Hepatitis G Virus: Prevalence in Blood Donors in Armed Forces

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Abstract

Background: A new RNA virus designated hepatitis G virus (HGV) was recently identified. Because HGV has less than 25% sequence or amino acid homology with hepatitis C virus (HCV) and other established Flaviviridae, it is considered to be a new genus in this growing family of hepatotropic viruses. Hepatitis G virus has been associated with hepatitis and is transmitted through parenteral and sexual route.

Material and methods: A study comprising 500 healthy voluntary blood donors (service personnel) was under taken to find out prevalence of HGV. HGV RNA was detected by reverse transcriptase – polymerase chain reaction (RT-PCR). Hepatitis B surface antigen (HbsAg) and antibody to HCV were detected by Enzyme Linked Immunosorbent Assay (ELISA).

Results: Thirteen donors (2.6%) were positive for HGV RNA. 17 donors (3.4%) were positive for antibody to hepatitis C virus (HCV) by ELISA. Co-infection of HGV with hepatitis B virus (HBV) was seen in 5 donors and with HCV infection in 2 donors. Co-infection of HGV, HBV and HCV was not seen in any donor.

Conclusion: So far there is no conclusive evidence that HGV produces hepatitis. But presence of HGV in hepatitis cases casts a doubt on this finding. Prevalence rate in blood donors may be helpful in future studies when the exact role of HGV is known.

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Key Words: Hepatitis G Virus; Blood donors; Armed Forces

Introduction

Hepatitis G virus (HGV) is a recently discovered virus transmitted by blood and blood products and by sexual route. Hepatitis A, B, C, D and E are well characterised with an unequivocal association with liver disease in human beings. The role of HGV in hepatitis is controversial [1]. HGV has a positive sense RNA genome, approximately 9.4 kb in length that contains a 458-nucleotide long 5' non-coding region (NCR), a single open reading frame (ORF) encoding a polyprotein of about 2900 aminoacids, and a 3' NCR of 315 nucleotides [2]. The detection of viral genome is carried out by RT-PCR for diagnosis of infection. We have attempted to determine the prevalence of HGV among healthy voluntary blood donors in Armed Forces of India.

Material and Methods

Five hundred voluntary blood donors were included in the study over a period of 2 years. 5 ml of blood sample was collected from each donor in a sterile container and allowed to clot at 4°C to prevent loss of RNA. Serum was separated and stored at -80°C till further tests.

Viral RNA was extracted by using Quiagen mini RNA columns (Germany) and stored at -80°C for further tests.

Manufacturer's instruction was followed for extraction of RNA.

RT-PCR for HGV was carried out as per methods described by Arankalle et al (3). Briefly, all serum samples were tested for the presence of HGV RNA in duplicate by nested RT-PCR. cDNA synthesis was carried out at 42°C for one hour using external antisense primers and reverse transcriptase enzyme (Bangalore Genei) using a thermal cycler (Lab System, Finland). This was followed by 35 cycles at 94°C for one minute, 55°C for two minutes and 72°C for three minutes for first round PCR and 30 cycles for the second round PCR using internal sense primers and Taq polymerase enzyme (Bangalore Genei). The PCR products were analyzed by 2% agarose gel electrophoresis and visualized using ethidium bromide. Presence of 343 base pair was considered positive for HGV RNA.

The primers used were - External sense primer (89-106) 5' AGG TGG TGG ATG GGT GAT 3', External anti-sense primer (513-492) 5' TGG CAC CCG CCCTCA CCC GAA 3', Internal sense primer (114-135) 5' TGGTAG GTC GTAAAT CCC GGT 3' and Internal anti-sense primer (457-436) 5' GGA GGT GGG TGG CT CAT GCTT 3'.

ELISA for HbsAg was carried out by using commercial ELISA kit (Abbot, U.S.A). Third generation ELISA kits (United Biotech, U.S.A) were used for detection of antibody to HCV.

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Table 1**Age and sex wise distribution of donors positive for various viral markers**

Viral markers	18-25 yrs		26-35 yrs		Age group 36-45 yrs		> 45 yrs		Total	
	M	F	M	F	M	F	M	F	M	F
	n=96	n=22	n=210	n=37	n=103	n=18	n=14	Nil	n=423	n=77
Positive for HGV RNA	2	Nil	5	2	3	Nil	1	Nil	11	2
Positive for HBsAg	3	1	8	1	2	1	1	Nil	14	3
Positive for antibody to HCV	2	Nil	2	Nil	1	1	Nil	Nil	5	1

Fig. 1 : Reverse transcriptase polymerase chain reaction confirming Hepatitis G virus

Results

Out of 500 samples, 13 (2.6%) were positive for HGV RNA as detected by presence of 343 base pair by RT PCR (Fig 1), 17 were positive for HbsAg and 6 were positive for HCV antibody (Table 1). Out of 17 samples positive for HbsAg, 5 were also positive for HGV RNA and out of 6 samples positive for HCV antibody, 2 were also positive for HGV RNA. None of the samples were positive for all three viral markers.

Age and sex wise distribution of HGV RNA, HbsAg and anti HCV antibody positivity are shown in Table 1. There was male predominance and majority of cases belonged to age group of 18-35 years.

Discussion

500 voluntary blood donors were tested for HGV RNA by RT-PCR. All the samples were also tested for HbsAg and antibody to HCV. 13 out of 500 (2.6%) were positive for HGV RNA. The result was confirmed twice by PCR. The prevalence of HGV observed was 2.6% in our study. In other countries prevalence ranges from 1-14% [4-7]. In a group of about 300 West Africans, 14% prevalence of HGV RNA was noted [7]. Among individuals with parenteral risk factors, the prevalence is much higher reaching 18% in haemophiliacs [4] and 16-33% in IV drug users [5,7,8]. The prevalence reported in other countries are South Africa 11% [9], Vietnam 7.4%(10) and Brazil 9%[11]. Adhani et al, Alter

et al and Smith JF had found 1-2% positivity in blood donors in U.S.A [12-14]. In a Korean study, HGV was detected in 1-2.5% of blood donors [15]. In Western India Arankalle et al reported no positivity of HGV RNA in 51 voluntary blood donors [3] whereas Jain et al in a study in Northern India found 24% blood donors positive for HGV RNA [16]. 2.6% positivity in our study is much less to that of Jain et al and higher than Arankalle et al. The above two Indian studies have shown a wide variation in two different parts of India. Armed Forces personnel being a mixed population drawn from all parts of the country, the prevalence of 2.6% probably represents true prevalence in the country.

Though more number of cases were seen in males and in age group of 18-35 years, the finding was not statistically significant. Co-infection of HGV with HBV and HCV suggests similar modes of transmission. HGV has been isolated in semen and spouses of infected persons have been found to be positive[17].

Out of 17 donors positive for HBsAg, 5 were also positive for HGV RNA showing a co-infection of 29.4%. Similarly 2 out of 6 donors positive for antibody to HCV were also positive for HGV RNA showing a co-infection of 33.3%. As both HBV and HCV are transmitted parenterally and by sexual route, it suggests that HGV also follows a similar transmission route. The co-infection rate of HGV with HCV is 3.4-24.4% depending on risk factors[17] and with HBV is 32%[18]. In our study the prevalence of HGV infection did not show any significant difference among blood donors positive for HBsAg and with antibody to HCV. The co-infection rate is comparable to other studies mentioned. No donor was positive for markers of all the three viruses i.e. HGV, HBVa and HCV though triple infection is reported[14].

So far there is no conclusive evidence that HGV produces hepatitis. But presence of HGV in hepatitis cases casts a doubt on this finding [14]. Prevalence rate in blood donors may be helpful in future studies when the exact role of HGV is known.

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