# HIGH PREVALENCE OF HGV COINFECTION WITH HBV OR HCV AMONG NORTHEASTERN THAI BLOOD DONORS

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Abstract. Hepatitis G viral (HGV) infection among northeastern Thai blood donors was determined by the nested RT-PCR technique. HGV RNA was amplified by the degenerated helicase primers for a product of the expected size of 83 base pairs were used in this study. Serum samples from 322 of three different categories of northeastern Thai blood donors were included in this study. There were 104 HBsAg and Anti-HCV seronegative blood donors (control group), 100 samples of HBs Ag seropositive blood donors (HBV infected group) and 118 serum samples from anti-HCV seropositive blood donors (HCV infected group). The results demonstrated that HGV RNA was not detected in the control group but was found in 10 individuals (10%) in the HBV infected group and 13 (11%) in the anti-HCV positive blood donors. The prevalences of HGV in both seropositive groups were significantly different from the control group ($p = 0.001$). HGV co-infection is highly prevalent among northeastern Thai blood donors who are infected with HBV or HCV. The results also reveal that blood donors seronegative for HCV and HBV are a low risk group for HGV infection.

## INTRODUCTION

Hepatitis G virus (HGV) or GB virus C (GBV-C) was independently named in a search for new hepatitis viruses. HGV is a blood born virus that is parentally transmitted (Schmidt *et al*, 1996). Severe hepatitis with HGV is rare (Yoshiba *et al*, 1995; Shimizu *et al*, 1997). Most infections are subclinical or mild. No causative association with liver disease has been established, therefore limited information about the clinical manifestations is available. HGV infection can be diagnosed by detecting RNA in a blood sample (Simons *et al*, 1995; Linnen *et al*, 1996). HGV RNA can be found in serum for 2-20 weeks after infection (Masuko *et al*, 1996; Alter *et al*, 1997; Shimizu *et al*, 1997; Yashina *et al*, 1997). Retrospective studies of stored sera show that HGV can cause a persistent infection for longer than 7 to 16 years (Masuko *et al*, 1996). The amount of HGV RNA in serum is about $10^4$ to $10^5$ copies, but only $10^2$ copies are usually found in liver tissue (Kudo *et al*, 1997). PCR amplification of HGV RNA in serum by reverse transcription (RT-PCR) has been performed since early discovery of the virus (Simons *et al*, 1995; Linnen *et al*, 1996). The frequency of HGV infection is high in patients with overt parenteral exposure to multiple blood products: 18% of anemic patients with multiple transfusions (Linnen *et al*, 1996), 26% of hemodialysis patients in Spain (Forns *et al*, 1997) and 12.5% in the US (Fong *et al*, 1997), 3.1 to 57.5% of hemophiliacs and the majority of hemophiliacs who were infected with HGV were also coinfected with HCV (Linnen *et al*, 1996; Masuko *et al*, 1996; Tsuda *et al*, 1996; Gerolami *et al*, 1997). Infection with HBV or HCV, but not HIV or HTLV-I, is very common in northeastern Thailand (Barusrux *et al*, 1995; Urwijitaroon *et al*, 1996; 1997; Barusrux *et al*, 1997). There have been no reports on the prevalence of HGV infection in this region.

In this study, the nested RT-PCR technique with degenerated helicase primers was used to amplify the HGV RNA in the serum to examine the prevalence of HGV infection among northeastern Thai blood donors.

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## MATERIALS AND METHODS

### Serum samples

Serum samples were collected from 322 northeastern Thai first time blood donors during 1999 to 2000 at the blood transfusion center, Srinagarind Hospital, Khon Kaen University, Khon Kaen, Thailand. There were three different categories of blood donors. The first group was the control group, which consisted of 104 sera from blood donors with seronegative HBsAg and Anti-HCV. The second group comprised of 100 sera from HBs Ag seropositive donors (HBV infected group). The third group consisted samples from 118 anti-HCV seropositive blood donors (HCV infected group).

### Serum RNA extraction

Serum samples were detected for HGV RNA by nested RT-PCR technique. RNA was extracted from 300 µl of sera with an extraction reagent containing guanidine thiocyanate and chloroform (SepaGene, RV-R, Sanko Junyaku, Japan) and dissolved in 9 µl of distilled water treated with DEPC. Then, 11.2 µl of reverse transcription mixture was added and incubated at 42ºC for 30 minutes to reverse transcribe RNA into cDNA.

### HGV RNA detection

The cDNA from the extracted RNA samples was used for the nested PCR. Degenerate primers (G8,G9,G10,G11) originally described to amplify the NS3 helicase region of the HGV genome by nested PCR were used (Yoshiba *et al*, 1995; Masuko *et al*, 1996). Two microliters of cDNA was used for first round amplification. The outer primers with G8 sense primers at position 4278 (5′-TATgggCATggHATHCCYCT-3′) and G9 antisense primers at position 4435 (5′-TCYTTgATgAT DgAACTgTC-3′) were used with the following thermal cycles: 94ºC for 30 seconds, 55ºC for 60 seconds, and 72ºC for 60 seconds for 35 cycles. One microliter of the first round PCR product was used for second round amplification. The inner primers with G10 sense primers at position 4335 (5′- CATTCVAAggCggAgTgYgA-3′) and G11 antisense primers at position 4417 (5′-TCYTTACCCCTRTAATAggC-3′) were used with the following thermal cycles: 94ºC for 30 seconds, 55ºC for 30 seconds, and 72ºC for 45 seconds for 30 cycles. The second round PCR product was added to 2% agarose gel. Current was run in the 0.5X TBE buffer at 100 volts for 20 minutes. Then, the gel was stained with ethidium bromide and observed under UV illumination. The positive target band was 83 base pairs. The prevalences of HGV among HBV and HCV infected groups versus the control group were studied. Chi-square with 95% confident intervals was used for statistical analysis.

## RESULTS

The amplified product of HGV RNA by nested RT-PCR of 83 base pairs is shown in Fig 1. HGV RNA was not found in the control group but was found in 10% of 100 HBV infected blood donors and 11% (13/118) of anti-HCV positive

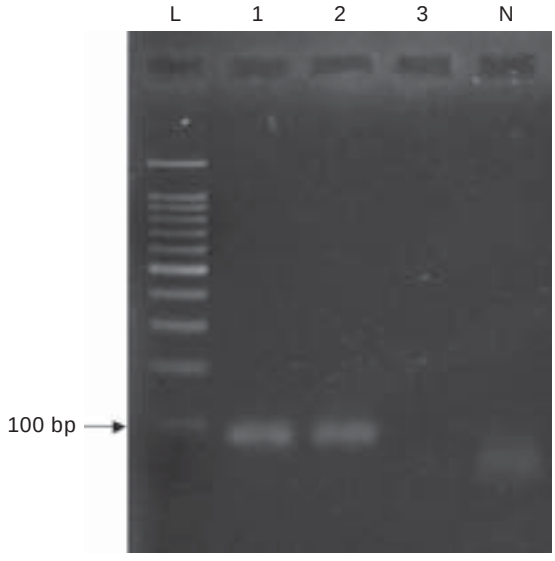


Fig 1–HGV RNA detected in northeastern Thai blood donors by the nested RT PCR technique, with the degenerated helicase primers that amplified a product of 83 base pairs. Amplicons of the PCR were analyzed by 2% agarose gel electrophoresis. Lane 1 shows positive HGV RNA from a HBV infected blood donor. Lane 2 shows HGV RNA in an anti-HCV positive sample. Lane 3 is a negative result in one control sample. The 100 bp DNA ladder and PCR negative control were also run in parallel (lane L and lane N, respectively).

Table 1
GBV-RNA among 322 northeastern Thai blood donor in three different categories.

| Blood donor catagories | HGV RNA | | |
|---|---|---|---|
| | Number | Positive (%) | p-value |
| Seropositive group | | | |
| - HBV infected | 100 | 10 (10.0) | 0.0006 |
| - HCV infected | 118 | 13 (11.0) | 0.0014 |
| Total | 218 | 23 (10.5) | 0.0013 |
| Seronegative (control group) | | | |
| - HBsAg, anti-HIV, anti-HCV negative | 104 | 0 (0) | |
| Total cases | 322 | 23 (7.1) | |

HGV = hepatitis G virus; HBV = hepatitis B virus, HCV = hepatitis C virus; HIV = human deficiency virus

blood donors. The difference between the HBV and HCV infected blood donors (infected group) are the control group was statistically significant (p=0.0006 and p=0.0014, respectively) as shown in Table 1.

## DISCUSSION

The helicase (NS3 region) primer set was used for HGV detection in this study because NS3 and 5′ UTR primers showed 98-100% sensitivity, whereas the E2 primer showed only 84% sensitivity in a previous report (Kao *et al*, 1997). The prevalence of HGV infection among blood donors has been reported to range from 0.9 to 10% around the world. It has been detected with different prevalence rates: 0.9% in Japan (Masuko *et al*, 1996), 2% in Taiwan (Wang *et al*, 1996), 0.7 to 2% in southern China (Wang and Jin, 1997; Wu *et al*, 1997), 1.4% in US (Alter *et al*, 1997), 1.9 to 4.7% in Germany (Heringlake *et al*, 1996; Schleicher *et al*, 1997), 3.2% in UK (Haydon *et al*, 1997), 4.2% in France (Loiseau *et al*, 1997) and 10% in Brazil (Bassit *et al*, 1998). HGV infection was detected in 1.7% of blood donors with normal serum alanine aminotransferase (ALT) levels and 1.5 to 1.9% of blood donors who were rejected due to ALT elevation (Linnen *et al*, 1996; Feucht *et al*, 1997). Most patients who were infected with HGV alone had normal ALT levels (Masuko *et al*, 1996). These data suggested that ALT level do not correlate with HGV infection.

Our study shows that HGV infection is highly prevalent among northeastern Thai blood donors co-infected with HBV (10%) or HCV (11%). This coinfection is probably related to similar modes of transmission. It is spread parenterally, the same as HBV and HCV (Aikawa *et al*, 1996; Jarvis *et al*, 1996; Alter *et al*, 1997; Kinoshita *et al*, 1997; Feucht *et al*, 1997; Shimizu *et al*, 1997; Wu *et al*, 1997). Therefore, those seronegative for HCV and HBV are at low risk for HGV infection. This result correlates with previous reports that found HGV co-infection in 10% with HBV infection and in 11-19% with HCV infection in Europe (McHutchison *et al*, 1997) and USA. (Linnen *et al*, 1996; McHutchison *et al*, 1997). Fortunately, unlike its cousin HCV, HGV has no clear association with a known disease state. A prospective study of post transfusion hepatitis (PTH) in Canada (Blajchman *et al*, 1993) showed the prevalence of HGV positivity in non A-C post-transfusion hepatitis was 15% (3/20), or 3 in 4,588 recipients. HGV RNA persisted for 5 years in one of the patients after recovery from PTH, but none of the three patients developed chronic liver disease. Another prospective study of PTH showed that 34 cases of 400 recipients became HGV positive after surgery. This included of 7 recipients who were co-infected with HCV, 2 recipients who were co-infected with HCV and HTLV-I and 25 recipients who were infected with HGV alone. In the HGV infected alone group, 3 cases had mild ALT elevation and 2 had peak ALT levels of 101 and 123 IU/l during the 6 month

follow-up. All the patients who were infected with HGV alone were asymptomatic without evidence of hepatitis during 1 to 8 years follow-up despite the persistence of HGV up to 8 years. The clinical courses in the 7 HCV coinfected recipients were similar to those infected with HCV alone (Wang *et al*, 1996). A prospective study of transfusion-associated hepatitis from the NIH (Alter *et al*, 1997) showed that 9 of 79 patients with transfusion-associated NANB hepatitis were HGV positive, 6 patients were coinfected with HCV and 3 patients infected with HGV alone. The disease in the three patients with HGV infected alone was mild and only one remained persistently viremic with elevated SGPT levels for 4 years before dying from unrelated causes. Hepatic injury in HCV/HGV co-infection has not been found to be different from HCV infection alone (Alter *et al*, 1997). All these reports support there is poor correlation between the presence of HGV and chronic liver disease, and coinfection is not associated with histological severity of liver disease.

Many reports suggest coinfection with hepatitis C virus may cause a more rapid progression of HIV, but co-infection with HGV, which is relatively common in HCV-infected patients, might delay progression of HIV disease (Tillmann *et al*, 2001; Xiang *et al*, 2001; Bjorkman *et al*, 2004; Williams *et al*, 2004; Kaiser and Tillmann, 2005). HIV replication was inhibited *in vitro* by the presence of HGV (Xiang *et al*, 2001).

In conclusion, transmission of HGV through blood transfusion can occur but may not need to be screened for in blood donors for the following reasons: 1) most infections occur as co-infection with other hepatitis viruses, such as HBV and HCV, 2) most of patients who are infected with HBV alone have little or no clinical disease, 3) the infection rarely causes postransfusion hepatitis, 4) the infection rarely induces chronic hepatitis, and 5) the HGV coinfection does not change the clinical course of other hepatitis viruses.

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