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# Prevalence of Hepatitis G Virus in Blood Donors and Recipients

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## ABSTRACT

We evaluated the prevalence of hepatitis G virus (HGV) infection in patients who had received multiple blood components and in blood donors and the possible coinfection with hepatitis C virus (HCV).

Eighty patients who had received multiple blood components and 70 eligible blood donors, as controls were included in this study. HGV RNA was determined by reverse polymerase chain reaction in serum. HGV-RNA was detected in three (3.7%) of patients and in one (1.4%) of controls. Statistical analysis showed no difference between the groups ( $p > 0.05$ ). HGV and HCV coinfection was not observed in both patient and control groups. Although the most important risk factor for HGV infection was found to be blood transfusions in various studies, this study showed that there is not significant relationship between blood components transfusion and HGV infection.

Key Words: Hepatitis G virus, Blood donors.

## ÖZET

### Kan Ürünü Verici ve Alıcılarında Hepatit G Virüs Prevalansı

Bu yazıda, çok sayıda kan ürünü alan hastalar ve vericilerinde hepatit G virüs prevalansını ve hepatit C virüs (HCV) enfeksiyonunu inceledik.

Çok sayıda kan ürünü alan 80 hasta ve kontrol grubu olarak 70 kan ürünü vericisi çalışmaya alındı. HGV RNA serumda PCR ile belirlendi. Hastaların üçünde (%3.7) ve vericilerin birinde (%1.4) HGV RNA pozitif bulundu. İstatistik analiz gruplar arasında fark göstermedi ( $p > 0.05$ ). Her iki grupta da HGV ve HCV enfeksiyon birlikteliği gösterilmedi. Her ne kadar pek çok çalışmada HGV enfeksiyonu için en önemli risk faktörü kan transfüzyonu olarak gösterilmekte ise de bu çalışmada bu ilişki gösterilemedi.

Anahtar Kelimeler: Hepatit G virüs, Kan ürünü.

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## INTRODUCTION

Infection with blood-borne viruses is common among patient who had received blood components. Recently, two independent laboratories have identified a newly proposed hepatotropic RNA viral agent named either GB virus-C (GBV-C) or hepatitis G virus (HGV). The amino acid sequences of these viruses share 95% homology and they are considered to be different isolates of the same virus in the Flaviviridae family sharing large structural and biological similarities with hepatitis C virus (HCV)<sup>[1,2]</sup>.

It now seems that the transmission of HGV is primarily by the parenteral route, especially by transfusion of contaminated blood components<sup>[1,3-9]</sup>. But, the epidemiology of HGV infection, including determination of modes of transmission of HGV must still be a subject of research to answer unresolved questions. This study was carried in order to determine the prevalence of HGV in selected groups of Turkish patients who had received multiple blood components and eligible blood donors.

## MATERIALS and METHODS

**Study population:** We screened the presence of HGV-RNA in the sera of 80 patients (mean age  $\pm$  SD: 46.0  $\pm$  18.4 years, range 14-80 years, 43M/34F) with different diseases (acute myeloblastic leukemia 12, acute lymphoblastic leukemia 11, chronic lymphocytic leukemia 5, Hodgkin's disease 6, non-Hodgkin's disease 12, myelodysplastic syndrome 3, multiple myeloma 3, aplastic anemia 2, hemophilia 5, gastric cancer 14, pancreatic carcinoma 2, colon cancer 4, ovarian carcinoma 3) who had received blood components. We also

analyzed sera from 70 age- and sex-matched healthy subjects, as control group (mean age  $\pm$  SD: 45.4  $\pm$  18.4 years, range 16-79 years, 39M/31F). The criteria for controls were that they evidenced no risk factor for blood-transmissible viruses and that they had never received a transfusion. At least one month had passed between the first transfusion and blood sampling. The average duration of previous transfusions was five years. The characteristics of all study population were showed in Table 1.

The exposure to blood components is expressed in blood units (1 blood unit is defined as 1 unit of packet red cells or platelets or fresh frozen plasma derived from a single donor). The median number of units transfused was 6.8 per patient (1-50 Units).

Sera from patients and controls were rapidly frozen and stored at -75°C, until required parallel assays. The status for coinfection with HCV and for the possibility of liver diseases was determined hepatitis C virus antibody (anti-HCV) in all.

Determination of HGV RNA by reverse polymerase chain reaction assay. HGV RNA was determined by RT-PCR with nested primers derived from conserved regions of the 5'-noncoding region of the HGV genome by the method reported elsewhere<sup>[10]</sup>.

RNA was extracted from each 100  $\mu$ l serum by using High Pure Viral Nucleic Acid (Boehringer, Mannheim, Germany). cDNA's were synthesized by using 5'-CCT ATT GGT CAA GAG AGA CAT-3' antisense primer (#G75) AMV reverse transcriptase (RT) enzyme in a thermal cycler (Techne PHC-3,UK) at 42°C for one hour. From these cDNAs, 5'-CAG GGT TGG TAG GTC GTA ATT CC-3' sense and mentioned

Table 1. Demographic and serologic profile of patients and controls

	Patients (n= 80)	Controls (n= 70)
Age mean $\pm$ SD (range)	46.0 $\pm$ 18.4 (14-80)	45.4 $\pm$ 18.4 (16-79)
Sex (M: F)	43:34	39:31
Number of transfusion (average)	6.8 Unit	0
HGV RNA positive	3 (3.7%)	1 (1.4%)
Anti-HCV positive	6 (7.5%)	2 (2.8%)
HGV RNA positive /Anti-HCV positive	0	0
ALT (> 41 IU/l)	6 (7.5%)	2 (2.8%)

HGV: Hepatitis G virus, anti-HCV: Antibody to hepatitis C, ALT: Serum alanine aminotransferase.

above #G75 antisense primers were used to synthesize first-round RT-PCR product of 242 bp size of HGV virus. From this first-round PCR product, 5'-GGT CAT CCT GGT AGC CAC TAT AGG-3' (#G134) sense and 5'-AAG AGA GAC AAT GAA GGG CGA CGT-3' (#G131) nested primers were used to amplify a second-round PCR product of 208 bp.

**Marker of hepatitis C:** The prevalence of anti-HCV simultaneously tested in the same group of patients and controls. Anti-HCV determined by a third-generation enzyme-linked immunoabsorbent assay (HCV EIA, Abbott Laboratories, North Chicago, Illinois). Conventional liver biochemical tests done using a multiple auto analyzer. The prevalence of abnormal liver function tests was defined as an elevated serum alanine aminotransferase (ALT; above 41 IU/liter, which was previously determined in routine biochemistry laboratory).

**Statistical analyses:** The one-way ANNOVA test was used to estimate the differences of age distribution among groups and between sexes. Differences in the frequency with which HGV RNA and anti-HCV were found in the study groups were using Chi-square test. Odds ratios were calculated to compare the odds of the group with the lowest prevalence and test-based 95% confidence intervals (CI) were done.

## RESULTS

The mean age and the sex ratio were not significantly different for these two groups (respectively,  $p > 0.05$ ;  $p > 0.05$ ). HGV RNA was detected in three of the patients, and in one of the controls. The prevalence of HGV RNA in patient group was not significantly higher than control group (3.75%, 1.4%; chi-square  $p > 0.710$ ). A patient who had received blood components shows a risk of bearing HGV RNA 2.68 times higher (95% CI: 0.274-26.451) than a matched control. Anti-HCV was detected in six (7.5%), of the patient group and in two (2.8%) of the control group. The prevalence of anti-HCV in patient group was not significantly higher in control group ( $p > 0.170$ ). A patient who had received blood components shows a risk of bearing anti-HCV 5.59 times higher (95% CI: 0.657-47.659) than a matched control. HGV and HCV coinfection was not observed in both patient and control groups.

Mean  $SD \pm$  values of serum ALT levels were found to be  $39.4 \pm 19.2$  IU/L in patient group and  $30.1 \pm 6.6$  IU/L in control group. When compared to those of control group, serum ALT levels were significantly

higher in patient group ( $p < 0.0001$ ). Serum ALT levels were detected above cut off value in 10 of the patients and six of the controls. Serum ALT was not elevated in both groups who have HGV RNA positivity. Serum ALT was elevated in two patients with anti-HCV, but the control with anti-HCV had normal ALT level.

## DISCUSSION

Despite limited diagnostic methods, researches have attempted to determine the prevalence of HGV infection in various populations. There is little information on the prevalence of HGV among various populations in the world. In the literature, the prevalence of HCV RNA among nonremunerated blood donor populations was reported 1.9%-2.3% in Germany, 2.6%-4.2% in France, 2.25% in the United Kingdom, 1%-1.7% in the United States, 1.6% in Southern Sweden, and 0.9%-1.2% in Japan<sup>[5,7-9,11-15]</sup>.

Several reports indicate even higher prevalence among residents in South America (9%) and Africa (10.4%)<sup>[16,17]</sup>. Moreover, the HGV infection was reported highly prevalent among the Jewish population in Uzbekistan (10.9%)<sup>[18]</sup>. In the study, the prevalence of HGV infection (1.4%) is similar of some others. The controversial data of authors may be due to different study design, geographical differences, or to in significant associations due to the small number of individuals examined.

In the literature, increased prevalence of HGV virus was reported in subjects such as haemodialysed patient (6.9%-16%), haemophiliacs (14.3%-35.2%), thalassaemics (35%), intravenous drug users (25%-28.8%) and patients with aplastic anemia (26.3%)<sup>[2,7,8,19-22]</sup>.

Such data suggest that the transmission of HGV is primarily by transfusion of contaminated blood components. Parenteral route was not the only source of HGV infection, however. The HGV RNA detection in the patients with no transfusion history and with no other risks for parenteral exposure could be related to the prevalence of HGV infection in the normal blood donor population<sup>[5,6,23]</sup>. Also, a high prevalence of HGV has been detected in nondrug-addict homosexuals (13.4%) and prostitutes (13.9%), which provide strong evidence of its spread via sexual intercourse<sup>[24]</sup>. This would suggest that HGV is transmitted by other unknown means, similar to HCV.

The prevalence of HGV RNA (3.75%) in patients receiving blood components in this study was higher than control (1.4%), but it was not statistically different ( $p > 0.05$ ). The prevalence of HGV RNA in patients receiving blood components in this study is lower than the prevalence reported in literature<sup>[19-22]</sup>. In a similar study at our country, the HGV RNA positivity was detected one of the 56 patients undergoing haemodialysis<sup>[25]</sup>.

If HGV appears to be broadly distributed geographically, its prevalence could differ both in patients who had received blood components and in blood donors in various geographic area, as is the case for other transfusion-transmissible hepatitis virus. The lower prevalence of HGV RNA in patients receiving blood components in this study could explain the low prevalence of HGV infection in our geographic area. Also, this difference may be related to number of units transfused. This study failed to show any relationship between number of blood transfusions and HGV-RNA positivity due to the limited number of patients in this study.

HGV infection can be diagnosed only by detecting viremia by reverse-transcription polymerase chain reaction. This assay is highly sensitive but has several intrinsic problems. The sensitivity of HGV assays depends on the choice of primers, save condition of serum samples, and presence or absence of contamination. These factors can result in both false-positive and false-negative results and might explain some of the reported differences in the prevalence of HGV infection in similar populations<sup>[3]</sup>.

We amplified 5'-UTR to this reason. The sensitivity of PCR assays using 5' UTR primers was 10 to 100 times more likely to detect HGV RNA than that of NS3 and E2 primers<sup>[26]</sup>.

The reported detection rates of HGV RNA among patients infected with HCV have revealed HGV co-infection or super infection in the 5 to 20%<sup>[6]</sup>. However, we found no HGV RNA in the anti-HCV positive group. Recent large-scale studies have found no causal re-

lationship between HGV and hepatitis, although there is a report associating a strain of HGV with fulminate hepatitis<sup>[27-28]</sup>. Many other studies have also shown that HGV has no impact in the clinical course of co-existent HCV infection<sup>[4,6,8,13,20]</sup>. In this series, HGV-infected subjects in both groups have a normal serum ALT. None of them had any signs or symptoms of liver disease. In this study we also did not show any relationship between HGV and liver injury.

In conclusion, although blood components are most important risk factor for the transmission of HGV infection, this study showed that the prevalence of HGV RNA in patients receiving blood components was higher (3.75%) than control (1.4%), but it was not statistically different. Further studies should be done to ascertain the geographical distribution in general population and in patients who had received blood components.

## REFERENCES

1. Moriyama K, Okamura T, Nakano S. Hepatitis GB virus C genome in the serum of aplastic anaemia patients receiving frequent blood transfusions. *Br J Haematol* 1997;96:864-7.
2. Brown KE, Wong S, Young NS. Prevalence of GBV-C/HGV, a novel "hepatitis" virus, in patients with aplastic anaemia. *Br J Haematol* 1997;97:492-6.
3. Cheung R, Keeffe EB. Hepatitis G virus: Clinical relevance and responsiveness to interferon. *Am J Gastroenterol* 1997;92:1957-61.
4. Alter HJ, Nakatsuji Y, Melpolder J, Wages J, Wesley R, Shih JW, Kim JP. The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. *N Engl J Med* 1997;336:747-54.
5. Yoshikawa A, Fukuda S, Itoh K, Kosaki N, Suzuki T, Hirakawa K, Nakao H, Inoue T, Fukuda M, Okamoto H. Infection with hepatitis G virus and its strain variant, the GB agent (GBV-C), among blood donors in Japan.

- Transfusion 1997;37:657-63.
6. Tan D, Matsumoto A, Conry-Cantilena C, Melpolder JC, Shih JW, Leuther M, Hess G, Gibble JW, Ness PM, Alter HJ. Analysis of hepatitis G virus (HGV) RNA, antibody to HGV envelope protein, and risk factors for blood donors coinfecting with HGV and hepatitis C virus. *J Infect Dis* 1999; 179:1055-61.
  7. Nübling CM, Bialleck H, Fursch AJ, Scharrer I, Schramm W, Seifried E, Schmidt U, Staszewski S, Lower J. Frequencies of GB virus C/hepatitis G virus genomes and of specific antibodies in German risk and non-risk populations. *J Med Virol* 1997;53:218-24.
  8. Feucht HH, Zöllner B, Polywka S, Knodler B, Schröter M, Nolte H, Laufs R. Prevalence of hepatitis G viremia among healthy subjects, individuals with liver disease, and persons at risk for parenteral transmission. *J Clin Microbiol* 1997;35:767-8.
  9. Loiseau P, Mariotti M, Corbi C, Ravera N, Girot R, Thauvin M, Portelette E, Mariette X, Roudot-Thoraval F, Benbunan M, Lefrère JJ. Prevalence of hepatitis G virus RNA in French blood donors and recipients. *Transfusion* 1997;37:645-50.
  10. Watanabe T, Ishiguro M, Kametani M, Sugai Y, Takakawa K, Akahane Y, Masuko K, Shimizu M, Kojima M, Fujita K, Tsuda F, Okamoto H. GB virus C and hepatitis C virus infections in hemodialysis patients in eight Japanese centers. *Nephron* 1997;76: 171-5.
  11. Cantaloube JF, Gallian P, Biagini P, Attoui H, Escher J, Zappitelli JP, Delord Y, de Micco P, de Lamballerie X. Prevalence of GB virus type C/hepatitis G virus RNA and anti-E2 among blood donors in Southeastern France. *Transfusion* 1999;39:95-102.
  12. Linnen J, Wages J Jr, Zhang-Keck ZY, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JW, Young L, Piatak M Jr, Hoover C, Fernandez J, Chen S, Zou JC, Morris T, Hyams KC, Ismay S, et al. Molecular cloning and disease association of hepatitis G virus: A transfusion-transmissible agent. *Science* 1996;271:505-8.
  13. Blair CS, Davidson F, Lycett C, McDonald DM, Haydon GH, Yap PL, Hayes PC, Simmonds P, Gillon S. Prevalence, incidence, and clinical characteristics of hepatitis G virus/GB virus C infection in Scottish blood donors. *J Infect Dis* 1998;178:1779-82.
  14. Bjorkman P, Widell A, Veress B, Verbaan H, Hoffmann G, Elmstahl S, Lindgren S. GB virus C/hepatitis G virus infection in patients investigated for chronic liver disease and in the general population in southern Sweden. *Scandinavian J Infect Dis* 2001;33:611-7.
  15. Masuko K, Mitsui T, Iwano K, Yamazaki C, Okuda K, Meguro T, Murayama N, Inoue T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M. Infection with hepatitis GB virus C in patients on maintenance hemodialysis. *N Engl J Med* 1996;334:1485-90.
  16. Bassit L, Kleter B, Ribeiro-dos-Santos G, Maertens G, Sabino E, Chamone D, Quint W, Saez-Alquezar A. Hepatitis G virus: Prevalence and sequence analysis in blood donor of Sao Paulo, Brazil. *Vox Sang* 1998;74:83-7.
  17. Tucker TJ, Louw SJ, Robson SC, Isaacs S, Kirsch RE. High prevalence of GBV-C hepatitis G virus infection in a rural South African population. *J Med Virol* 1997;53:225-8.
  18. Kato T, Mizokami M, Nakano T, Kondo Y, Ohba K, Orito E, Ueda R, Mukaide M, Gurtsevitch V, Syrtsev A, Ruzibakiev R, Abdurakhnamov M, Yamashita M, Hayami M. High prevalence of GB virus C/hepatitis G virus infection among the Jewish population in Uzbekistan. *Virus Research* 1997;48:81-7.
  19. Hayashi J, Furusyo N, Sawayama Y, Kishihara Y, Kawakami Y, Ariyama I, Etoh Y, Kashiwagi S. Hepatitis G virus in the general population and in patients on hemodialysis. *Dig Dis Sci* 1998;43:2143-8.
  20. Lamproye A, Delwaide J, Vaira D, Maggipinto G, Gérard C, Sondag-Thull D, Rentier B, Rorive G, Belaiche J. Prevalence of hepatitis G virus in a haemodialysis unit. *Acta Gastroenterologica Belgica* 1999;62:13-5.
  21. Kondili LA, Chionne P, Dettori S, Badolato MC, Grosso A, Vania A, Rapicetta M. GB virus/hepatitis G virus exposure in Italian pediatric and young adult thalassemic patients. *Infection* 2001;29:219-21.
  22. Wu RR, Mizokami M, Cao K, Nakano T, Ge XM, Wang SS, Orito E, Ohba K, Mukaide M, Hikiji K, Lau JY, Lino S. GB virus C/hepatitis G virus infection in Southern China. *J Infect Dis* 1997;175:168-71.
  23. Feucht HH, Schröter M, Zöllner B, Polywka S, Laufs R. Age-dependent acquisition of hepatitis G virus/GB virus C in a nonrisk population: Detection of the virus by antibodies. *J Clin Mic* 1999;37:1294-7.
  24. Rubio A, Rey C, Sánchez-Quijano A, Leal M, Pineda JA, Lissen E, del Rocio V, Spain S, Hess G. Is hepatitis G virus transmitted sexually? *JAMA* 1997; 277:532-3.
  25. Mass MR, Sağlamkaya U, Kubar A, Çolakoğlu M, Ateşkan Ü, Nalbant S, et al. Hepatitis G virus infection among patients undergoing hemodialysis. *Gut* 1997;41(Suppl 3):A130 (6<sup>th</sup> UEGW Poster Presentations).
  26. Kao J, Chen D. GB virus-C/hepatitis G virus infection in Taiwan: A virus that fails to cause a disease? *J Biomed Sci* 1999;6:220-5.
  27. Yoshida M, Okamoto H, Mishiro S. Detection of the GBV-C hepatitis virus genome in serum from patients with fulminant hepatitis of unknown etiology. *Lancet* 1995;346:1131-2.

28. Fiordalisi G, Zanella I, Mantero G, Bettinardi A, Stellini R, Paraninfo G, Cadeo G, Primi D. High prevalence of GB virus C infection in a group of Italian patients with hepatitis of unknown etiology. *J Infect Dis* 1996;174:181-3.

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