

INVITED REVIEW GB VIRUS C/HEPATITIS G VIRUS AND OTHER PUTATIVE HEPATITIS NON A - E VIRUSES

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SUMMARY

The identification of the major agents causing human hepatitis (Hepatitis A, B, C, D and E Viruses) was achieved during the last 30 years. These viruses are responsible for the vast majority of human viral hepatitis cases, but there are still some cases epidemiologically related to infectious agents without any evidence of infection with known virus, designated as hepatitis non A - E. Those cases are considered to be associated with at least three different viruses: 1 - Hepatitis B Virus mutants expressing its surface antigen (HBsAg) with altered epitopes or in low quantities; 2 - Another virus probably associated with enteral transmitted non A-E hepatitis, called Hepatitis F Virus. Still more studies are necessary to better characterize this agent; 3 - Hepatitis G Virus or GB virus C, recently identified throughout the world (including Brazil) as a *Flavivirus* responsible for about 10% of parenteral transmitted hepatitis non A-E. Probably still other unknown viruses are responsible for human hepatitis cases without evidence of infection by any of these viruses, that could be called as non A-G hepatitis.

KEYWORDS: hepatitis G; hepatitis F; GBV-C; HGV; HFV; non A-E hepatitis; HBV mutants.

Three different phases can be distinguished in the history of the discovery of viruses causing human hepatitis. During the first period, comprising the late 60's and early 70's, hepatitis A and B viruses were identified^{7,22} by immunological methods associated to primate transmission experiments. The second period started when it was established that there were hepatitis cases of probable viral etiology without evidences of infection by any known virus^{3,23,28,33}. These cases were included in a clinical entity, denominated hepatitis non-A, non-B.

Several groups from different countries started to research the viruses causing hepatitis non-A, non-B cases. In 1989, using molecular biological techniques, Hepatitis C Virus (HCV), the major etiological agent of parenteral transmitted hepatitis non-A, non-B cases was

identified^{13,37}. In 1990, Hepatitis E Virus was identified as the major etiological agent of enteral transmitted hepatitis non-A, non-B cases⁶⁴.

According to some authors, in up to 20% cases of parenteral transmitted non-A, non-B hepatitis, no previous known virus could be detected. These cases were denominated hepatitis non-A, non-B, non-C, non-D, non-E (hepatitis non A - E)⁴.

Indeed, the existence of more than one virus involved in hepatitis non-A, non-B cases have been suggested by previous experiments with chimpanzees^{8,30,79} and by the observation that some patients developed more than one episode of hepatitis non-A, non-B^{28,33,48,49,52,73}. Results from chimpanzee transmission ex-

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periments suggested that at least two different viruses with different susceptibilities to ether / chloroform were involved in hepatitis non-A, non-B⁸.

The third period on the research of hepatitis non A-E viruses started inside this picture and three different research lines have been developed, that will be described below.

HEPATITIS NON A - E CAUSED BY HEPATITIS B VIRUS VARIANTS

Some authors have previously suggested that some hepatitis non-A, non-B cases could be caused by HBV variants^{29,67}. Recently, two different mutants have been characterized in association of these cases.

Hepatitis B Virus harboring mutations in the major HBsAg epitope *a* have been detected in newborns from HBsAg seropositive mothers submitted to perinatal prophylaxy^{55,81} and in liver transplant recipients treated with anti-HBs monoclonal antibodies⁴⁵. These mutant viruses evade the neutralizing antibodies elicited by vaccination. Furthermore, these mutants are hardly detected by commercial available ELISA kits using specific monoclonal antibodies driven only to the epitope *a*^{10,11} and have been associated with post- transfusional hepatitis^{12,63} and fulminant hepatitis^{11,65,77}. ELISA tests using monoclonal antibodies driven to other HBsAg epitopes^{10,11} and the development of vaccines containing other HBV antigens will allow the detection and prevention of infection by these mutants.

HBV mutants with deletions in the X region of the viral genome have also been associated with HBsAg seronegative hepatitis cases. The expression of HBsAg is highly reduced in these mutants, but the virus retains its infectivity. These viruses are hardly detected by serological assays and even by PCR, if primers directed against the X region were used for amplification. These mutants can be detected by PCR using primers driven to other genomic regions and were found in thalassemic polytransfused children²⁴ and in dialysed patients^{25,75}. Some authors have designated these cases as "Hepatitis F"⁷⁵, but the classification of this virus as another HBV mutant seems more appropriate.

ENTERAL TRANSMITTED HEPATITIS NON A - E - HEPATITIS F VIRUS(?)

A novel agent denominated Hepatitis F virus associated with enteral transmitted hepatitis non A-E was reported in 1994, associated with 60% of sporadic non-A, non-B cases in India¹⁷. This agent was a DNA virus with 27-34 nm particles, 20 kbp double strand genome. Although feces from human patients containing this vi-

rus were capable of inducing hepatitis in rhesus monkeys, further studies are still necessary to characterize this agent and to correlate it with human hepatitis. Indeed, epidemiological studies suggest that another agent is also associated with enteral transmitted human hepatitis⁶.

PARENTERAL TRANSMITTED HEPATITIS NON A - E - GB VIRUS C OR HEPATITIS G VIRUS

The search for viruses causing parenteral transmitted hepatitis non A-E returns as a major research field after the description of Hepatitis C Virus. In 1991, the analysis of 10 cases with severe non A-E hepatitis suggested that a paramyxovirus could be the causative agent of this picture, as syncytial giant-cell hepatitis diagnosis was established histopathologically and evidences for paramyxoviral infection were found by electronic microscopy and serology⁵⁹. Although this picture was tentatively called as Hepatitis G¹⁹, no more reports about this discovery were found. Furthermore, a fine molecular characterization of this agent was never described.

The detailed characterization of a new agent involved in parenteral transmitted hepatitis non-A, non-B designated as hepatitis G virus or GB virus C was recently reported by two different groups. The history of its discovery, the major features and the prevalence of this virus among different populations throughout the world will be discussed below.

HISTORY

This agent was initially detected in 1967 by studies on the transmission of human viral hepatitis to white lipped, hairy faced tamarins (*Saguinus fuscicollis* and *S. nigricollis*) or crested, barefaced, cotton top tamarins (*S. (Oedipus) oedipomidus*)¹⁴. Serial transmission of hepatitis with 2 among 5 different inocula was achieved intravenously. One of the inocula employed was the serum of a 34 years-old surgeon, initials G.B., collected in the third day of jaundice. Hepatitis was also induced in marmosets using intramuscular¹⁵ and oral inoculation of this serum⁴⁴.

Further experiments, using ultrafiltration and cross challenging, demonstrated that this serum contained a virus distinct from Hepatitis A Virus, that was called agent GB^{15,32}. Cross challenge studies suggested that the GB agent was also distinct from the major virus causing non-A, non-B hepatitis in humans (that was lately identified as Hepatitis C Virus)^{35,73}. The human non-A, non-B serum used as inoculum in one of these studies^{35,36} was latter the source for the identification of hepatitis G virus, as will be described.

On the other hand, some authors have described the presence of marmoset hepatitis viruses and the development of hepatitis in cotton-topped tamarins marmosets (*S. (Oedipus) oedipomidus*) even in the absence of any inoculation^{56,57}. However, subsequent studies demonstrated that hepatitis in marmosets appeared only after inoculation of plasma from cases with acute hepatitis and did not showed activation of any non human latent virus^{15,31,44}. These latter results were more recently confirmed using colony born tamarins (*Saguinus labiatus*); hepatitis was observed only after inoculation of plasma or liver tissue from previously inoculated animals and no case of spontaneous hepatitis was reported among the tamarins³⁵.

The confusing data on the human or marmoset origin of the GB agent have let aside the research on this subject for many years. In 1976, after the definition of hepatitis non-A, non-B as a clinical entity, two different viral particles have been found by electronic microscopy in samples infected by the agent GB. Parvovirus-like particles with 20-22nm in diameter were visualized in infectious sera from white lipped marmosets (*Saguinus sp.*)². Another group described particles with 34-36nm in diameter in liver homogenates from marmosets (*Saguinus sp.*) at the peak of acute GB infection¹⁸.

GB VIRUS C (GBV - C)

The identification of the agent GB was only recently achieved using molecular biological methods. During the last few years, new experiments of hepatitis transmission to tamarins (*Saguinus labiatus*) were performed and the agent was identified using the Representational Difference Analysis (RDA) technique⁴³. Eleven clones derived from nucleic acids present only in the infected tamarins were obtained. These clones were not present in genomic DNA from tamarins, humans, *Escherichia coli* or *Saccharomyces cerevisiae*. RNA molecules around 8.3 kb were found in the plasma from infected tamarins by Northern blot analysis using these clones as probes. Other hybridization assays and sequencing extension showed that two different Flavivirus-like genome were present in infected tamarins. These viruses are distinct, but with restricted homology to HCV and were designated as GB viruses A and B⁷⁰.

Further transmission studies to tamarins showed that all animals inoculated with GB virus B (GBV-B) exhibited an elevation in liver enzymes after inoculation while such elevation did not occur when GB virus A (GBV-A) was the only agent detected in the serum. These data suggested that GBV-B is the probable causative agent for hepatitis in tamarins⁶⁸.

Proteins corresponding to the NS3 and NS4 regions of the viral genome were expressed in *Escherichia coli*

and utilized for the detection of specific antibodies by ELISA in different human populations. Antibodies were detected in 19.9% West Africa individuals, 1.5% North American blood donors and 14% North American intravenous drug users⁷¹. However, GBV-A and GBV-B could not be detected in any of these patients³⁶. A related virus was detected in one patient from West Africa by PCR with degenerated primers for the helicase region (allowing the detection of different virus from the *Flaviviridae* family). Analysis of the amplified product suggested that this sequence was from a virus more close related to GBV-A than GBV-B, or any of the HCV genotypes and was designated as GB virus C (GBV-C). Further screening using GBV-C specific primers resulted in the detection of this new virus in more 7 out of 42 West African serum samples that had antibodies against GBV-A and/or GBV-B recombinant proteins⁷¹.

Sequence from the initial GBV-C PCR product was extended and recombinant proteins were expressed in *E. coli* and used in ELISA to detect GBV-C immunoreactive sera. To verify the presence of this virus among non A-E hepatitis cases, 161 patients were screened with ELISA using recombinant antigens derived from GBV-A, GBV-B or GBV-C and 26 (16.1%) were immunoreactive. Using a PCR protocol to increase the detection of GBV-C variants among these patients, more 4 positive cases were identified, one from East Africa and three from North America⁷¹.

HEPATITIS G VIRUS (HGV)

Another group have also described the isolation of virtually the same virus, designated as hepatitis G virus (HGV), from the serum of a chronic non-A, non-B hepatitis patient (PNF2161). Interestingly, this patient was initially believed not to be infected with HCV using first generation immunoassay, but second generation HCV immunoassay and PCR lately demonstrated that PNF2161 was infected with HCV. A λ gt11 expression library was constructed using cDNA amplified by the use of a modified amplification technique designated Sequence Independent Single Primer Amplification (SISPA)⁴². After immunoscreening, the immunoreactive clones were analysed and the sequences obtained were either HCV-related or sequences that did not match any in the GenBank database. PCR primers designed from these non matching sequences revealed an exogenous clone (designated as 470-20-1) present in the cloning source plasma but not in healthy control subjects, *E. coli* or *S. cerevisiae*⁴².

Using an anchored PCR approach, multiple overlapping clones were generated and sequenced to create a 9392 nucleotide sequence encoding a polyprotein of

2873 aminoacids. Interestingly, the aminoacid sequence encoded by the immunoreactive clone 470-20-1 was not present in the polyprotein, but as a part of a small open reading frame (ORF) of 119 aminoacids encoded in the complementary strand. Antibodies against this clone were also found in several other non A-E hepatitis cases, so the negative strand may be expressed during infection⁴².

Using the sequences from patient PNF2161, a similar sequence was found in another patient, R10291, an asymptomatic individual with a history of intermittent elevations in liver enzymes, without markers for HBV or HCV infection. HGV variant from R10291 is predicted to encode a slightly larger polyprotein of 2910 aminoacids, as PNF2161 contains a virus with a single nucleotide deletion that introduces a frame shift resulting in the shorter continuous ORF⁴².

Comparison of these HGV sequences with the GenBank database revealed highly significant similarity first to GBV-C (85.5% and 100% identity in the NS3 region for nucleotide and aminoacid, respectively), second to GBV-A (43.8% identity) and then to GBV-B and HCV. Limited similarity was observed with the genera Pestivirus and Flavivirus, both belonging to the family *Flaviviridae* and with plant viruses, as tobacco necrose virus and tomato bushy stunt virus^{42,54}.

These data are indicative that HCV, GBV-A, GBV-B, GBV-C and HGV belong to a distinctive group of hepatitis associated viruses within the family *Flaviviridae*. Conserved motifs typical of this family were found in the RNA-dependent RNA-polymerase (RDRP), helicase and chymotrypsin like protease⁴².

A diagnostic PCR was developed for the detection of hepatitis G virus (HGV) that was detected in different populations from the USA, Europe, Australia, Japan and South America, as shown in the Table 1. In two cases, transfusion transmission and association with the development of clinical overt hepatitis was especially documented: HGV was detected only after blood transfusion, slightly earlier than the ALT rise and persisted for at least one year after infection⁴².

VIROLOGY

Comparisons of the entire genomic sequences of GBV-C and HGV showed aminoacid sequence identity at 95% (85% at nucleotide level), constituting independent isolates of the same virus. Neither GBV-C or HGV shows identity greater than 32% with GBV-A, GBV-B or HCV. They belong to a discrete group of viruses within the family *Flaviviridae*. This group can be delineate into

three lineages: GBV-A and GBV-C/HGV, GBV-B and HCV. The newly identified virus may be designated as GBV-C or HGV until the Committee on Viral Taxonomy and Nomenclature placed them into their appropriate family and genus⁸². In this review, this virus will be designated as GBV-C/HGV.

The genomic organization of GB viruses A, B and C have been analysed in detail (Figure 1)^{40,50}. These viruses have RNA genomes of more than 9,000 nucleotides containing a single large ORF that encodes the polyprotein precursor. The genomes are organized much like other pestiviruses and flaviviruses, with genes predicted to encode structural and non-structural proteins located at the 5' and 3' ends, respectively. Amino acid sequence analysis of the large precursor reveals the presence of protease, helicase and replicase motifs characteristics of the *Flaviviridae* family. The degree of sequence divergence between GBV-A and GBV-B and other *Flaviviridae* members suggests that they are representatives of two new genera within this family⁵⁰.

GBV-C is most similar to GBV-A, being 48% identical at the aminoacid level, while only 29 and 28% identical to HCV and GBV-B, respectively. Comparison of HCV and GBV-C reveals several regions of local identity between these two sequences, the greatest identity occurring within the NS3 region coding for helicase (as obtained when comparing GBV-C either to GBV-A or GBV-B) and within the N-terminus of the putative NS3 (serine protease) region and in the putative NS5B (RNA dependent RNA polymerase) region⁴⁰. The presumed potential proteolytic processing sites of the polyprotein for HCV, GBV-A, -B and -C by viral and host proteases seems to be conserved. The diversity observed between GBV-C and the closest HCV genotype is 5.3 times higher the maximal distance between any of the HCV genotypes⁴⁰.

The most striking feature of GBV-A and GBV-C is the absence of core-like proteins, distinguishing them from all other members of *Flaviviridae* family. Site-specific mutagenesis and N-terminal sequencing located the sites of translation initiation immediately upstream of the putative signal sequence for GBV E1 envelope glycoproteins. The 5' NTR of these viruses contains unique secondary structures that are involved in translation initiation recognized as internal ribosome entry site (IRES). Furthermore, efficient translation required the inclusion of GBV coding sequences, that contain a very stable stem loop structure. The level of activity of IRES is very low, what might act to reduce recognition of the infection by the immune system and thus promote viral persistence⁷².

Using recombinant proteins expressed in *E. coli*, antigenic regions were identified in the putative Core, NS3, NS4 and NS5 proteins from GBV-A, -B and -C: three epitopes in GBV-A, five epitopes in GBV-B and four epitopes in GBV-C. These sites were identified using a pool of convalescent tamarins and five different human sera against 25, 19 and 28 recombinant antigens derived from GBV-B, GBV-A and GBV-C, respectively⁶⁰. Although some antigenic regions were identified (Core, NS3 and NS5 for GBV-B; NS3 and NS5 for GBV-A; NS3, NS4 and NS5 for GBV-C), the response from each individual sample to each different antigen was quite variable, and tests with these antigens are still far from the standardization of reliable immunological assays for antibody detection. Recently, a new ELISA was developed to the detection of anti-E1 antibodies, that seems useful as a marker of previous GBV-C/HGV infection, but not to the detection of the uremic phase.

The nucleotide sequences from the different GBV-C/HGV isolates suggests that a high variability can be observed among the different sequences from GBV-C/HGV, although longer sequences including other genome regions are needed for more conclusive data. Using the Lasergene software (DNASTAR, Madison, USA), one of us found 78.9 to 100% similarity comparing the first 8 GBV-C isolates from Africa and North America among each other⁷¹ and 72.7 to 85% similarity among Japanese isolates involved in fulminant hepatitis⁷⁸ and these first 8 isolates⁶². These data are under evaluation by our group, but such sequence differences could relate to different genotypes associated with the severity of the infection in different patients and geographical regions, as previously suggested by other authors^{34,46,66}.

TRANSMISSION

Transfusion-transmission of GBV-C/HGV was firstly reported in two patients who were not initially infected that received blood units during surgery and became GBV-C/HGV positive. In this paper, it was also shown that the prevalence of infection was higher in population at parenteral exposure risk than in volunteer blood donors⁴².

Molecular evidence for transmission of GBV-C/HGV by blood transfusion was also shown in two GBV-C/HGV positive patients in Germany, one with severe aplastic anemia and other with end-stage liver disease. Each patient have received a previous transfusion from an PCR GBV-C/HGV positive donor. Sequence analysis showed at least 98% homology at the nucleotide level among samples from the respective donors and receptors⁶⁹.

In Japan, among 49 patients with HCV associated chronic liver disease with IVDU (but no blood transfusion) history, 12 (24%) were GBV-C/HGV infected, while only 9 (7%) out of 128 HCV associated chronic liver disease and no history of drug abuse were GBV-C/HGV infected. Tattooing may not efficiently transmit this virus, as GBV-C/HGV was found in only 1 (6%) out of 17 men with HCV associated liver disease but no IVDU history¹.

The parenteral transmission of GBV-C/HGV led to the investigation of the presence of this virus in blood derived products: 7 to 40% of commercial plasma pools used for the production of blood derivatives were GBV-C/HGV PCR positive, especially the pools from American origin (33 to 40%) when compared to the European origin (7 to 10%). Intravenous globulins also can be contaminated with this virus: 14 (88%) of 16 batches of a commercial product involved in HCV transmission were GBV-C/HGV positive by PCR⁵³.

An increased risk for GBV-C/HGV infection was also found among confined patients with leprosy. Sequences from the isolates suggested that some of them might be infected with the same viral strain, that would reflect a horizontal transmission of GBV-C/HGV within a closed society²⁰.

Vertical transmission of GBV-C/HGV was also verified. Nine (14.8%) out of 61 pregnant women had detectable viremia, six (20%) belonged to the 30 HCV co-infected women and three (17.6%) to the 17 HIV-1 co-infected groups. Six (66.7%) of HGV infected women have an IVDU history. Vertical transmission was found in three (33.3%) among nine infants, from 2 HCV and 1 HIV coinfecting mothers²⁶.

CLINICAL PICTURES

GBV-C/HGV was first described in 7 individuals from a population where no clinical data were available. In the same work, this virus was also detected in more 4 cases among 126 non A-E hepatitis cases. The clinical diagnosis of the four positive cases were acute hepatitis (two), hepatitis associated aplastic anemia and a history of hepatitis in an intravenous drug user that was also positive for HCV-RNA⁷¹. Serial samples were available from two of these individuals. In one of them, viraemia persisted for at least 20 days after the normalization of serum ALT. In the other, serology against GBV-C were positive during the symptomatic phase of hepatitis and aplastic anemia. Viral RNA was also detected except in the sample collected at presentation⁷¹.

GBV-C/HGV was also detected in 3 out of 6 cases of fulminant hepatitis non A - E in Japan⁷⁸. These 3 pa-

tients had histories suggesting previous exposure to or infection with unknown hepatitis viruses. No antibodies or nucleic acids for any previously known hepatitis virus could be detected in these patients, as confirmed by serology and PCR⁴⁷. On the other hand, some authors have not found this virus in fulminant hepatitis non A-E in Britain⁶⁶ or even in Japan³⁸. Genetic differences among the viral present in different geographic areas might explain these results, as already discussed.

Patients on maintenance hemodialysis are at increased risk for GBV-C/HGV infection, as 3.1% (16/519) were infected against 0.9% (4/448) healthy blood donors in Japan. In these patients, the virus produced persistent infections, mainly transmitted by transfusion but also by other means (as the virus was found in one patient with no history of transfusion). None of the 16 GBV-C/HGV positive patients had evidence of active liver disease. Therefore, in Japan, at least in patients on maintenance hemodialysis (known to have compromised immune responses) this virus was not associated with liver diseases⁴⁶. There might be some virus variants with crucial mutations in genomic regions not yet determined responsible for severe clinical picture found in some GBV-C/HGV infected cases⁷⁸.

Prevalence of GBV-C/HGV infection in patients on maintenance hemodialysis was higher in France: 35 (57.5%) out of 61 patients have viremia detectable by PCR. Furthermore, GBV-C/HGV was associated with hepatitis, as in all four patients presenting elevated serum alanine aminotransferase concentrations, GBV-C/HGV was the sole human hepatitis virus detected. The presence of viral genome correlated significantly with the duration of haemodialysis and receipt of previous transfusion¹⁶.

GBV-C/HGV and HCV RNAs were detected in 12 (5.2%) and 41 (18%) out of 229 leprosy patients in Japan, respectively. Nine patients infected with GBV-C/HGV alone had aminotransferase levels lower than the three patients co-infected with HCV or the 38 patients infected with HCV only, suggesting that the infection with GBV-C alone would not induce hepatic injuries as severe as HCV infection²⁰.

GBV-C/HGV seems to be an important agent implicated in a significant number of non A-E hepatitis in Italy: this virus was found in 35% (11/31) of acute and 39% (7/18) of chronic cases studied. Only one out of 100 control subjects (healthy blood donors) was infected. Among the 18 GBV-C/HGV positive patients, 3 had IVDU histories and 1 had a previous blood transfusion. Furthermore, the alignment of the predicted translation products identified two recurrent aminoacid substitutions

in 6 patients, suggesting the possible existence of at least two different GBV-C subtypes in this population²⁷.

GBV-C/HGV was found in two young adult male patients with hepatitis associated aplastic anaemia by two different groups^{9,30}. Of note, one of the first reported cases infected with this virus had also this clinical picture⁷¹. The association of hepatitis associated aplastic anaemia and GBV-C/HGV infection requires further confirmation, as this would add a new dimension to its public health profile.

A prospective study of transfusion-transmitted GB virus C infection in Taiwan found 40 (10%) GBV-C/HGV viremic patients among 400 adults who underwent cardiac surgery. The risk of transmission was estimated to be 0.46% per donor. Viremia was detected 1 week after transfusion and may persist for 8 years. However, clinical and laboratorial evidences for hepatitis is not identified in 25 patients infected with GBV-C/HGV alone. A longer follow up of these patients seems necessary to analyse the appearance of possible sequelae. In eight patients with post transfusion hepatitis, only one was positive for GBV-C/HGV RNA⁷⁶.

The use of carefully selected primers for the detection of GBV-C/HGV was recently shown. When consensus primers were designed according to the most conserved regions of previous isolates, the sensitivity of the method was significantly improved. GBV-C/HGV was detected in 10 (versus 4 with previous primers) out of 76 hepatitis non A-E cases and in 10 (versus 4 with previous primers) out of 62 blood donors with indeterminate HCV serology results⁴¹. An additional advantage of the consensus primers is that they do not cross react with HCV⁴¹, as we have also observed⁶².

Most of the data already published were based on amplification reactions using primers from the NS3 region, except the data describing the molecular cloning of Hepatitis G Virus⁴². Primers derived from the NS5 region were used to evaluate HGV prevalence in several populations, as discussed above (Table 1). Currently, a commercial detection system is available for GBV-C/HGV detection using two sets of primers covering the NS5 and 5'NCR regions. There is much expectancy on the sensitivity of 5'NCR primers, because, if GBV-C/HGV sequences heterogeneity behaves like HCV, these primers might be the more sensitive, as they cover the most conserved region. The use of primers from these two regions have been recently reported but it was not found a large difference in sensitivity^{51a}. The use of 5'NCR sequence is also proposed for GBV-C/HGV genotyping⁵¹.

This virus was also recently found in Brazil, in 1 among 13 cases of hepatitis non A-E, using the primers

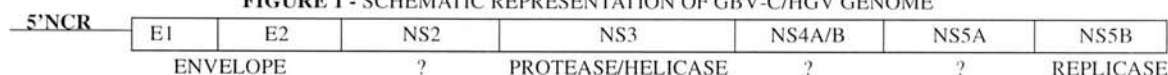
TABLE 1

HGV in different populations. Populations: AH, acute hepatitis; BD, blood donors; CH, chronic hepatitis; FH, fulminant hepatitis; H, hepatitis; HCC, hepatocellular carcinoma; IVDU, intravenous drug users; NB, newborns; OHS, open heart surgery; PBC, primary biliar cirrhosis;. PTH, post transfusional hepatitis.

Population	origin	patients total	GBV-C/HGV +	ref.
LIVER DISEASES				
PTH non A-E	US	12	2(16.7%)	42
	AU	1	1	42
CH non A-E	SA	48	6(12.5%)	42
	IT	18	7(39%)	27
	BR	13	1(7.7%)	61
AH non A-E	BR	80	6(7.5%)	62
	US	38	5(13.1%)	42
H non A-E	IT	11	35(39%)	27
	EU	12	1(8.3%)	42
FH non A-E	US	76	4(5.3%)	71
	US	76	10(13.2%)	41
	JA	6	3(50%)	78
CH HCV +	JA	6	0	34
	GB	6	0	66
CH HCV +	EU	96	18(18.7%)	42
	BR	10	2(20%)	62
	JA	128	9(7.0%)	1
CH HCV+, tattoo	JA	17	1(6.0%)	1
CH HCV+, IVDU	JA	49	12(24.5%)	1
CH HBV +	EU	72	7(9.7%)	42
HCC	EU	30	2(6.7%)	42
H alcoholic	EU	49	5(10.2%)	42
H auto-immune	EU	53	5(9.4%)	42
PBC	EU	58	1(1.7%)	42
PARENTERAL EXPOSURE RISK:				
Hemofilics	EU	49	9(18.3%)	42
Polytransfused	EU	100	18(18%)	42
IVDU	EU	60	20(33.3%)	42
	US	13	2(15.4%)	41
Hemodialysis patients	JA	519	16(3.1%)	46
Hemodialysis patients	FR	61	35(57.5%)	16
NB, infected mothers	GE	9	3(33.3%)	26
OTHER GROUPS:				
BD (accepted)	US	769	13(1.7%)	42
BD (rejected - ALT >45 UI/ml)	US	709	11(1.5%)	42
HIV +	GE	100	9(9%)	53
BD	IT	100	1(1%)	27
BD (accepted)	JA	60	0	20
BD	JA	448	4(0.9%)	46
HCV seroindeterminants	US	62	10(16.1%)	41
Leprous patients	JA	229	12(5.2%)	20
OHS - donors	TA	200	4(2%)	76
OHS - receptors	TA	400	40 (10%)	76

Origin: AU, Australia; BR, Brazil; EU, Europe; FR, France; GB, Great Britain; GE, Germany; IT, Italy; JA, Japan; SA, South America; TA, Taiwan; US, United States.

FIGURE 1 - SCHEMATIC REPRESENTATION OF GBV-C/HGV GENOME



initially described⁶¹. Further studies carried out by our group, using a modified technique and the new consensus primers, identified 6 GBV-C/HGV positive patients out of 80 cases of hepatitis non A-E. The rate of (GBV-C/HGV)/HCV mixed infection is also considerable in Brazil, as 2 out of 10 HCV positive chronic hepatitis cases were also GBV-C/HGV positive. Among the 6 patients infected only with GBV-C/HGV, severe liver disease was found in three (hepatic cirrhosis), two of them with severe liver failure and only one have another reported cause that could be implicated for this clinical picture⁶².

Some authors argue that the evidences for the association of GBV-C/HGV and hepatitis are not consistent based on the following arguments: 1 - Although more severe cases of Hepatitis G have been observed in some patients with community acquired hepatitis, the persistent viremia after clinical and biochemical recovery suggests that these patients may have been chronic carriers who then had an unrelated acute hepatitis; 2 - Prevalence of GBV-C/HGV was not different in the first study involving blood donors with normal and elevated ALT levels, casting serious doubts on whether this virus truly causes liver disease in healthy blood donors; 3 - The coincidence of post transfusion hepatitis and the GBV-C/HGV infection can be explained by the simultaneous infection with another virus; 4 - The vast majority of GBV-C/HGV infected patients have no evidence of associated liver disease; 5 - The prevalence of GBV-C/HGV in Europe, Japan and United States in hepatitis non A-E patients was higher than in blood donors but similar to those in control patients with non viral liver disease; 6 - GBV-C/HGV accounts for only a minority of cases of hepatitis without a defined viral cause; there are three to six times more cases of non A-G hepatitis^{5,58}.

Certainly, there are no prospective studies that document histologically the progression from acute infection through various stages of chronic hepatitis to the development of cirrhosis, hepatocellular carcinoma or end-stage liver disease. Until this demonstration, GBV-C/HGV may be an innocent bystander in a process caused by an unknown virus or some non viral event. Clearly, additional studies with careful assessment of risk factors for infection and evaluation of diseases associated with infection will be required before the causative association between GBV-C/HGV and acute liver failure can be clarified⁵.

The inapparent clinical symptoms and signs do not preclude the necessity for further screening for this virus, as serious sequelae could happen in only a small proportion of patients with chronic viral infections, as happens for parvovirus B19 and HTLV-I. Serologic assays for markers of this infection could reveal its true frequency and may be used for clinical diagnosis and perhaps, for donor screening⁷⁶.

Further studies should be developed to evaluate the prevalence of this virus around the world, especially in our country, among hepatitis non A-E cases and the general population. The results of such studies will define which procedures should be adopted to control the spread of this virus in the population.

Anyway, as GBV-C/HGV accounts at best for a minority of all cases of unexplained liver disease, the existence of still other viruses involved in clinically important human hepatitis cases are quite probable. There are many evidences of a virus resistant to chloroform that might be involved in parenteral transmitted human hepatitis.

RESUMO

O Vírus da Hepatite G e outros possíveis vírus causadores de hepatites não-A, não-E

A identificação dos vírus responsáveis pela vasta maioria dos casos de hepatite (Vírus das Hepatites A, B, C, D e E) foi realizada durante os últimos 30 anos. Entretanto, existem ainda alguns casos epidemiologicamente relacionados com agentes infecciosos, nos quais não se encontra nenhuma evidência de infecção por nenhum vírus conhecido. Estes casos foram designados de Hepatites não A-E e são atualmente relacionados com pelo menos três diferentes vírus: 1 - Mutantes do Vírus da Hepatite B, que expressam o antígeno de superfície (AgHBs) com epitopos alterados ou em baixas concentrações. 2 - Um outro vírus, denominado Vírus da Hepatite F, foi associado com as hepatites não A-E de transmissão entérica. Entretanto, maiores estudos são ainda necessários para sua melhor caracterização. 3 - O Vírus da Hepatite G ou Vírus GB-C foi recentemente identificado em diferentes regiões do mundo (incluindo o Brasil) como um Flavivírus responsável por cerca de 10% dos casos de hepatites não A-E de transmissão parenteral. Provavelmente, ainda outros vírus responsáveis pelas hepatites humanas serão encontrados em casos de hepatites sem marcadores de infecção por nenhum destes vírus.

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