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Occurrence & nucleotide sequence analysis of hepatitis G virus in patients with acute viral hepatitis & fulminant hepatitis

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***Background & objectives*: Association of hepatitis G virus (HGV) with acute viral hepatitis (AVH) and fulminant hepatitis (FH) is not clearly understood.This study was designed to asses the occurrence of HGV infection and its relationship with other hepatotropic viruses in patients with FH and AVH and also to determine the nucleotide sequence of HGV isolates.**

***Methods*: The study included 100 patients of FH and 125 of AVH on the basis of clinical examination, liver function test and serology for hepatitis A, B, C and E virus. HGV RNA was detected by reverse transcriptase-polymerase chain reaction (RT-PCR) and direct sequencing for 4 randomly selected samples followed by phylogenetic analysis.**

***Results*: Of the 100 patients with FH, 30 were negative for hepatitis viruses A, B, C and E by serology (non A - non E) while 60 were negative in the AVH group. In the non A- non -E hepatitis group, HGV was positive in 16.66 per cent (5/30) cases of FH, 10 per cent (6/60) cases of AVH and 6 per cent (6/100) of healthy controls. The difference in HGV seropositivity between FH and AVH patients was statistically not significant compared to healthy controls, while HBV and HCV infections were significant. The four isolates sequenced seemed to be of same type and close to Chinese strain of HGV (Y13755.1 Y13756.1 Y15407, and U67782) on phylogeny.**

***Interpretation & conclusion*: In HGV infection was not found to be clinically significant as well as non-pathogenic in the patients of FH and AVH and appeared to be an innocent bystander in the course of the disease. The four sequenced HGV isolates showed close pairing with Chinese strains.**

Key words Hepatitis G virus - phylogenetic analysis - sequencing - viral hepatitis

Hepatitis G virus (HGV) infection is thought to be a blood borne virus. However, little is known about the epidemiology, transmission, replication site, and disease inducing capacity of this virus. Association of HGV with fulminant hepatitis (FH) and acute viral hepatitis (AVH) is still controversial and its clinical significance is to be clearly understood. HGV infection has been reported in the patients with AVH, chronic hepatitis, fulminant hepatitis, haemodialysis, intravenous drug abuser and blood donors around the world[1].

HGV is a positive sense single stranded RNA genome, approximately 9.4 Kilo base and belongs to *Flaviviridae* family. Though HGV genome is related to

hepatitis C virus (HCV), it is too divergent to be classified as the genotype of HCV[2]. It is well established that HCV exhibits significant genetic heterogeneity not only between different patients but also in the same individual and the same is expected in HGV[3]. Sequence variation in HGV ranges from 0.5 to 20.7 per cent at the nucleotide level, and from 0 to 16.5 per cent at the amino acid level[4]. HGV has considerable degree of genetic heterogeneity, the NS3 region of HGV has sequence divergence of 10-20 per cent.

Reports from Argentina and Italy showed 30.6 and 39 per cent seroprevalence of HGV respectively in fulminant hepatitis patients[5,6]. A study from Japan reported HGV in 50 per cent of non A non E hepatitis patients with FH; the higher prevalence may be because of small sample[7].

In view of paucity of information about HGV infection in the patients with FH and AVH from northern India, this study was designed to assess the occurrence and clinical relevance of hepatitis G virus isolates in patients with acute viral hepatitis and fulminant hepatitis and to compare their nucleotide sequences with other HGV isolates of gene bank.

Material & Methods

A total of 100 consecutive patients (mean age: 29.57 ±15.33 yr) of FH and 125 patients (mean age: 27.86±9.73 yr) of AVH attending the Medical OPD and wards of Lok Nayak Hospital, New Delhi, India during the period October 2002 to July 2004 were included in the study. The control group included 100 apparently healthy individuals (male:female 78:22; mean age 31.24±7.41 yr) from the blood bank. 5 ml of blood was collected and separated serum samples were stored at -20°C deep freezer till analysis. The patients were included on the basis of predesigned proforma with relevant information with respect to case history, risk factors, clinical examination, liver function test and serological tests for HBsAg, IgM HBc, HBeAg, IgM HAV, IgM HEV and anti HCV. The written informed consent was obtained from all the patients and the study was approved by the ethical committee of the institution. Serum samples were screened for HBsAg using 3rd generation ELISA kit (Biokit; S.A., Spain), IgM HBc and HBeAg using ELISA kit of RADIM SpA Italy, IgM HAV and IgM HEV using ELISA kit of Medical Biological Services S.R.L. Italy, and anti HCV antibody was tested by 3rd generation ELISA kit (General Biological Corporation, Taiwan). The serum samples of healthy controls were only screened for HBsAg and anti HCV. The HGV assay was done by reverse transcriptase polymerase chain reaction (RT-PCR). Total RNA was extracted from 100 µl serum by acid-guanidinium-phenol-chloroform as descried earlier[8-10]. The HBV DNA, HCV RNA and HGV RNA were amplified using PCR and RT-PCR respectively as described elsewhere[8,10,11]. The sequencing of the positive HGV PCR product (cDNA) was done from the Macrogen Inc, Korea on commercial basis. The sequences were aligned using Neighbour joining method of Clustal W software (1.82) multiple alignments of the European Bioinformatics Institute, UK for phylogeny[12].

Results & Discussion

HGV infection was seen in 16 per cent (16/100) cases of FH, 10.4 per cent (13/125) of AVH and 6 per cent (6/100) of controls. The difference in the occurrence of HGV was not statistically significant is the patients of AVH and FH compared to controls. However, the seroprevalence of HBV and HCV was found to be significantly higher ($P<0.05$) in patients as compared to healthy controls (Data not shown). In the non A-non E cases of FH and AVH, the HGV positivity was seen in 16.66 (5/30) and 10 per cent (6/60) of the cases, respectively.

Clinical significance of hepatitis G virus in the patients of AVH and FH studied in different countries showed conflicting reports. In our earlier studies, we have reported the HGV infection in 14.3 per cent cases of AVH, 11.4 per cent cases of FH, 4 per cent in general population, 46.6 per cent in commercial blood donors and 6 per cent cases of chronic renal failure on haemodialysis[8-11,13]. Varying HGV seropositivity has been shown in different studies from India. Panda *et al*[14] reported 8.3 per cent HGV infection while Arankelle *et al*[15] showed 3.58 per cent patients with FH positive for HGV infection. Dawson *et al*[16] reported 42.7 per cent seroprevalence of HGV in non A non E hepatitis patients with FH which was comparable to a study from Japan[16]. Heringlake *et al*[17] *et al* from Germany reported 50 per cent of FH patients positive for HGV. HBV DNA was not detected in any of the serologically negative cases. However, HCV, RNA was detected in one serologically negative case. Hepatitis E virus infection was observed in 49 per cent (49/100) cases of FH and 35.2 per cent (44/125) cases of AVH. Hepatitis A virus infection was seen in 9 per cent (9/100) cases of FH and 10.4 per cent (13/125) cases of and AVH. This shows the predominant HEV virus infection in the cases of AVH and FH. The co-infections of HGV observed with hepatitis A, B and E

in the patients of AVH and FH may be because of common mode of transmission.

Liver function profile of HGV positive and negative cases was comparable. The aminotransferase level that believed to be correlated with the disease activity, was found to be comparable in HGV positive and negative cases of AVH and FH (data not shown). The liver function profile of patients with only HGV and those co-infected with HEV was also comparable. Studies from US, Japan, Brazil, and China have not indicated any clinical significance of HGV infection in the patients of fulminant hepatitis[6,18,19]. Our results also supported the same. Patients with only HGV infection had normal liver function profile and also clinically asymptomatic on six month follow up. No mortality was observed in the patients of AVH including HGV positive cases. However, of the 16 HGV positive patients of fulminant hepatitis, 8 died. Of the 5 HGV infected non A non E cases of FH, 2 expired. These results suggest that HGV may not have any pathogenic role in the course of acute viral hepatitis and fulminant hepatitis.

The four selected HGV isolates, two each from FH and AVH were sequenced. On analysis of 101-nucleotide sequence of NS3/ helicase region, all the four HGV isolates nucleotide sequence showed pairing to each other when subjected to clustal W (1.82) multiple alignments with other sequences of NS3/helicase region available in the genbank (NCBI). Inference could not be drawn regarding genotype because the very small fragment (~100 bp) was sequenced. The NS3/helicase region is not considered to be ideal for genotyping of the hepatitis G virus[19,20]. Studies on NS3 region of HGV have been reported from the Spain, Germany, China, USA and UK[21-24]. The variation amongst HGV isolates across several continents was between 10-30 per cent while the variation of HCV isolates between geographic regions is greater than 30 per cent across the whole genome[21]. This may be due to weak selection pressure of the HGV Indian isolates Ind 1 HGV-DQ076328, Ind 2 HGV- DQ076329 of FH and Ind 3HGV-DQ076330, Ind 3 HGV-DQ076331 of AVH patients. All four Indian isolates showed close pairing to each other on phylogeny suggestive of the same type (Fig.). These HGV Indian isolates showed pairing with the Chinese (U67782, Y15407, Y13755.1, Y13756.1) isolates in phylogeny compared with the sequences of Spain, Germany, USA and UK (NCBI Server). The isolates of China U67782 and Y15407 are of prototype showing pairing with the Indian isolates.

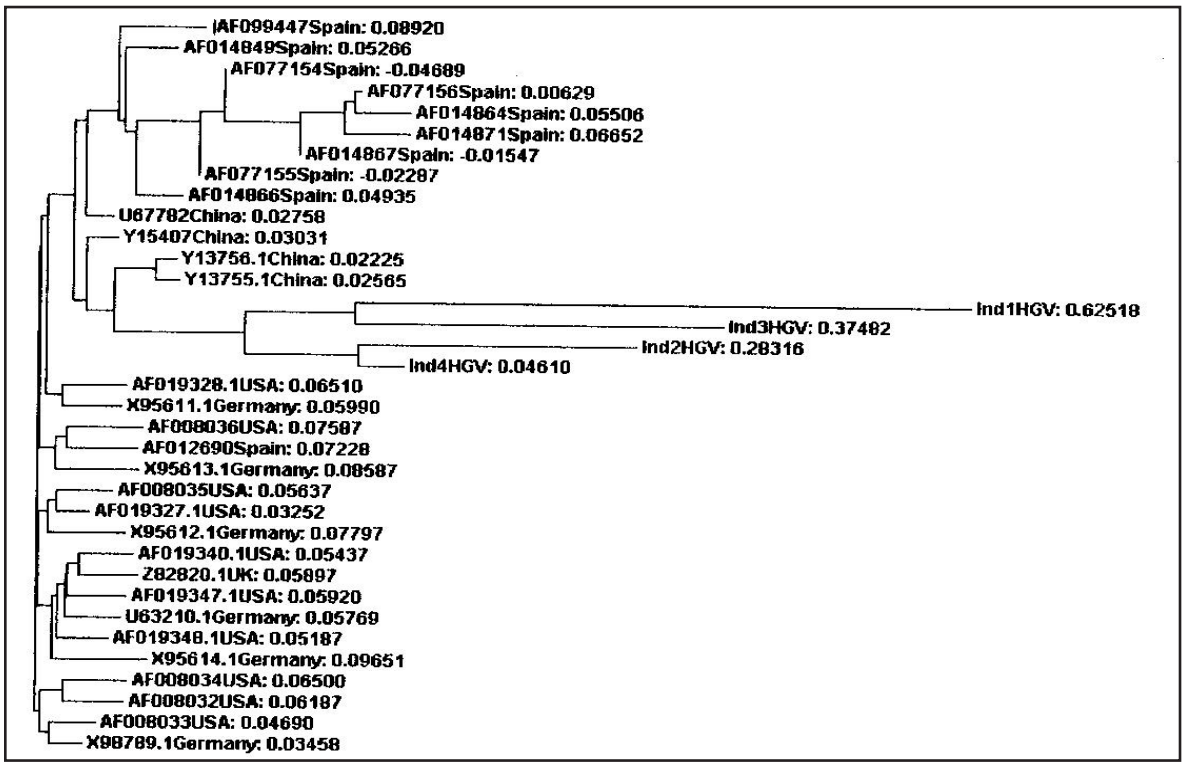

Fig. Phylogenetic tree of four Indian HGV sequences with the other sequences of China, Spain, Germany, USA, and UK (prepared by the Clustal multiple alignment software of European Bioinformatics Institute, UK.

In conclusion, the HGV and its co-infection with other hepatotrophic viruses do not seem to have a pathogenic role, and the clinicians need not to look for the presence of HGV infection in non A-E cases of FH and AVH. The HGV infection appears to be clinically silent and innocent bystander in the course of FH and AVH. HEV continues to be major viral infection in these patients. The geographical basis of phylogeny predicts that the Indian isolates may be of prototype and close to Chinese strain.

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