

HEPATITIS G VIRUS / GB VIRUS C IN BRAZIL. PRELIMINARY REPORT

J.R.R. PINHO(1,2), M.L. CAPACCI (3), L.C. da SILVA (3,4), F.J. CARRILHO (3), C.A. SANTOS (2), V. PUGLIESE (3),
B. GUZ (5), J.E. LEVI (2,4), C.A.F. BALLARATI (2) & A.P. BERNARDINI (2,3)

SUMMARY

Hepatitis G virus/ GB virus C is a novel flavivirus recently detected in hepatitis non A-E cases. In this study, the presence of this virus in chronic non-B, non-C hepatitis patients was evaluated using GBV-C specific PCR and this virus was detected in one out of thirteen patients. This patient has presented a severe liver failure, has lived for a long time in the Western Amazon basin and no other cause for this clinical picture was reported. The impact of the discovery of this new agent is still under evaluation throughout the world. The study of the prevalence of this virus among chronic hepatitis patients and healthy individuals (as blood donors) will furnish subsides to evaluate its real pathogenicity.

KEYWORDS: Hepatitis G; GB virus; Hepatitis non A - E; HGV

INTRODUCTION

After the identification of Hepatitis C Virus^{6,14} and Hepatitis E Virus²⁰, there were still many cases of acute and chronic hepatitis without evidence of infection by any known virus. These cases were designated as hepatitis non-A, non-B, non-C, non-D, non-E, or simply, hepatitis non A-E³. Evidences for other viruses causing human hepatitis were found in previous animal experiments, showing multiple infections in animals after inoculation with human sera^{4,11,25} and from patients with more than one episode of non-A, non-B hepatitis^{9,12,17,18,19,23}.

In 1967, serial passages of hepatitis were observed in tamarins primarily inoculated with the serum from a 34 years-old surgeon called G.B., collected in the 4th day of jaundice⁷. In 1976, virus - like particles were identified in the tamarins sera^{2,8}. Finally, in 1995, using the Representational Difference Analysis (RDA) amplification method¹⁶, the genome of two viruses, designated GBV-A and GBV-B, were cloned²¹.

Immunological assays were developed using recombinant virus proteins expressed in *Escherichia coli*. Although many samples were reactive to these tests, these viruses were not found in humans, but a close related virus, designated GBV-C, was detected by PCR using degenerated primers in African and North American patients²². Another group detected virtually the same virus using similar molecular biological methods in different populations from North and South America, Australia and Europe¹⁵.

The aim of this study is to evaluate if this novel agent is found among chronic non-B, non-C hepatitis patients in our country, using a GBV-C specific PCR²².

MATERIAL AND METHODS

Patients - Thirteen patients with previous diagnosis of chronic non-B, non-C hepatitis were attended at Department of Gastroenterology, Faculdade de Medicina de São Paulo and at the Hospital do Servidor Público Estadual, São Paulo, SP, Brazil.

(1) Serviço de Virologia do Instituto Adolfo Lutz, São Paulo, SP, Brasil.

(2) Laboratório Bioquímico Jardim Paulista, São Paulo, SP, Brasil.

(3) Departamento de Gastroenterologia da Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil.

(4) Instituto de Medicina Tropical de São Paulo, São Paulo, SP, Brasil.

(5) Hospital do Servidor Público Estadual de São Paulo, São Paulo, SP, Brasil.

Correspondence to: João Renato Rebelo Pinho, Instituto Adolfo Lutz, Av. Dr. Arnaldo, 355, 01246-902, São Paulo, SP, Brasil.

These patients were clinically diagnosed as chronic non-B, non-C hepatitis according to the following parameters: raised serum Alanine aminotransferase (ALT) levels for more than one year, no evidence of previous infections by Hepatitis B and C Viruses (assessed by determination of anti-HCV, HBsAg and anti-HBc) and absence of other reported causes for chronic hepatitis, such as alcohol, drug abuse, hereditary and autoimmune diseases. The most relevant clinical, pathological, epidemiological and laboratorial data from these patients are depicted in the TABLE. All patients were asked about exposure to known risk factors (alcohol abuse, intravenous drug use, blood transfusion, surgery, tattoo, acupuncture, homosexuality, health care work, familial diseases, origin from endemic areas of hepatitis).

Serology - anti-HCV, anti-HBc and HBsAg were assayed using commercial available kits (Abbott Laboratories, Chicago, IL, USA).

Polymerase Chain Reaction (PCR) - after RNA extraction by the guanidine isothiocyanate method⁵, detection of HGV/GBV-C was accomplished using specific PCR, as previously described by SIMONS *et al.*²², with minor modifications.

After reverse transcription, the samples were submitted to nested PCR. In the first amplification round, the cDNA was amplified by the following cycles: denaturation at 94°C for 1 min; annealing at temperatures decreasing from 55° to 41°C at 3 cycles/ C, for 42 cycles, followed by 10 cycles at 40°C (2 min); extension at 72°C for 3 min; using primers ns3.1-a2 (5' CVA TRG TRA WRG MGG GTC MAG G 3') and ns3.2-s1 (5'ATG GTI AII GTN GGR TCH ARR 3') (C = cytosine, A = adenine, G = guanine, C = cytosine, T = thymine, R = A or G, Y = C or T, M = A or C, K = G or T, W = A or T, H = A, C or T, V = A, C or G, N = G, A, T or C).

In the second amplification round, 5 microliters from the first round were amplified by 35 cycles of 94°C (1 min), 55°C (1 min) and 72°C (1 min), using primers GB-C-s1 (5' GAC GTT GGT GAG ATC CCC TT 3') and GB-C-a1 (5' CGA AGT TTC CTG TGT ACC C 3'). Positive samples showed a 238 bp band after agarosis gel electrophoresis. The PCR product was sequenced in an automated DNA Sequencer (ABI, model 373A) and the sequence was compared to other previously described using the LASERGENE program (DNASTAR).

All the samples were also submitted to PCR amplification for the detection of HBV¹³, HCV¹⁰, GBV-A and GBV-B²¹.

RESULTS

Serology - All patients were confirmed as negative for anti-HCV, anti-HBc and HBsAg.

PCR - All samples were negative for HBV, HCV, GBV-A and GBV-B sequences.

One sample was positive after GBV-C PCR, showing the expected 238 bp band (FIGURE). Nucleotide sequence analyses confirmed this signal to be derived from GBV-C, since 74-83% and 71-72% homology was observed with the sequences described by SIMONS *et al.*²² and YOSHIBA *et al.*²⁴, respectively.

DISCUSSION

Hepatitis non A-E were observed throughout the world and HGV/GBV-C was found in 2/12, 6/48 and 1/12 hepatitis cases in the USA, South America and Europe, respectively¹⁵. This virus was also found in Japan^{1,24}, Africa²² and Australia¹⁵.

In this paper, we describe the detection of this virus in 1 out of 13 chronic non-B, non-C hepatitis cases in Brazil. This patient has lived for a long time in the Western Amazon basin and has presented severe chronic liver failure that contraindicated liver biopsy. No other possible cause for this clinical picture was reported.

To our knowledge, this is the first time that this virus is detected in a Brazilian patient. The PCR technique used in this study may probably be improved using primers derived from the 5' non coding region, that is expected to be more conserved among the different viral isolates, as described for HCV²⁴. In this way, the frequency of HGV/GBV-C infection among chronic non-B, non-C patients may be higher than described here.

The impact of the discovery of this virus is still under evaluation. HGV/GBV-C transfusion transmission was temporally associated with ALT peak and development of post transfusion hepatitis in some cases¹⁵ and this agent has also been associated with fulminant hepatitis cases in Japan²⁴. On the other hand, the high prevalence of this virus among healthy blood donors¹⁵ raises some doubts about its pathogenicity. We may speculate that infection with this virus does not manifest as clinical hepatitis in the majority of individuals, as seen with other hepatitis viruses.

In conclusion, we describe in this paper the finding of GBV-C/HGV in one chronic non-B, non-C hepatitis case in Brazil. This study confirms the presence of this virus in our

TABLE 1
Characterization of patients with clinical diagnosis non-B, non-C hepatitis

Name	Age	Sex	Liver Histology	Epidemiology	anti-HBc	HBsAg	anti-HCV	ALT (x UNL)
E.M.C.	55	M	CPH	(-)	(-)	(-)	(-)	2.89
C.B.A.	65	F	LC	(-)	(-)	(-)	(-)	1.79
E.A.M.	49	F	CSH	(-)	(-)	(-)	(-)	1.08
R.O.	21	M	CAH	(-)	(-)	(-)	(-)	2.32
C.S.	41	M	Steatosis	(-)	(-)	(-)	(-)	1.72
M.L.C.	38	M	SH	(-)	(-)	(-)	(-)	1.42
N.K.	59	M	LC	open heart surgery	(-)	(-)	(-)	1.02
S.N.A.	41	F	LC	(-)	(-)	(-)	(-)	1.80
W.R.S.	62	M	LC	(-)	(-)	(-)	(-)	2.00
C.O.S.	29	M	Steatosis	(-)	(-)	(-)	(-)	1.45
L.D.	67	M	LC	(-)	(-)	(-)	(-)	1.12
M.P.C.	42	M	Non-available*	origin from hepatitis endemic area	(-)	(-)	(-)	1.49
M.M.	30	M	Steatosis	(-)	(-)	(-)	(-)	1.15

LIVER HISTOLOGY - CPH (chronic persistent hepatitis), LC (liver cirrhosis), CSH (chronic septal hepatitis), CAH (chronic active hepatitis), SH (Steatohepatitis); EPIDEMIOLOGY - all patients were asked about exposure to known risk factors; anti-HBc, HBsAg and anti-HCV were assayed as described in MATERIAL AND METHODS. (-) = negative. ALT (Alanine aminotransferase) is expressed as x UNL (times Upper Normal Limit). The GBV-C/HGV positive case (M.P.C.) is highlighted by bold. *Severe liver failure contraindicated biopsy in this patient.

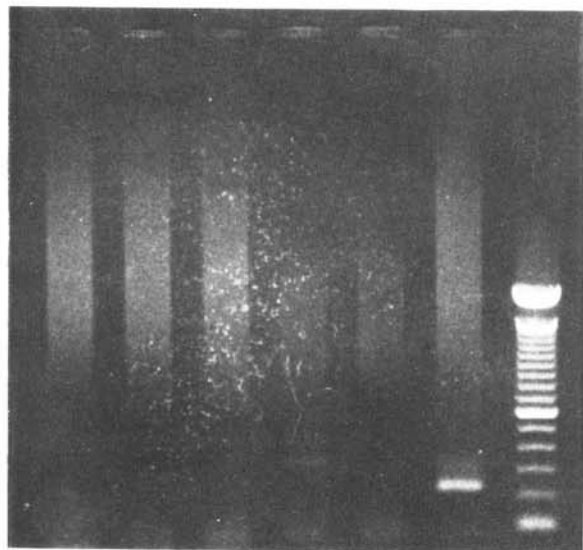


Fig. 1 Detection of hepatitis G virus/GB virus C by PCR. Samples from non-B, non-C chronic hepatitis patients were submitted to specific PCR and electrophoresed in 2% Agarosis gel stained with Ethidium bromide. From left to right lane 1 to 5 - negative samples, lane 6 - GBV-C positive sample, and lane 7 - molecular weight standard (100 bp ladder).

country. Further studies should be performed to establish the prevalence of this virus in larger populations, involving chronic hepatitis patients and healthy individuals (as blood

donors). These studies will certainly be necessary to evaluate the real pathogenicity of this virus.

RESUMO

Vírus da Hepatite G / Vírus GB-C no Brasil

O vírus da Hepatite G ou vírus GB-C é um novo vírus recentemente descoberto em casos de hepatites não A-E. Neste estudo, casos de hepatite crônica não-B, não-C foram testados com uma reação de amplificação específica para GBV-C. Este vírus foi detectado em 1 entre os 13 casos estudados. Este paciente apresentava insuficiência hepática severa, tinha habitado por vários anos na Amazônia Ocidental e nenhuma outra causa para este quadro clínico havia sido relatada. O impacto da descoberta deste novo agente está ainda sendo investigado. O estudo da prevalência deste vírus entre pacientes com hepatite crônica e entre indivíduos sadios (como, por exemplo, doadores de sangue) fornecerá subsídios para o estabelecimento de sua real patogenicidade.

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