirrhosis | HCC |
| Hematocrit (%) | 41.97 ± 4.85 | 35.55 ± 5.35 | 33.01 ± 4.39 |
| ALT (6-36 U/l) | 177.43 ± 107.37 | 84.94 ± 72.47 | 92.40 ± 24.14 |
| AST (14-33 U/l) | 109.86 ± 74.87 | 90.00 ± 49.18 | 138.80 ± 65.81 |
| GGT (5-38 U/l) | 107.17 ± 87.98 | 134.57 ± 213.50 | 70.12 ± 21.75 |
| AP (20-90 U/l) | 88.61 ± 39.03 | 134.78 ± 198.44 | 147.33 ± 99.79 |
| Albumin (32-55 g/l) | 41.73 ± 4.79 | 33.92 ± 8.10 | 36.13 ± 7.47 |
| Bilirubin (3.4-17.1 μmol/l) | 22.51 ± 33.35 | 47.93 ± 38.41 | 17.54 ± 4.98 |
| Prothrombin time (second) | 14.32 ± 2.39 | 16.25 ± 2.97 | 16.80 ± 1.88 |
| HCV-RNA (MEq/ml) | 19.1558 ± 5.8243 | 11.4192 ± 6.7915 | 12.1923 ± 8.4817 |

ALT = Alanine aminotransferase, AST = Aspartate aminotransferase ,
GGT = γ-glutamyl transpeptidase , AP = Alkaline phosphatase

Table 3
Baseline data in patients with chronic hepatitis C with and without HGV co-infection (n=82).

| Baseline data | Groups of patients | |
|---|---|---|
| | HGV-RNA positive | HGV-RNA negative |
| No. of patients | 5 | 77 |
| No. of Male : Female | 2: 3 | 43: 34 |
| Age (mean ± SD, year) | 36.8 ± 10.0 * | 46.5 ±10.9 |
| Blood transfusion; N (%) | 3 (60.0%) | 41 (53.2%) |
| Intravenous drug use; N (%) | 1 (20.0%) | 3 (3.9%) |
| History of transfusion (mean ± SD, year) | 6.4 ± 2.8 | 8.1 ± 4.6 |
| ALT (mean ± SD, U/l) | 116.40 ± 36.01 | 181.53 ± 109. 17 |
| AST (mean ± SD, U/l) | 99.80 ± 37.92 | 110.53 ± 76.81 |
| GGT (mean ± SD, U/l) | 89.0 ± 77.12 | 108.66 ± 89.21 |
| ALP (mean ± SD, U/l) | 100.60 ± 50.69 | 87.77 ± 38.39 |
| HCV-RNA (mean ± SD, MEq/ml) | 13.2343 ± 9.3382 | 16.5811 ± 11.9662 |
| HCV genotype; N (%) | | |
| Type II | 4 (80.0%) | 53 (68.8%) |
| Type III | 1 (20.0%) | 20 (26.0%) |
| Mixtypes II and III | 0 | 4 (5.2%) |
| Anti-HBs positive ; N (%) | 4 (80.0%) | 41 (53.25%) |

* = Statistically differences with p = 0.01

Table 4

Baseline data of 27 treated patients, according to therapeutic responses of interferon.

| Parameters | Types of therapeutic responses | | |
|---|---|---|---|
| | Sustained responders | Relapses | Non-responders |
| No. of patients (%) | 6 (22.2%) | 9 (33.3%) | 12, (44.4%) |
| Age (mean ± SD, year.) | 39.0 ± 4.5 | 47.7 ± 11.6 | 49.8 ± 7.6 |
| Male : Female | 3 : 3 | 7 : 2 | 10 : 2 |
| ALT (mean ± SD, U/l) | 302.83 ± 72.02 | 211.67 ± 120.42 | 183.67 ± 77.51 |
| AST (mean ± SD, U/l) | 123.00 ± 71.68 | 120.89 ± 67.19 | 114.17 ± 48.18 |
| GGT (mean ± SD, U/l) | 161.25 ± 83.08 | 118.75 ± 105.59 | 142.06 ± 105.14 |
| HCV-RNA (mean ± SD, MEq/ml) | 19.1663 ± 12.7158 | 23.5326 ± 15.8349 | 28.7284 ± 18.7065 |
| HCV genotype; N (%) | | | |
| Tpye II | 2/6 (33.3%) | 7/9 (77.8%) | 9/12 (75.0%) |
| Type III | 4/6 (66.7%) | 2/9 (22.2%) | 2/12 (16.7%) |
| Mixtypes II and III | 0 | 0 | 1/12 (8.3%) |
| HGV positive (N) | 2 | 1 | 0 |

Table 5

Baseline data in 27 treated patients with and without HGV co-infection.

| Parameters | Groups of patients | |
|---|---|---|
| | HGV-RNA positive | HGV-RNA negative |
| Number of patients | 3 | 24 |
| Age (mean ± SD, year.) | 34.0 ± 10.67* | 42.00 ± 9.37 |
| Sex ratio (Male: Female) | 1 : 2 | 19 : 5 |
| ALT (mean ± SD, U/l) | 168.0 ± 59.34 | 217.46 ± 121.92 |
| AST (mean ± SD, U/l) | 119.0 ± 48.18 | 128.35 ± 57.09 |
| HCV-RNA (mean ± SD, MEq/ml) | 18.8568 ± 12.3695 | 26.9865 ± 19.3787 |
| HCV genotype; N (%) | | |
| Tpye II | 2/3 (66.66%) | 16/24 (66.66%) |
| Type III | 1/3 (33.33%) | 7/24 (29.16%) |
| Mixtypes II and III | 0 | 1/24 (4.16%) |
| Therapeutic response (N ) | | |
| SR : RE : NR | 2 : 1: 0 | 4 : 8 : 12 |

SR = sustained responders, RE = Relapse, NR = non responders.

All 6 sustained responders including 2 patients with HGV co-infection, remained negative for HCV-RNA up to the 12-month follow up. At the 48-month follow-up, 2 sustained responders (one with HGV co-infection) became HCV-RNA positive despite normal ALT levels. Two patients, one each in the relapse and non-responders group developed HCC at the 24 and 46-month follow-up, respectively.

## DISCUSSION

Patients with chronic liver disease often harbor more than one hepatitis agent, in particular parenterally transmitted viruses. In the present study, the majority (62.4%) of patients with HCV infection had been either exposed to blood transfusion, major surgery or were intravenous drug users. Patients with HCC had a significantly more common exposure to blood transfusions (90%) as well as longer duration of blood transfusions (15.6±6.2 years) compared to patients with liver cirrhosis or CH. These findings suggest that blood transfusion represents an important mode of HCV infection and is related to progression of chronic liver disease. The majority of the studied patients (62.4%) were positive for anti-HBs and anti-HBc and 4.3% of all patients were co-infected with HGV. Between patients with and without antibodies to HBV, there were no significant differences as to levels of ALT or HCV-RNA. And the proportions of patients with antibodies to HBV infection were not significantly different among these chronic liver disease groups. Thailand is an endemic area for HBV infection where 5-10% of the population harbor HBsAg (Pramoolsinsap *et al,* 1986). Although patients with HBsAg were excluded from the present study, prevalence of only antibodies to HBV is high in patients with chronic HCV infection.

The pathogenic differences of HCV genotypes is not fully known but many studies have suggested that genotype II/1b is associated with more advanced liver disease and with the development of liver cirrhosis and HCC (Shimotohno *et al,* 1995; Yotsuyanagi *et al,* 1995; Kobayashi *et al,* 1996; Lopez-Labrador *et al,* 1997). In the present study, the majority of patients with all types of chronic liver disease were infected with HCV genotype II/1b (50-70%) including 4/5 patients with HGV co-infection. Our results concur with reports from East Asia where HCV type II/1b is most prevalent among blood donors in Japan (77%), China (69%), Taiwan (60.1%) and Hong Kong (71%) (Takada *et al,* 1993; Zhang *et al,* 1995; Wu *et al,* 1997). And HCV type III/2a is the second most common genotype found

in Japan (17%) and China (13%) (Takada *et al,* 1993; Zhang *et al,* 1995).

Hepatitis G is principally a parenterally transmitted virus. A high prevalence of HGV viremia has been found in blood transfusion recipients and in drugs users (Linnen *et al,* 1996; Alter *et al,* 1997b; Aikawa *et al,* 1996; Diamentis *et al,* 1997). In the present study, 4/5 patients with HGV co-infection were exposed to either blood transfusion, major surgery or were intravenous drug users. HGV is often found as a co-infection with HCV and occurs in 3.3-18.7% of patients with chronic liver disease (Pramoolsinsap, 1998; Linnen *et al,* 1996; Jeffers *et al,* 1996; Nakatsuji *et al,* 1996), with infection rates usually 2-10 fold higher than those among healthy blood donors from the same geographic areas (Pramoolsinsap, 1998). In the present study, the infection rates of HGV in chronic liver disease (4.3%) are similar to those in healthy blood donors (5%). The prevalence of HGV in our patients with chronic liver disease related to HCV infection is similar to 3.3% reported from Taiwan and Korea (Kao *et al,* 1997; Park *et al,* 1997) but lower than 6.6-11.2% reported from Japan and China (Tanaka *et al,* 1996; Wang and Jin, 1997). The 5' UTR region of HGV investigated in the present study is usually well conserved among members of the flaviviridae family (Lau *et al,* 1995; Bukh and Miller, 1994). However, the HGV sequences are highly variable and some isolates may have escaped detection by the primers (Masuko *et al,* 1996).

A concurrent HGV infection influencing disease severity of HCV infection (Sugai *et al,* 1997; Suarez *et al,* 1997) has not been supported by many reports (Tanaka *et al,* 1996; Pawlotsky *et al,* 1996; Bralet *et al,* 1997; Martinot *et al,* 1997; Francesconi *et al,* 1997; Kubo *et al,* 1997; Sáiz *et al,* 1997; Nakatsuji *et al,* 1996). In the present study, the average ALT levels and HCV-RNA levels of all 5 patients with HGV co-infection were lower compared to patients with HCV infection alone, although these differences were not significant. These findings correspond with a study from Japan in which no evidence of viral interference between HGV and HCV was detected (Nakatsuji *et al,* 1996) and with a report from Spain in which no significant differences between HGV-infected and non-infected patients with HCV infection were found in relation to liver function tests, liver histology and HCV genotype (Saiz *et al,* 1997).

Concurrent HCV and HGV infections have been reported in all types of liver disease and a study from Japan has found that HCV-RNA is less frequently detected in patients with CH compared to those with liver cirrhosis or HCC (Sugai *et al,* 1997). HGV viremia has been reported in 10% of liver carcinoma patients in Taiwan (Kao *et al,* 1997) but in none of 213 patients with HCC in another study from Japan (Kubo *et al,* 1997). In the present study, detection of HGV-RNA was confined to patients with CH thus being similar to many other previous reports which suggest that HGV infection has no impact on the clinical course of coexistent HCV infection (Alter *et al,* 1997a,b; Tanaka *et al,* 1996; Pawlotsky *et al,* 1996; Bralet *et al,* 1997; Martinot *et al,* 1997; Francesconi *et al,* 1997; Kubo *et al,* 1997; Saiz *et al,* 1997). All our 5 patients with HGV co-infection were significantly younger (36.8±10.0 years) compared to patients without HGV viraemia (46.5±10.9 years). Although HGV-RNA may persist for several years without liver dysfunction (Lefrere *et al,* 1997), our findings may represent either an early or more frequent clearance of HGV infection at a younger age (Francesconi *et al,* 1997; Goeser *et al,* 1997). A study in drug users has found an inverse correlation between HGV infection and the duration of intravenous drug consumption (Diamentis *et al,* 1997), suggesting that a high proportion of HGV-infected persons may clear the virus and develop protective antibodies.

Therapeutic responses to interferon in chronic hepatitis related to HCV infection are variable. In the present study, 55.5% of treated patients responded to the therapy but only 22.2% were sustained responders. In comparison to relapse patients or non-responders, patients with sustained response were younger and had lower HCV-RNA levels but these were not significantly differences. The majority of sustained responders were infected with HCV genotype III/2a (66.7%) while genotype II/1b was found in the majority of the other two groups. These findings correspond with other reports in which younger age, low viral load and HCV genotype III/2a have been found independently associated with long-term response (Kobayashi *et al,* 1996; Bukh and Miller, 1994; Goeser *et al,* 1997; Yamada *et al,* 1995; Martinot-Peignoux *et al,* 1995; Marcellin *et al,* 1997).

HGV is sensitive to interferon, particularly if the pre-treatment viral load is low (Bralet *et al,* 1997; Sáiz *et al,* 1997; Nagayama *et al,* 1997). Interferon treatment has resulted in a decline or

disappearance of HGV-RNA with normalization of ALT levels (Kararayiannis *et al,* 1997) in patients with either HBV or HCV infections. However, a study in Japan found that only 2 of 9 interferon treated patients show long term disappearance of HGV after discontinuation of the therapy (Tanaka *et al,* 1996). Whether HGV infection may modify the response to interferon therapy in chronic HCV infection is currently unknown but hepatic inflammation in these patients appears to depend on HCV replication (Tanaka *et al,* 1996; Bralet *et al,* 1997; Martinot-Peignoux *et al,* 1995). Normalization of ALT levels has been found to correlate with the loss of HCV-RNA, regardless of persistence or loss of HCV-RNA (Martinot *et al,* 1997; Nagayama *et al,* 1997). In the present study, all 3 patients with HGV co-infection who received interferon responded to treatment, 2 were sustained responders and one had relapses of HCV-RNA and HGV-RNA after 24 weeks of treatment. However, all these 3 patients were relatively young (mean age = 34±10.7) and had low levels of ALT and HCV-RNA.

Many reports on co-infections with HGV and HCV are from low prevalence areas where less than 5% of the general population harbor HGV and the numbers of HGV infected patients are often too limited for firm conclusions. The controversial findings of HGV infection are partly attributed to differences in HGV-RNA levels, HGV genotype (Khudyakov *et al,* 1997) or host factors, *eg* age, genetic background, coexistent infections and underlying liver pathology. Detection of antibody to HGV is yet to be developed but will reveal its prevalence of past exposure and facilitate the understanding of the natural history and pathogenicity of HGV infection. The results of the present study correspond with much current evidence and suggest that HGV co-infection does not interfere with clinical severity, disease progression or therapeutic response of HCV infection.

## ACKNOWLEDGEMENTS

We are grateful to the Thailand Research Fund, Senior Research Scholar Program for supporting the HGV-RNA detection. We would like to thank the staff of the National Blood Center, Thai Red Cross for providing the blood donor specimens.

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