Hepatitis G Virus Infection in Healthy Individuals, Acute Viral Hepatitis and Persons at Risk for Parenteral Transmission

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Abstract

Background: Hepatitis G virus (HGV), transmitted mostly by parenteral route, has been under investigation for its role as an agent for viral hepatitis. This study was carried out to find out the prevalence of HGV in healthy individuals, multi-transfused patients with acute viral hepatitis and those under going dialysis.

Method: The study included 200 healthy individuals and 180 patients, comprising acute viral hepatitis (100 cases), multi-transfused patients (50 cases) and patients undergoing dialysis (30 cases). HGV RNA and Hepatitis C virus (HCV) RNA was detected by reverse transcription and polymerase chain reaction (RT-PCR) in all. Viral marker studies for hepatitis A, B and E were carried out by ELISA in acute viral hepatitis cases. In healthy individuals, in patients with multiple transfusions or those undergoing dialysis, marker studies for HBV and HCV were carried out.

Result: The prevalence of HGV in healthy individuals was 2.5% (5/200), in non A-E hepatitis 3% (3/100), in multi-transfused patients 4% (2/50) and in patients undergoing dialysis 6.67% (2/30). There was no significant difference in the prevalence rate of HGV infection in healthy individuals and in patients with non A-E hepatitis.

Conclusion: Depending on prevalence rate, HGV could not be implicated as cause of acute viral hepatitis. Persons with parenteral risk factor (multiple blood transfusions and those undergoing dialysis) had higher prevalence rate as compared to healthy individuals.

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Key Words: Hepatitis G virus; Polymerase chain reaction; Viral hepatitis; Parenteral transmission.

Introduction

Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Hepatitis E virus (HEV) are the commonest causative agents of acute viral hepatitis throughout the world. HAV is responsible for 30-50%, HBV for 40-50% and HCV for 5-20% of this infection [1]. The causative agent is unknown in approximately 4-10% cases of viral hepatitis [2]. A new virus named Hepatitis G virus (HGV), mainly transmitted through parenteral route, has been detected recently by molecular biology method [3]. It belongs to the Flaviridae family and is closely related to HCV [4]. The role of HGV as a causative agent of viral hepatitis is controversial. Though the virus is present in non A-E hepatitis, it is unlikely to cause hepatitis [5]. This study was carried out to determine the prevalence and distribution of HGV in healthy individuals, patients with acute viral hepatitis and in persons at risk for viral transmission through parenteral routes.

Materials and Method

The study population comprised 200 healthy individuals, 100 acute viral hepatitis (diagnosed clinically and biochemically), 50 multi-transfused individuals (> 5 units of blood) and 30 patients undergoing dialysis.

10 ml of blood was collected, serum separated at 4°C, made into aliquots and stored at -80°C. Viral markers, IgM Anti HAV for hepatitis A virus, HBsAg and IgM Anti HBc for hepatitis B virus, IgM Anti HEV for hepatitis E virus, HCV RNA detection by reverse transcription and polymerase chain reaction (RT-PCR) for hepatitis C virus and HGV RNA detection (RT-PCR) for hepatitis G virus were tested for cases of acute viral hepatitis. In other subjects HBsAg and RT-PCR for HCV and HGV was carried out.

Serological markers (HBsAg, IgM Anti HAV, IgM Anti HBc, IgM Anti HEV) was carried out by ELISA using commercial kits (United Biotech, USA).

RT-PCR for 5' non-coding region (5' NCR) which is highly conserved region of HCV, was carried out as mentioned in the Manual of 3rd National Molecular Biology workshop, [6] with minor modifications using extraction of RNA by columns. Briefly RNA was extracted from the serum sample by using

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RNA mini-column for virus (Qiagen, Germany). Extracted RNA was stored at -80°C till further use. Complementary DNA (cDNA) was synthesized using the anti-sense primer and reverse transcriptase enzyme in the thermocycler (Lab system, Finland). Cycles were carried out at $43^{\circ}\text{C} \times 59$ minutes, $99^{\circ}\text{C} \times 5$ minutes and hold at 4°C .

cDNA synthesis was followed by PCR using cDNA as template with sense primer and Taq polymerase enzyme in the thermocycler. Cycles were carried out at $94^{\circ}\text{C} \times 1$ minute followed by 40 cycles of $94^{\circ}\text{C} \times 30$ sec, $59^{\circ}\text{C} \times 30$ sec, $72^{\circ}\text{C} \times 1$ minute and $72^{\circ}\text{C} \times 7$ minutes and hold at 4°C .

Primer sequence

Sense primer - $5' \text{GCC GAC ACT CCA CCA TAG TAG A} -3'$

Anti-sense primer - $5' \text{GGT GCA CGG TCT ACG AGA CCT} -3'$

PCR product was visualised by 2% agarose gel electrophoresis with ethidium bromide under UV light. Appearance of 323 base pair DNA band coinciding with positive control was considered positive test (Fig 1). Known positive control and distilled water (in place of serum) was used as negative control.

RT-PCR for HGV was carried out using the protocol as mentioned by Arankale et al [7]. Briefly, RNA was extracted by using Qiagen columns as mentioned above for HCV RT-PCR. Nested RT-PCR was carried out for $5'$ NCR by using following primers:

External sense primer (89-106) - $5' \text{AGG TGG TGG ATG GGT GAT} -3'$

External anti sense primer (512-492) - $5' \text{TGG CAC CCG CCC TCA CCC GAA} -3'$

Internal sense primer (114-135) - $5' \text{TGG TAG GTC GTAAAT CCCGGT} -3'$

Internal anti sense primer (457-436) - $5' \text{GGA GCT GGG TGG CCT CAT GCTT} -3'$

cDNA synthesis was carried out at 42°C for one hour using the external anti sense primer. This was followed by 35 cycles at 94°C for 1 minute, 55°C for 2 minutes and 72°C for 3 minutes for first round PCR and 30 cycles for the second round PCR. The PCR products were analysed by 2% agarose gel electrophoresis and visualised by using ethidium bromide incorporated into the gel. Positive control (obtained from National Institute of Virology, Pune) and distilled water in place of serum as negative control was used every time. Presence of 343 base pair in PCR product was considered positive (Fig 2). All positive samples were also subjected to repeat RT-PCR for reproducibility and confirmation. PCR reagents were supplied by Bangalore Genei, Bangalore.

Results

Of the 200 healthy individuals screened, seven cases were positive for HBV, two for HCV RNA and five for HGV RNA.

Of the 100 acute viral hepatitis cases, 34 were due to HAV, 38 due to HBV, five due to HCV and six due to HEV (Table 1).

Of the 50 patients with multiple transfusions, none was positive for HBsAg or HCV RNA while two were positive for HGV RNA. In patients undergoing dialysis, none was positive

Fig. 1

Fig. 2

for HBsAg, one was positive for HCV RNA and two were positive for HGV RNA. Overall HGV RNA was positive in four cases (Table 2).

In healthy individuals, out of seven positive for HBsAg, three (42.86%) were also positive for HGV RNA. In the same group, out of two positive for HCV RNA, one (50%) was also positive for HGV RNA. Only one individual was positive for HGV RNA alone.

Among viral hepatitis, HGV RNA was positive in seven out of 38 (18.42%) with HBV infection and two out of five (40%) with HCV infection. Three were positive only for HGV RNA. No co-infection of HGV with HAV/HEV was seen. Co-

Table 1

Positivity of viral markers for various hepatitis viruses in acute viral hepatitis cases and in healthy individuals

Category	Total	HAV	HBV	HCV	HEV	HGV
Acute viral hepatitis	100	34	38	5	6	12
Healthy individuals	200	-	7	2	-	5

Table 2

Positivity of HBV, HCV and HGV markers in multi transfused individuals and patients undergoing dialysis

Category of patients	Total number	HBV	HCV	HGV
Multiple transfusion	50	Nil	Nil	2
Dialysis	30	Nil	1	2
Total	80	Nil	1	4

infection of HGV and HCV was seen in one patient undergoing dialysis (Table 3). Triple infection (HBV, HCV, HGV) was not seen in any subject.

Discussion

The newly isolated hepatitis G virus (HGV) is transmitted mainly through parenteral route, though vertical and sexual transmission occur to a lesser degree [3,5,8]. As the mode of transmission of HGV is similar to that of HBV and HCV, persons at risk of acquiring hepatitis B/C infection are also at the risk of acquiring HGV infection. Various studies have shown that, though HGV is present in hepatitis cases it is not implicated as an aetiological agent of non A-E hepatitis, and persistent infection with HGV though common, does not lead to chronic hepatitis [3,5,9,10].

In 2.5% (5/200) healthy individuals HGV RNA could be detected by RT-PCR. The prevalence in healthy individuals and in healthy blood donors ranges from 0-24% [5,7,11-16]. Arankale et al [7] in their study did not find a single healthy voluntary blood donor positive for HGV RNA in western India, whereas in Northern India, the prevalence is reported to be 24% [11]. Prevalence rate of 2.5% in our study of armed forces personnel may be due to the mixed population of troops and their families.

In acute viral hepatitis cases, 12% (12/100) were found to have HGV RNA. Of these, three (3%) cases

were positive for HGV RNA only (in non A-E hepatitis cases) while nine had co-infection with HBV/HCV. Prevalence of HGV infection in acute viral hepatitis ranges from 3.4% in USA to 39% in Italy [5,13]. Our study shows no significant difference of HGV RNA positivity in healthy individuals (2.5%) as compared to 3% in non A-E hepatitis cases ($P > 0.05$). This suggests that probably HGV is not a cause of hepatitis in these groups.

A higher positivity rate (4%) was seen in individuals with multiple transfusions and in persons undergoing dialysis (6.67%). As the pilot samples of the blood units were not tested for the presence of HGV RNA, it can not be conclusively proved that these individuals acquired infection through blood transfusion. But epidemiologically a higher rate of positivity as compared to healthy individuals suggests that persons with multiple transfusion and dialysis are at risk for parenteral transmission of HGV. Positivity of HGV RNA has been reported to be 21.1% in blood transfusion recipients and 6.8% to 21.2% in patients undergoing dialysis. In haemophilia patients it is as high as 35.2% [5,8]. Significantly higher HGV RNA positivity among haemophiliacs and dialysis patients confirms the importance of transmission of HGV through parenteral route.

Co-infection of HGV with HBV has been reported to be 32% in USA [5] and more than 50% in Africa [14]. Of the total 45 samples positive for presence of HBV (healthy individuals and viral hepatitis cases), 10 (22.2%) had detectable HGV RNA. Co-infection with HBV was seen in three out of seven (42.86%) healthy individuals and seven out of 38 (18.4%) cases with acute viral hepatitis B. Higher rate of co-infection as compared to acute viral hepatitis cases may be due to persistent viraemia in asymptomatic individuals with hepatitis B infection, or may be due to different mode of transmission in these individuals. Co-infection with HCV ranged from 3.4%-24.4% [5,9]. A co-infection rate of 57.1% in our study may be due to small number of HCV positive (seven) cases. As hepatitis C is mostly transmitted through parenteral route similar to that of HGV, a high co-infection rate is not unusual. Though co-infection rate of HGV with HAV has been reported

Table 3

Co-infection of HGV with other Hepatitis viruses.

Category	Co-infection of HBV and HGV	Co-infection of HCV and HGV	Co-infection of HAV and HGV	Co-infection of HEV and HGV	Only HGV infection
Healthy individuals	3	1	-	-	1
Acute Viral hepatitis	7	2	Nil	Nil	3
Multiple transfusion	Nil	Nil	-	-	2
Dialysis	Nil	1	-	-	1
Total	10	4	-	-	7

to be 25% by Alter et al [9], we did not find a single case co-infected with HAV/HEV. HAV and HEV are exclusively transmitted through faeco-oral route, where as HGV mainly follows parenteral route of transmission. In the study by Alter et al, cases of hepatitis with intra venous drug use were also included and that may be the reason for higher positivity rate.

We have shown that known risk factors for HCV and HBV infection are linked to high prevalence of HGV infection. Horizontal transmission via blood is an important route to acquire HGV infection besides vertical and sexual transmission.

Conflicts of Interest

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