REVIEW ARTICLE

NOVELITY IN GB VIRUS C / HEPATITIS G VIRUS AND ITS CONTROVERSY

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Several novel human RNA viruses were identified in 1995-96 and were partially characterized that apparently can cause acute and chronic hepatitis both in monkeys and humans. These new viruses are related to the flavivirus hepatitis C. It is known to be distinct from other human hepatitis viruses (A, B, C, D, E). Three viruses, identified by investigators at Abbott Labs, have been termed GB-A, GB-B and GB-C. GB-A and GB-B are likely tamarin viruses whereas GB-C infects humans only. All these three viruses are the isolates of the same virus termed HGV, which is positive stranded RNA virus. The genomic sequences of these viruses have been determined by different researchers, which were found to be 1600 nucleotide long. Another group at Genelabs Technologies has identified and determined the complete genomic sequence of a virus they termed hepatitis G virus (HGV). Based on genomic sequence comparisons HGV is probably the same as GB-C. It is a highly controversial virus regarding pathogenecity, mode of transmission and site of replication.

INTRODUCTION

HEPATITIS

'Hepatitis' means 'inflammation of liver'. It can be caused by many types of infections (viral, bacterial, fungal, T.B. etc), toxic drugs, poisons, alcoholism, vascular disorders and immune system diseases. The major causative agent, nowadays, for hepatitis are viruses and out of them, hepatitis viruses commonly known as hepatotrophic viruses, are the most common causative agents causing hepatitis all around the world[1,2]. Hepatitis caused by hepatotrophic viruses has affected humans for centuries[3,4] and was first described by Hypocrites over 2000 years ago[5,6].

Despite the development of effective vaccines to prevent hepatitis A and B, viral hepatitis caused by hepatotropic viruses continues to be a common problem. The Centre for Disease Control and Prevention (CDC) reports an annual incidence of 25 cases per hundred thousand (100,000) population of acute viral hepatitis[7]. The true incidence rate is estimated to be five times higher than the one reported by CDC[8,9]. Probable reasons for this disparity are under reporting and misdiagnosis.

Hepatotropic viruses, the most common cause of acute viral hepatitis, can be grouped under two main categories. First category is the one, which is transmitted through feco-oral route, and Hepatitis A Virus and Hepatitis E Virus are included in this category. Second category includes the hepatitis viruses transmitted through parenteral route (through blood) and sexual routes; they include Hepatitis B Virus, Hepatitis C Virus, Hepatitis D Virus and Hepatitis G Virus.

HEPATITIS G VIRUS

HISTORY

HGV, a novel flavivirus which is identical to GB Virus C, was originally discovered in a surgeon with hepatitis of non A and non B origin in 1995 and later identified in animals to be different from hepatitis A, B, C, D and E respectively. It was named as GB virus C after the initials of the surgeon, which were GB[10]. In 1996, another group claimed finding another hepatitis virus, which was named as hepatitis G virus (HGV)[11,6]. In 1996, it was identified that both GB virus C and HGV are the different isolates of the same virus and that both share same structure which is nowadays commonly known as HGV[10,12].

STRUCTURE

HGV is a positive, single stranded RNA virus that shares about 25–40 % homology with HCV[13,14]; containing approximately 9400 nucleotides, have a genomic organization resembling that of flaviviridae (Hepacivirus). Genome structure is 9,.4-kb RNA and virus particle is probably 30–60 nm. Morphology is enveloped and incubation period is 30–120 days[15]. HCV and HGV are transmitted from person to person by the same methods, that are parenteral and sexual routes, and above all 1 out of every 5 hepatitis-infected patients from HCV also carries HGV[1,16].

HGV genome organization was found to be similar to that of HCV with a single open reading frame and 5' and 3' untranslated regions. In addition analysis of the predicted amino acids sequences indicated the presence of structural and non-structural proteins as well as a number of putative proteolytic cleavage sites in a relative position found in HCV. So, it can be assumed that HGV replicates through negative strand RNA and hence its presence can be regarded as a direct evidence of viral replication[17].

PREVALENCE OF HGV INFECTION

In Acute Hepatitis

Cryptogenic 3–20 %
HBsAg positive 5–25 %
Anti HCV positive 10–30 %
Fulminant and Sub-acute Hepatic Failure more than 50 %

In Chronic Hepatitis / Cirrhosis

Cryptogenic 3–40 %
HBsAg positive 2–20 %
Anti HCV positive 10–40 %

In General Population:

Prevalence in general population is 3–10 %

In Parenteral Risk Group

In parenteral risk groups the prevalence rate reaches more than 25%.

In a joint study conducted in four different hospitals of Czech Republic, the prevalence among the IV drug users is 2/3rd of HGV RNA positive patients[15].

ROLE OF HGV AS HEPATITIS VIRUS

Hepatitis G virus's (HGV) transmissibility and persistence of viraemia has been established by animals and human studies but its clinical significance as a hepatitis virus has become increasingly controversial[l8,19]. Viraemia can be detected in about 5% of American donors and like HCV the virus was thought originally to be transmitted primarily by parenteral routes[13,14]. However, Viraemia is detectable in 6–7% of non-transfused American children[20] and other studies failed to confirm an increased risk with parental exposure[21,22].

The role of HGV as a hepatitis virus also remains controversial[23], however some early reports suggested an association between HGV viraemia and fulminant hepatitis[24] but others have not confirmed this association[22].

Similarly, some groups claim that this virus has shown its replication in liver and hence hepatotropic[25,17] but there are many reports that suggested lymphocytes, bone marrow and spleen rather than hepatocytes to be the site of replication of HGV[26]. The major obstacle in the study of HGV replication site is the lack of availability of multiple tissue samples from the infected individual.

It has been said that there is minor role of HGV in the aetiology of liver disease and little pathogenic effect on the liver. In a 35-year-old health care worker assessed over a long period of time to evaluate the changes over time in HGV RNA viral load. The patient did not have any positive history of drug abuse, blood transfusion, familial liver

disease and no co infection with HAV, HBV and HCV. After at least 6 years (and probably more than 14 years) of persistent HGV infection, no significant necroinflammation or progressive liver damage developed in the patient. This study argues against a major role for HGV as a cause of chronic liver disease[22].

However in another clinical study shows four different stages of the clinical course of the infection with HGV[23].

a. Asymptomatic

b. Light Acute Hepatitis

When the laboratory values and histological changes are quickly back in normal status.

c. Persistent Infection

When the laboratory values and histological changes persist.

d. Chronic Infection

When histological finds are of that of steatosis with persistent or chronic active hepatitis with fibrosis or Cirrhosis.

Most of the patients show minor elevation in aminotransferase levels that last until the clearance of HGV RNA[15].

ROUTES OF TRANSMISSION

HGV is transmitted through intravenous, sexual routes and perhaps also via perinatal transmission. Further chronic liver disease does not ensue due to HGV and liver is not the primary site of HGV replication[17]. Studies from the Centre of Disease Control and Prevention has shown that approximately 18% HGV RNA patients are present among the patients newly diagnosed as non-A, non-B hepatitis in United States of America. Most of these 18%, (approximately 80%) are also co-infected with HCV[27].

However, there are some other studies, which reported no cases of HGV in patients of fulminant hepatitis. It is also seen that 1.6% of volunteer blood donors are seropositive for HGV RNA[27].

Heuft and co-workers, from Germany, reported that out of 23 blood transfusion recipients, who received blood from two HGV affected long term blood donors, 15 patients (65%) were found HGV RNA seropositive. Seven out of remaining 8 showed anti-E2 response, previous HGV infection with spontaneous clearance of the virus. In the last one recipient neither HGV RNA nor anti-E2 response can be detected. Moreover, out of these 23, none developed post transfusion hepatitis[28].

Viraemia of high titres and mode of delivery are closely associated with mother to infant transmission of HGV. Lin et al have checked 25 infants (2.1%), who were HGV RNA positive, out of 2046 mothers for 1-year. Thirteen infants (52%) were viraemic and infection became persistent in all. None of the mothers was delivered by elective C-Section. In comparison, the other 12 infant's mothers, who remained HGV RNA negative, 10 mothers had lower HGV RNA titres but the two mothers with high titres opted for elective C-Section[29].

Moaven suggested that HGV RNA can be transmitted through mother to baby. He tested the infant of one mother through RT PCR, who was HGV RNA positive, and found the baby negative for HGV RNA at birth but at the age of 4–6 weeks the samples were found to be positive for HGV RNA[30].

SITE OF REPLICATION OF HGV

The extreme 5' terminal sequences of HGV, containing elements essential for regulation of viral gene expression and replication have not been determined. By cloning the extreme 5' terminal sequences of viral genome from the serum of the three Taiwanese patients, 2 Americans and 1 West African using RNA-ligase-mediated RACE (rapid amplification of the cDNA ends) procedure, supported the following hypothesis:

1. The extreme 5' end of HGV viral genome has been cloned.

2. There are different genotypes related to geographic separation.

3. The viral translation and replication mechanisms may be similar to that of HCV and pestivirus.

This data shed light on the mechanism of viral replication but the site of replication of HGV is still not found[31].

Both HCV and HGV can replicate in bone marrow and the actual prevalence of infection may be under estimated if serum samples are used[32]. Although HGV is unlikely to be a primary hepatotropic virus but its site of replication remains unclear[26].

By using RT-PCR, viral RNA negative strand is searched in various autopsy tissues of two patients who died due to the end stage liver disease. Negative strand was detected in spleen and bone marrow of both the dead patients and negative strand was present in the lymph node of one of the two dead patients[33]. Recent progress demonstrates that HGV replicates in lymphocytes, bone marrow and spleen rather than hepatocytes[32].

Different cell lines, haemopoetic, hepatocyte-derived cell lines, primary human lymphocytes, inoculated with virus, were used with strand specific RT-PCR and looked for the evidence of viral antigen production by using a rabbit polyclonal antibodies against a viral envelope protein. The negative strand RNA was detected in CESS and Meg 01 cell line up to 40 days and after that no viral RNA could be detected, despite over 90% cell viability as shown through tryphan blue dye exclusion. Moreover no E2 antigen was detected on day 0 but could be detected afterwards in all the cell cultures that were only positive for positive strand RNA. So, detection of positive strand shows inoculated virus only[20]. From this study it has been hypothesized that haemopoetic blood cells or vascular endothelial cells were good candidates for the site of viral replication. It has also been demonstrated that the virus could be passaged to fresh cells. This is also been indicated that the viral replication is occurring in Meg 01 cell lines because the antigens can be only detected in Meg 01 cell line through immunoflorescence.

In 1998, Laskus *et al*[34] performed a study in which they used Tth based strand specific RT-PCR to search the negative strand RNA of HGV in autopsy tissues samples of four patients with AIDS and blood mononuclear cells from six other HIV positive patients. Negative strand HGV RNA was detected in 3 out of 4 bone marrow samples, 2 out of 2 spleen samples and 1 out of four liver samples[34]. This study does not support HGV as hepatotropic virus and its replication in liver, even in the presence of AIDS, is very low or absent. However, cell lineage supporting viral replication in bone marrow or spleen remains to be determined.

In another study serum and bone marrow samples from 48 patients from haematologic outpatient clinic was analysed for HGV and HCV RNA. HCV RNA was detected in 18 (38%) and 15 (31%) and HGV RNA was detected in 6 (13%) and 9 (19%) of serum and bone marrow samples. In 3 patients, HGV was detectable in bone marrow but not in serum[32].

Daudi cells are burkitt's lymphoma cell lines which are reported to be capable of supporting productive infection of HCV. These cell lines, when became resistant to interferons during continual cultivation after HCV infection, coded as H-903, was used as host cells for replication of HGV. The virus RNA was detected in the culture by RT-PCR for more than 130 days after inoculation, while it was detected only for 44 days in parental interferon-sensitive Daudi cells. Productive infection of HGV in H-903 system was confirmed by serially inoculating supernatants from the infected cultures into uninfected cells. The viral E2 antigen was detected by immunoflorescence in the cells inoculated with the fifth passage of HGV. The presumed capsid-coding region of the viral genome in the

inoculum, in the serially passaged virus or in the virus produced by a long-term culture, was only 16 aminoacids long, suggesting that HGV with a short core sequence was replication competent[35].

It is quite evident from different studies that HGV a new virus discovered in 1996 shares 25–40% homology with HCV. Co-infection with HCV is higher than the other hepatitis viruses. High prevalence rate is found in IV drug users, age group mostly infected is young people and the incidence in males is more than the females.

Its role as a hepatitis virus as well as its site of replication is still controversial and according to different studies, it is more likely to be bone marrow, spleen or lymphocytes rather than liver. The role of HGV infection depending on acute or chronic hepatitis is not fully defined.

Nowadays there are many different schools of thoughts about this virus. It is not even included in the possible etiologic agent for viral hepatitis, in some literature. It is indicated that we should think about its clinical meaning because it cannot still be excluded from the possibility of pathogenic activity in relationship to co-infections. The different aspects of this virus and its infective course should be studied completely before jumping to conclusions.

There is no doubt that a lot has been done about this virus after its discovery till now but still there is a long way ahead of us before we can totally exclude the possibility of dangerous course of infectivity of this virus.

155 million Americans and same ratio all around the World are exposed to Hepatitis Virus—but you are not to know. More than half the planet is now infected. You know all about AIDS, an illness that you will most likely never get. However, you may already be infected by HGV shots and unfortunately nobody knows what harm it can cause.

REFERENCES

1. Sack and Etzkorn. Hepatitis virus alphabet: an introduction to hepatitis viruses A through G. Borland: Aroover Art; 2001 pp320-4.
2. Aikawa T, Sugai Y, Okamoto H. Hepatitis G infection in drug abusers with chronic hepatitis C [letter]. New J Med 1996;334:195-6.
3. Koff RS. Diseases of the liver. 7th ed. Lippincott (NY): Raven Publishers; 1993.
4. Linnen J, Wages J Jr, Zhang-Keck ZY, Fry KE, Krawczynski KZ, Alter H, et al. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science 1996;271:505-8.
5. Alexander IM. Viral hepatitis: primary care diagnosis and management. SpringNet – CE Connection 1998.
6. Martinot M, Marcellin P, Boyer N, Detmer J, Pouteau M, Castelnau C, et al. Influence of hepatitis G virus infection on the severity of liver disease and response to interferon-alpha in patients with chronic hepatitis C. Ann Intern Med 1997;126:874-81.
7. The Council of State and Territorial Epidemiologists. Mobidity and mortality weekly report, recommendation of ACIP. CDC 1991;45:883-4.
8. Noskin GA. Prevention, diagnosis and management of viral hepatitis. Arch Fam Med 1995;4:923-34.
9. Masuko K, Mitsui T, Iwana K, Yamazaki C, Okuda K, Meguro T, et al. Infection with hepatitis GB virus C in patients on maintenance hemodialysis. N Eng J Med 1996;334:1485-90.
10. Anonymous. The Hepatitis G Virus : HGV. Tulane University Med J 1998. www2.tulane.edu/~dmsomder/WWW/335/Reading.html.
11. Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, et al. Isolation of novel virus like sequences associated with human hepatitis. Nat Med 1996;1:565-9.
12. The Management of viral hepatitis. Proceedings of Canadian Association for Study for the Liver Consensus Conference; 1999 Mar 15-19; Montreal, Quebec.
13. Fried MW, Khudyakov YE, Smallwood GA, Cong M-E, Nichols B, Diaz E, et al. Hepatitis G virus co-infection in liver transplantation recipients with chronic hepatitis C and nonviral chronic liver disease. Hepatology 1997;25:1271-5.
14. Seipp S, Wahl R, Mueller H, Stremmel W, Theilmann L Goeser T. Sequence analysis of hepatitis GB virus C isolates from 14 patients. Virus Res 1996;46:81-8.
15. Strakrle V, Bucek J, Konig J, Pokorny A. The Occurrence of HGV, clinical and histopathological finds, therapy, in patients with suspicion of diagnosed hepatitis. 1998 www.hepnet.com/hepg/strakrle99.html-101k
16. Stransky J. The discovery of hepatitis G virus. Casopis Lekaru Ceskych 1996;135:99-101.
17. Laskus T, Radkowski M, Wang LF, Vargas H, and Rakela J. Lack of evidence for hepatitis G virus replication in liver of patients co-infected with hepatitis C and G viruses. J Virol 1997;71:7804-6.
18. Pessoa MG, Terrault NA, Detmer J, Kolberg J, Collins M, Hassobal HM et al. Quantitation of hepatitis G and C viruses in the liver: evidence that hepatitis G virus is not hepatotropic. Hepatology 1998;27:877-80.
19. Tanaka E, Alter HJ, Nakatsuji Y, Shih W-K, Kim JP, Matsumoto A et al. Effect of hepatitis G virus infection on chronic hepatitis C. Ann Int Med 1996;125:740-3.
20. Handa A, Brown KE. GB virus C/hepatitis G virus replicates in human haematopoietic cells and vascular endothelial cells. JGV 2000;81:2461-9.
21. Cantaloube JF, Gallian P, Biagini P, Attoui H, Escher J, Zappitelli JP, et al. Prevalence of GB virus type C/Hepatitis G virus RNA and Anti E2 among blood donors in south-eastern France. Transfusion 1999;39:95-102.
22. Marco VD. Long, benign course of hepatitis G virus infection [letters]. Ann Int Med 1997;67:23.
23. Mushahwar IK, Zuckerman JN. Clinical implication of GB virus C. J Med Vir 1998;56:1-3.
24. Yoshiba 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.

25. Seipp S, Schiedel M, Hofmann WJ, Tox U, Theilmann L, Goeser T et al. Hepatotropism of GB virus C: GBV-C replication in human hepatocytes and cells of human hepatoma cell lines. J Hep 1999;30:570-9.
26. Karger SAG. GB Virus C/Hepatitis G Virus. Intervirology 1999; 42: 185-195.
27. DiBisceglie AM. Hepatitis G Virus Infection: A Work in Progress. Annals of Internal Medicine 1996; 125: 772-773.
28. Heuft HG, Berg T, Schreier E, Kunkel U, Tacke M, Schwella N et al. Epidemiological and clinical aspects of hepatitis G virus infection in blood donors and immunocompromised recepients of HGV – contaminated blood. Vox Sang 1998;74:161-7.
29. Lin HH, Kao JH, Yeh KY, Liu DP, Chang MH, Chen PJ et al. Mother to infant transmission of GB virus C/hepatitis G virus: the role of high – titred maternal viremia and mode of delivery. J Infect Dis 1998;177:1202-6.
30. Moaven LD, Tennakoon PS, Bowden DS, Locarnini SA. Mother to baby transmission of hepatitis G virus. Med J Aust 1996;165:84-5.
31. Hsieh SY, Yang PY, Chen HC, Liaw YF. Cloning and characterization of the extreme 5' – terminal sequences of the RNA genomes of GB Virus C/HGV. Proc Natl Acad Sci (US) 1997;94(7):3206-10.
32. Radkowski M, Kubicka J, Kisiel E, Cianciara J, Nowciki M, Rakela J et al. Detection of active hepatitis C virus and hepatitis G virus/GB virus C replication in bone marrow in human subjects. Blood 2000;95:3986-9.
33. Radkowski M, Wang LF, Cianciara J, Rakela J, Laskus T. Analyisis of hepatitis G virus/GB virus C quasispecies and replication sites in human subjects. Biochem Biophys Res Commun 1999;258:296-9.
34. Laskus T, Radkowski M, Wang L, Vargas H, Rakela J. Detection of hepatitis G virus replication sites by using highly strand-specific Tth-based reverse transcriptase PCR. J Virol 1998;72:3072-5.
35. Shimizu YK, Hijikata M, Kiyohara T, Kitamura Y, Yoshikura H. Replication of GB virus C (hepatitis G virus) in interferon – resistant Daudi cells. J Virol 1999;73:8411-4

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