Analysis of GB virus C infection among HIV-HCV coinfected patients

Análise da infecção pelo vírus GB-C em pacientes com coinfecção VIH-VHC

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ABSTRACT

The aim of this study was to evaluate the effect of GB virus C on laboratory markers and histological parameters among HIV-seropositive patients coinfected with HCV. Lower degrees of hepatic lesions were observed in the triple-infected patients, in comparison with HIV-HCV coinfected patients who were negative for GBV-C RNA.

Key-words: GB virus C. Hepatitis G virus. Hepatitis C virus. HIV. Coinfection.

RESUMO

O objetivo do estudo foi avaliar o efeito da infecção pelo vírus GB-C em marcadores laboratoriais e parâmetros histológicos em pacientes HIV soropositivos coinfectados com VHC. Menor grau de lesão hepática foi observado nos pacientes com tripla infecção em comparação aos pacientes coinfectados com VIH-VHC negativos para GBV-C RNA.

Palavras-chaves: Vírus GB-C. Vírus da hepatite G. Vírus da hepatite C. VIH. Coinfecção.

GB virus C (GBV-C) is a flavivirus closely related to hepatitis C virus (HCV), with amino acid sequence homology of approximately 30%[7]. GBV-C[14] and hepatitis G virus (HGV)[8] are independent isolates of the same RNA virus. GBV-C was initially reported to be associated with post-transfusion hepatitis in humans[8]. However, several research groups demonstrated there was no evidence of associations between GBV-C infection and hepatitis[1]. The liver-specific pathogenicity and tropism of GBV-C remain to be clarified. While some research groups found the GBV-C genome in hepatocytes[13], other groups reported that GBV-C is not hepatotropic[6 10]. In vitro studies have shown that GBV-C is able to infect peripheral blood mononuclear cells (PBMC)[19]. HIV-seropositive patients coinfected with GBV-C presented longer AIDS-free survival and higher CD4+ T-cell counts, compared with HIV-monoinfected patients[18 20]. Some studies have speculated that GBV-C might interfere with HIV and have suggested that this flavivirus may have a favorable effect on the course of HIV disease[18 19].

GBV-C infection is relatively common and has worldwide distribution. A study carried out in the City of São Paulo found that 5.1% of the general population were GBV-C positive, while 5.2-9% of Brazilian blood donors tested positive for GBV-C RNA[12]. Studies evaluating American populations found that 0.8-1.4% were positive for GBV-C RNA, whereas 1.8-3.2% were GBV-C infected in Europe[11]. Active GBV-C infection is demonstrated by viremia (RNA), while anti-E2 antibodies indicate resolved infection. Thirty to 65% of HIV-seropositive patients are positive for anti-E2 GBV-C antibodies and, in most cases, viral clearance occurs over time. Although simultaneous presence of GBV-C RNA and E2 is rare, detection of both of these markers is possible and could indicate a transition state[17].

Because of similar transmission routes, coinfection between GBV-C and HIV is common. Epidemiological studies have indicated that 39% of HIV-seropositive patients are viremic for GBV-C and 47% present anti-E2 antibodies[15]. The rates of coinfection in heterosexual individuals or injection drug users (IDUs) range from 14 to 17.5%, while 17.7% of homosexual men are viremic[9].

The information regarding liver histology is still conflicting and no data on triple infection (HIV-HCV-GBV-C) are available. Within this context, our study evaluated the effects of GBV-C on histopathology, among HIV-HCV coinfected patients, and the possible influence of GBV-C on HIV and HCV viral loads.

The study included 20 HIV-seropositive patients presenting chronic coinfection with HCV who underwent liver biopsy at Hospital São Paulo, Brazil. Patients were prospectively included between May 2006 and May 2007 and the study was approved by the Ethics Committee of UNIFESP (CEP 1296/05).

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The inclusion criteria were as follows: age between 18 and 70 years; and HIV-seropositive with HCV coinfection. Patients were excluded if they had other associated hepatic diseases. The technical procedures were carefully explained to all patients. After signing a written informed consent, a blood sample was collected and the laboratory and virological analyses were carried out.

Demographic data such as gender, age and risk factors for HIV and HCV transmission were collected. Data regarding medication were also collected. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) were measured and analyzed by using the AU640™ Chemistry Immuno-Analyzer (Olympus®). Flow cytometric immunophenotyping (FACSCalibur™, BD, USA) was used to determine the CD4 and CD8 T-cell counts. The HCV viral load was determined by means of real-time PCR (TaqMan) and the lowest detectable limit of HCV viral load was 200 copies/ml. HCV was genotyped by means of genome sequencing of PCR products, followed by phylogenetic analysis. Quantitative determination of HIV-RNA was carried out using the branched DNA HIV-1 RNA 3.0 assay (Bayer), with a detection range from 50 to 500,000 copies/ml. GBV-C RNA was detected in plasma samples by using an in-house RT-PCR assay[2]. IgG antibodies against the envelope protein E2 of GBV-C were detected using an ELISA assay (µPLATE Anti-HGenv, Roche Diagnostics).

Liver biopsy was indicated for all HCV RNA-positive patients, independent of the levels of the liver enzymes for defining the prognosis and treatment[4]. The 20 patients whose biopsies are presented in this report were those sequentially performed during the study period. The slides were stained with hematoxylin-eosin, Masson's trichrome, Perl's stain and silver for reticular fibers (Gomory's stain). Liver biopsies with a diagnosis of chronic hepatitis or nonspecific reactive hepatitis were semi-quantitatively analyzed according to the stage and grade using the criteria described by Gayotto et al[5]. A single pathologist who was unaware of the clinical data analyzed all the liver biopsy slides.

The patients were classified into three groups according to their GBV-C infection status: HIV-HCV coinfected patients who tested positive for anti-E2 antibodies (HIV-HCV-GBV-C E2), triple-infected patients (HIV-HCV-GBV-C RNA) and patients who were neither positive for GBV-C RNA nor positive for anti-E2 antibodies (HIV-HCV). Variables were compared between groups by means of the Mann-Whitney test, using the SPSS software, version 13.0. A significance level of 0.05 (α = 5%) was adopted as significant.

The mean age of the cohort was 43.0 ± 6.2 years; 60% (12/20) were men and 40% (8/20) of these had had parenteral exposure. The mean length of time with the diagnosis of HIV infection was 8.3 ± 3.7 years, 85% (17/20) of the cohort were undergoing antiretroviral therapy and 85% (17/20) presented undetectable HIV RNA levels. The CD4 and CD8 T-lymphocyte counts were relatively high, with a mean of 522.8 ± 246.2 cells/mm³ and 912.1 ± 506.6 cells/mm³, respectively. The mean value of ALT was 63.3 ± 55.0 U/l, AST 48.0 ± 32.5 U/l and GGT 104.7 ± 94.1 U/l. The mean HCV viral load among the 20 patients coinfected with HIV-HCV was 4.56 ± 1.64 $\log^{10}$, while two showed HCV-RNA viral loads below the lower limit of detection (< 200 copies/ml). Genotyping data demonstrated that HCV genotype 1a was present in 40% (8/20) of the cohort and genotype 1b in 20% (4/20).

Among the twenty biopsied patients, 70% (14/20) presented negative results for GBV-C (E2 or RNA) markers and 30% (6/20) presented at least one marker for GBV-C infection. These included 15% (3/20) with positive ELISA tests (GBV-C E2) and 15% (3/20) who were positive for PCR (GBV-C RNA). The triple-infected patients in the biopsied group (HIV-HCV-GBV-C RNA) presented undetectable HIV viral loads.

There were no statistical differences in HCV viral load or between any of the liver enzymes levels among the groups, possibly because of the limited number of patients. Two of the three patients with triple infection presented HCV genotype 1a. **Table 1** shows the characteristics of the cohort and referral criteria for classification. The histopathological characteristics of 16 patients with nonspecific reactive hepatitis and chronic hepatitis were evaluated and are shown in **Table 2**. The remaining four patients presented steatosis (1/20), steatohepatitis (1/20), normal liver (1/20) or insufficient material (1/20) and were not evaluated.

TABLE 1

Histopathological diagnosis of the biopsied patients (n=20).

Histopathological characteristics	n	%
Non-specific reactive hepatitis	4	20.0
Chronic hepatitis		
chronic hepatitis with absent interface hepatitis	4	20.0
chronic hepatitis with mild interface hepatitis	6	30.0
chronic hepatitis with moderate interface hepatitis	2	10.0
Steatosis	1	5.0
Steato-hepatitis	1	5.0
Normal liver	1	5.0
Material of narrow size range for histological analysis	1	5.0

We compared the groups of biopsied patients to evaluate whether there was any evidence that GBV-C had some beneficial effect. As shown in **Table 2**, our results demonstrated that none of the groups presented grade 4 staging or periportal and parenchymatous activities. Mild liver disease and lower degree of inflammation were observed in the triple-infected group (grades 0-2), histologically compared with the HIV-HCV coinfected patients (grades 0-3).

A similar study was carried out by Strauss et al[16] among HCV-GBV-C patients, in which GBV-C RNA was detected in six of the 22 patients with liver biopsy. Although there were no statistical differences between the groups, they observed a lower degree of inflammation in the HCV-GBV-C coinfected group and this interaction did not demonstrate any reason for more aggressive hepatic lesions. Another study on 158 HIV-HCV coinfected patients showed a significant association between the presence of GBV-C RNA (36%) and lower severity of HCV-related liver disease[3].

Despite our small group of patients, we observed that patients with active GBV-C replication (RNA) presented a lower degree of histological lesions. Further studies with a larger number of biopsied HIV-HCV coinfected patients are needed in order to provide more conclusive results regarding the effect of GBV-C on disease progression among HIV-HCV coinfected patients.

TABLE 2

Staging and necroinflammatory activity of chronic hepatitis and non-specific reactive hepatitis in co-infected patients with HIV-HCV compared with HIV-HCV-GBV-C RNA/ E2 patients.

Histopathological (n=16)	HIV-HCV (n=10)	HIV-HCV-GBV-C RNA (n=3)	HIV-HCV-GBV-C E2 (n=3)
Staging			
0	4	0	1
1	5	2	2
2	1	1	0
3	0	0	0
4	0	0	0
Peri-portal activity			
0	6	1	1
1	0	0	0
2	2	2	2
3	2	0	0
4	0	0	0
Parenchimatous activity			
0	2	0	1
1	3	3	0
2	4	0	2
3	1	0	0
4	0	0	0

HIV: human immunodeficiency virus, HCV: hepatitis C virus, GBV-C: GB virus C, RNA: ribonucleic acid.

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