Journal of Clinical Laboratory Analysis 14:70–72 (2000)

GB Virus C in Patients With Liver Disease of Unknown Etiology

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To assess the prevalence of GBV-C in patients suffering unknown liver disease we have investigated the GBV-C-RNA in serum of 54 patients: 10 with acute and 32 with chronic non-A-E hepatitis (16 active and 16 persistent), 10 with hepatocellular carcinoma, 2 diagnosed with hepatic fulminant failure, and 91 healthy blood donors (control). PCR with primers from NS3 helicase region was performed and the product was identified by a double strand DNA enzyme immunoassay. GBV appears to infect 40 and 31% of acute or chronic non-A-E hepatitis respectively. Also the GBV genome was found in 1 in 10 samples of hepatocarcinoma, in 2 cases of fulminant hepatitis, and in 1 in 91 of the control group. In spite of these results the role of GBV in the etiology of liver diseases has to be analyzed in more comprehensive studies. J. Clin. Lab. Anal. 14:70–72, 2000.

Key words: GBV-C; PCR; non-A-E hepatitis

INTRODUCTION

As early as 1967, Deinhardt et al. published serial transmission experiments with suspected infectious sera of patients with unknown hepatitis to tamarins (1–4). But only recently, a putative agent for human non-A-E hepatitis has been identified by molecular biological techniques and designated GB virus type C (5). Recently, another human non-A-E virus has been described and designated hepatitis G virus (HGV), but due to the similarity of aminoacid and nucleotide sequences to those of GBV-C, it is considered to be a distinct genotype of the same virus (6). It has a genomic organization resembling the Flaviviridae family; however, phylogenetic analysis has shown that it is not an HCV variant (7,8). Further studies are needed to ascertain the epidemiology and clinical significance of this new virus in human hepatitis and the possible treatment.

RNA of GBV-C can be determined by reverse-transcriptase (RT) polymerase chain reaction (PCR) with primers deduced from hypothetic helicase region and has been detected in patients with several pathologic conditions and diverse risk factors (6,9).

The aim of this study is to evaluate the GBV-C presence in a group of patients suffering a liver disease of unknown etiology.

MATERIALS AND METHODS

The group of patients enrolled in this study included a total of 54 subjects suffering liver disease of unknown but suspected viral etiology. Acute or chronic hepatitis by A, B, C, D, and E viruses, cytomegalovirus, and Epstein-Barr virus were excluded by serologic tests. To this end, anti-HAV (IgG/IgM), anti HBc (IgG/IgM), anti-HBs, anti-HBe, HBsAg, HBeAg, anti-HDV (IgG/IgM), anti-HCV, and anti-HEV were studied by ELISA. Additionally, HBV-DNA and HCV-RNA were searched in serum by commercial PCR tests. Also, cytomegalovirus and Esptein-Barr virus infection were excluded by testing the presence of anti-CMV (IgG/IgM), Ag CMVp65 in sera and anti-VCA-EBV (IgG/IgM) by ELISA.

ANA, SMA, and LKM antibodies were negative when tested by commercial immunofluorescence test (Kallestad, Quantafluor, Sanofi Diagnosis Pasteur, S.A. France). There was no history of patient drug use (paracetamol, alcohol, etc.) or exposure to toxins.

They were then diagnosed with acute viral (classical symptoms and signs as well as elevated ALT values: ALT > 500 u/l) and 32 with chronic non-A-E hepatitis (more than 6 months of elevated serum ALT-levels—1.5 times higher than normal values and liver histology), from which 16 were classified as active and 16 as persistent. Also, sera from 10 patients with hepatocellular carcinoma (by liver biopsies) and 2 with fulminant hepatitis of unknown origin were included in the study. These patients were male, without risk factors, and their illness lasted 10–15 days, all with a fatal outcome. The findings were: ALT maximun 792/650; time of prothombin 60/71 sec; serum bilirubin 25/18 mg/%; arterial pH minimum 7.1/7.2 and serum 3.5/5 mg/%. We also included a control group of 91 sera from healthy blood donors who had normal ALT levels (age 34 ± 5.3 years, 69% female, from the blood donor center).

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Received 29 December 1997; Accepted 2 November 1999

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

RNA was extracted from 100 µl of serum with TriPure Isolation Reagent (Boehringer, Mannheim, Germany) and reverse transcription was performed using random hexanucleotides and 200 units of the enzyme Superscript II (Gibco BRL, Life Technologies, Gaithersburg, MD). Then, the cDNA was subjected to amplification by PCR with degenerated primers derived from NS3 helicase region (9). The sequences of sense (H1) and antisense (H2) primers were respectively: 5′-TTA TGG GCA TGG HAT HCC YC-3′ and 5′-CCR TCY TTG ATG ATD GAR CTG TC-3′(R = A or G; Y = C or T; D = A or G or T; H = A or C or T). The protocol for amplification consisted of 45 cycles and each cycle included denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. PCR-product detection was carried out using DNA enzyme immunoassay (DEIA) as previously described (9,10) and the 5′ biotinylated degenerated probe 5′-TTC TGC CAY AAR GCK GAG TGY GARG-3′(R = A or G; Y = C or T; M = A or C; K = G or T) which is hybridized at 50°C.

RESULTS

The GBV genoma was observed in 31.4% of the patients studied in our report, all of them suffering liver disease of unknown but with suspected viral etiology as shown in Table 1, while in our control group only 1.09% was positive for RNA-GBV ($P < 0.001$). The individual from control group with RNA-GVB positive in serum had no clinical features or biochemical alterations. It includes a female, 35 years old, without apparent risk factors.

GBV appears to infect patients with either acute or chronic hepatitis without statistical difference (40 vs. 31%); also, we did not find any differences between sera from patients suffering persistent or active chronic hepatitis (31 vs. 31%). In serum from the two patients with fulminant hepatitis of unknown cause which were selected retrospectively for our study, GBV genomic RNA was detected by PCR and finally, GBV-RNA was identified in 1 (1/10) patient with idiopatic hepatocellular carcinoma. Therefore, we have not found differences in patients with different liver diseases, except that the frequency of RNA-GBV was quite lower in the patients with hepatocellular carcinoma than in those with the other liver diseases. The role of GB virus type C in the etiology of fulminant hepatic failure as well as in positive hepatocellular carcinoma but also in acute or chronic hepatic infection must be analyzed in more comprehensive studies.

TABLE 1. Prevalence of GBV-RNA in several groups of patients with liver disease

	GBV-RNA+ (n)	%
Non-A-E acute hepatitis (n = 10)	4	40
Non-A-E chronic hepatitis (n = 32)		
Chronic active (n = 16)	5	31
Chronic persistent (n = 16)	5	31
Fulminant hepatitis (n = 2)	2	100
Hepatocellular carcinoma (n = 10)	1	10
Healthy blood donors (n = 91)	1	1

DISCUSSION

Approximately 10–20% of hepatitis cases are of unknown etiology but recently, a possible agent for human nonA-E hepatitis has been described and named GBV-C (5,11). Its RNA can be detected by the reverse-transcription polymerase chain reaction (PCR) with primers deduced from the helicase-like region. We have evaluated whether GBV-C may be an agent contributing to acute and chronic liver disease which should be considered in the diagnostic evaluation of patients presenting with liver disease of unknown etiology.

The presence of GBV genoma in 31.4% of our patients suggests that this virus may be one of the causative agents of liver disease. Our results in acute and chronic (active and persistent) non-A-E hepatitis are higher than the ones reported by Linnen and Jeffers (6,12), although they are very similar to those from Fiordalisi et al. (9). Because the percentage of GBV-C positivity found in our control group is quite similar to that found by Fiordalisi et al., discrepancies could be attributed to a higher sensitivity of our method, a different geographic distribution of the virus, or even the variability of GBV-C; one method could more easily detect GBV-C variants that escape to the other method.

The results that we obtained from two cases of fulminant hepatic failure suggest the importance of GBV-C in the etiology of this disease as previously reported by others (13–15) and it even seems to be implicated in hepatocellular carcinoma (6,12,16).

Further investigations concerning epidemiological studies and genomic organization are necessary to understand if the new virus plays a significant role in liver diseases of unknown etiology. Studies of patients both before the onset of liver disease and after it is established would provide evidence that GBV-C can be directly implicated. The coinfection of hepatitis G virus and HBV or HCV is frequent (17–19). Also, no core or very short gene has been identified (11–20). As described in the references indicated, the initial methionine for protein translation is located at the star of the putative E_1 gene signal sequence, and it indicates that the positive-strand RNA genome does not encode a very short capsid protein. The implications of not finding a capsid within the GBV-C/HGV proprotein are unknown. A possible explanation is that the virus uses a cellular protein as its capsid, but another explanation is that productive GBV-C/HGV infection may require a helper virus.

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