Original Article

Hepatitis G virus infection among Iraqi patients with Chronic liver diseases

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Summary:

Background: The hepatitis G virus(HGV), also called hepatitis GB virus, as a member of the Flaviviridae family distantly related to hepatitis C virus (HCV), Little is known about the frequency of HGV infection, the nature of the illness, or how to prevent it. What is known is that transfused blood containing HGV has caused some cases of hepatitis. They infect humans, but are not known to cause human disease. This virus can be transmitted efficiently by blood transfusion and by other parenteral mechanisms. Transient and long lasting infections with HGV have been documented in man.

Patients and methods: HBs Ag, Anti-HCV IgG and Anti-HGV IgG were detected by Enzyme-Linked Immunosorbent Assay (ELISA).HCV RNA on the other hand, has been detected using PCR technique in the serum of 75 Iraqi patients with chronic liver diseases in comparison to 15 healthy individuals.

Results: HGV infection was detected in 25% of blood donors, 30% of chronic hepatitis C, 25% of chronic hepatitis B, and 20% of cryptogenic chronic liver disease. HGV infected patients tended to be younger than non-infected patients but no differences concerning sex, possible source of infection, clinical manifestations, biochemical and virological parameters, or severity of liver lesions were found.

Conclusions: The percentage of HGV infection in chronic liver disease seems to be relatively high in our area 19 out of 90cases (21.11%). Infection with HGV does not seem to play a significant pathogenic role in patients with chronic liver disease related to chronic HBV or HCV infection, or in those with cryptogenic chronic liver disease.

Key words: HGV, chronic liver disease, blood donors.

Introduction:

Hepatitis G virus and GB virus C (GBV-C) are RNA viruses that were independently identified in 1995, and were subsequently found to be two isolates of the same virus (1,2). They are member of the Flaviviridae family and are phylogenetically related to hepatitis C virus, but appear to replicate primarily in lymphocytes, and poorly if at all in hepatocytes (3, 4). GBV-A and GBV-B are probably Tamarin viruses, while GBV-C infects humans (4). Infection with this virus has, however, also been detected in a high proportion of patients with idiopathic fulminant hepatitis (5) suggesting that HGV may be highly pathogenic in some cases. This possibility is still open to question since other studies have not disclosed a significant prevalence of HGV infection in this condition (5). Parenteral, sexual and vertical transmission of GBV-C have all been documented, and because of shared modes of transmission, individuals infected with HIV are commonly co-infected with GBV-C. Among people with HIV infection, the prevalence of GBV-C viraemia ranges from 14 to 43% (6,7,8,9).Some studies have suggested that co-infection with GBV-C will actually slow the progression of HIV disease(8,9).There is also controversy about the role of HGV infection in the pathogenesis of chronic liver disease. Preliminary surveys of patients with chronic liver disease showed that HGV is often detected in patients chronically infected with other hepatotropic viruses such as the hepatitis B virus (HBV) or HCV or both but more rarely in patients with cryptogenic liver disease (8,10). Some studies have, however, found a relatively high prevalence of HGV infection in patients with cryptogenic chronic hepatitis, suggesting that this virus may be important in this condition (11, 12, and 13). Approximately 2% of healthy American blood donors are viraemic with GBV-C, and up to 13% of blood donors have antibodies to E2 protein (anti -E2 envelope), indicating prior infection (14, 15, and 16).To elucidate further the pathogenic role of HGV in chronic liver disease we investigated the percentage of HGV infection and its associated features among patients with different chronic liver diseases and in a group of blood donors.

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Patients and Methods:

Patients:

This study was conducted during the period of February 2008 to January 2009.

A total of 55 Iraqi patients with chronic liver disease and 20 volunteer blood donors were included in this study.

Apparently healthy control group with a total number

of 15 who have no history or clinical evidence of chronic liver disease or any other chronic disease were selected as normal control (friends, staff), they are age and sex matched . According to clinical, serological and histopathological features, patients were separated into the following categories:

Chronic hepatitis C This group included 20 patients (13 male and 7 female; mean age 45 years, range 18-69). Chronic hepatitis C was assessed by sustained elevation of ALT, histologically proved chronic inflammation of the liver, and positive tests for anti-HCV antibodies and for HCV-RNA in serum. Other causes of chronic liver disease were excluded.

Chronic hepatitis B This group included 20 patients (15 male and 5 female; mean age 38 years, range 9-62) with chronic hepatitis B virus infection, as assessed by long lasting hepatitis B surface (HBsAg) and elevated ALT. Liver biopsy was performed in all cases.

Cryptogenic chronic liver disease This group included 15 patients (5 male and 10 female; mean age 39 years, range 14-65) years. All presented with abnormalities of liver function tests consisting mainly of elevation of aminotransferase serum levels 1.5 times above the upper normal limit lasting for at least one year. None were obese or alcoholic, and all denied use of potentially hepatotoxic drugs. All had negative tests for HBsAg, anti-HCV, HCV RNA, and autoantibodies (antinuclear, antismooth muscle, antimitochondrial, and antiliver and kidney microsomes). Serum levels of iron, ferritin, caeruloplasmin, and α1-antitripsin were normal in all cases. Liver biopsy showed chronic hepatitis in 7 cases, portal and periportal fibrosis in 3 cases and micronodular cirrhosis in 5 cases.

Blood donors: Twenty consecutive volunteer blood donors (18 male and 2 female; mean age 30 years, range 22-58) were studied at the time of their first donation.

Laboratory investigations:The sera were tested for HBs Ag, Anti-HCV IgG and Anti-HGV IgG using Enzyme-Linked Immunosorbent Assay (ELISA) in Teaching Laboratories in Baghdad Medical city. Technique used human IgG Fc as the antigen coated the microwells plate and isotype-specific horse antibodies coupled to radish peroxidase; color was developed which turns yellow when the reaction was stopped with sulfuric acid, results were expressed as the optical density using a microwell plate reader with single wave length450nm.(ATLAS MEDICAL,Cambrige,CB4 4WX,UK)

HCV RNA in serum was determined by reverse transcription (RT) and amplification by PCR was carried in Al-Karama Teaching Hospital Laboratory and Central Public Health Laboratory. RNA was extracted from 100µl using RNA extraction kit(Ribo-Sorb RNA/DNA extraction kit ,REF:K-2-1, Sacace Biotechnologies,Italy) . Complementry DNA(cDNA) was synthesized from 10 µl of extracted RNA by30 minutes incubation at 37ºC with 0.5 µl of Molony leukaemia virus reverse transcriptase ,for each obtained cDNA sample a 20 µl of RT-buffer .Specific PCR amplification and hybridization of the 5'NCR of HCV genome by HGV-primers(5'-CACTATGGTGG→GTCTTAAG-3' , 5'GCGCACGGTCCA→CAGGTGTT-3') was carried out using a commercial kit(HCV 240/440 IC, REF:V-1-50R ,Lot No.TK045200 ,Sacace Biotechnologies ,Italy,) according to manufacturer's instructions. One positive and one negative control were included in each run. the samples were considered positive for HCV RNA if a band of 240bp could seen on 2% agarose gels with ethidium bromide, bands were cut out, and DNA was extracted and analyzed by automated sequencing. (5,6)

Other data included in this study (biochemical liver function tests, liver histopathology,autoantibodies)collected from Laboratory reports for each patient during the follow up period .

Statistical Analysis:

Comparisons between groups were made by the X^2or Fisher's exact test .A p-value less than 0.05 was considered significant.

Results:

HGV INFECTION IN PATIENTS WITH CHRONIC HEPATITIS C HGV IgG was found in 6 out of 20 patients (30%). Although the prevalence of HGV infection in this group was higher than in blood donors the difference did not reach statistical significance. No significant differences between HGV infected and non-infected patients were observed concerning the demographic features, the presumed source of infection, the biochemical or hematological abnormalities or the degree of severity of liver lesions (table-1)(table-2).

HGV INFECTION IN PATIENTS WITH CHRONIC HBV INFECTION HGV IgG was detected in 5 out of 20 patients (25%) with chronic hepatitis B infection, comparison of HGV infected and non-infected patients did not show significant differences concerning demographic features, biochemical and severity of liver lesions, (table-1)(table-3).

HGV INFECTION IN PATIENTS WITH CRYPTOGENIC CHRONIC LIVER DISEASE HGV IgG was detected in 3 of 15 patients (20%). Those patients are asymptomatic with abnormal liver enzymes and lasting many years after recovery from an episode of fulminant hepatitis of unknown etiology. Two patients had received blood products at that time, while a possible source of infection was not identified in third patient. Their liver biopsy showed evidence of chronic hepatitis with mild activity and minimal fibrosis. Further analysis was not possible due to the small number of HGV infected patients detected in this group. HGV INFECTION IN BLOOD DONORS In this study 5 out of 20(25%) cases were HGV IgG positive among asymptomatic apparently healthy blood donors whereas, non of healthy control group were positive to HGV IgG.

Table-1: Distribution of HGV -IgG among study groups

Study groups	HGV-IgG Negative cases No. (%)	HGV-IgG positive cases No.(%)	Total No. (%)
Blood donors	15(75)	5(25)	20(22.2)
Chronic hepatitis B	15(75)	5(25)	20(22.2)
Chronic hepatitis C	14(70)	6(30)	20(22.2)
Cryptogenic chronic liver diseases	12(80)	3(20)	15(16.7)
Control group	15(100)	0(0)	15(16.7)
Total	71(78.89)	19(21.11)	90(100)

Table-2: Demographic features and other pathological changes in HGV infection among patients with chronic hepatitis C

Characteristics	HGV-IgG positive (n=6)	HGV-IgG negative (n=14)	p-Value
Age (y)	42 (14)	43 (12)	0.25
Sex (male/female)	4/2	9/5	0.08
Presumed source of infection			0.44
Blood transfusion	3 (15 %)	6(30 %)	
Unknown	3 (15 %)	8(40 %)	
*AST (IU/l)	95 (80)	92 (61)	1.06
*ALT (IU/l)	186 (151)	151(115)	0.08
*glutamyl transferase (IU/l)	32 (15)	52 (44)	0.36
*Bilirubin (mg/dl)	1.0 (0.7)	0.9(1.01)	0.25
Degree of histological severity			0.17
Mild hepatitis	1 (5 %)	4(20 %)	
Moderate hepatitis	2(10 %)	3(15 %)	
Severe hepatitis	1 (5 %)	3(15 %)	
Cirrhosis	2 (10%)	4(20 %)	

***mean of serum enzymes**

Table-3: Demographic features and other pathological changes in HGV infection among patients with chronic hepatitis B

Characteristics	HGV-Ab positive (n=5)	HGV-Ab negative (n=15)	p-Value
Age (y)	32 (5)	36 (13)	0.52
Sex (male/female)	3/2	12/3	0.3
Presumed source of infection			
Blood transfusion	3 (15%)	5 (25%)	
Unknown	2 (10%)	10 (50%)	
*AST (IU/l)	63 (29)	70 (54)	0.12
*ALT (IU/l)	111 (91)	125 (118)	0.06
*glutamyl transferase (IU/l)	28 (7)	45 (47)	0.15
*Bilirubin (mg/dl)	0.6 (0.4)	0.8 (0.3)	0.09
Degree of histological activity			1.17
Mild hepatitis	0 (0%)	3(15%)	
Moderate hepatitis	1 (5%)	4 (20%)	
Severe hepatitis	1 (5%)	5 (25%)	
Liver cirrhosis	2 (10%)	3 (15%)	

***mean of serum enzymes**

Discussion:

HGV, or GBV-C can cause acute and chronic liver infection in man (12). The recent development of sensitive laboratory techniques for determination of HGV RNA sequences in clinical specimens has led to intensive investigation of the frequency and meaning of HGV infection in different clinical conditions. Despite many efforts, however, important aspects of the epidemiology and pathogenicity of HGV infection and its role in acute and chronic liver diseases still remain obscure. In the current study we investigated the presence of HGV IgG in patients with a variety of chronic liver diseases and in a group of volunteer blood donors. The main demographic, epidemiological, clinical, and histopathological features in HGV infected and non-infected patients were analysed. It is now clear that patients heavily exposed to blood and blood products, such as haemophiliacs, thalassaemics, and liver transplanted patients (17,18), as well as those at high risk of parenteral exposure, such as patients on haemodialysis (19,20) and intravenous drug users (3, 21) have the

highest prevalence of HGV infection. These data suggest that parenteral exposure plays an important role in the transmission of HGV. This study showed, however, that a substantial proportion of HGV infected patients with chronic liver disease did not have a history of overt parenteral exposure. Furthermore, irrespective of the clinical or histological diagnosis, most of these patients also had evidence of infection by other parenterally transmitted hepatotropic viruses, such as HCV, HBV, suggesting that HGV may also spread through non-apparent parenteral exposure.(12) Data on the pathogenic effects of HGV are controversial. HGV infection is the only agent identified in some patients with acute sporadic non-A, non-E hepatitis, idiopathic fulminant hepatitis (7,22), or cryptogenic chronic liver disease (14). Studies in blood donors and in haemodialysis patients (4, 23) however, have clearly shown that HGV infection is frequently found in subjects without clinical or biochemical evidence of liver disease. In a recent study in haemodialysis patients, HGV infected patients did not usually present with liver abnormalities except if coinfected with other hepatotropic viruses (24). Mild elevation of ALT is often found in HGV infected blood donors (25). In this study, a minimal elevation of ALT was observed in one of the four blood donors in whom HGV IgG was detected in serum, but this abnormality was also observed in a similar proportion of HGV non-infected donors and may be a non-specific finding. Infection has been reported in 10-20% of adult with chronic hepatitis B&C indicating that co-infection is a common occurrence. (17) In agreement with previous observations (18, 25) infection with HGV did not appear to increase the severity of liver lesions in patients with chronic liver disease resulting from HBV or HCV infection. On the other hand, there is increasing evidence indicating that coexisting HGV infection does not appear to modify the response to interferon in patients with chronic hepatitis C (18, 26). These data support the hypothesis that HGV coinfection does not play a relevant role in the pathogenesis of HBV or HCV induced chronic liver disease. Further evidence against the pathogenicity of HGV was recently provided by a study of patients with acute post-transfusional hepatitis, showing that combined infection with HGV and HCV did not produce more severe hepatitis than infection with HCV alone (27) Although HGV infection often becomes chronic, chronic hepatitis does not appear to develop in subjects persistently infected with HGV alone, as recently shown in patients with acute non-A, non-E hepatitis (28)and that most isolated instances of HGV infection are not associated with acute or chronic liver injury(21).

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