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HCV/HTLV Coinfection: Does HTLV-1 Interfere in the Natural History of HCV-Related Diseases?

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Hepatitis C virus (HCV) and human T-lymphotropic virus type 1 (HTLV-1) coinfection occurs in many regions. However, few studies have focused on the natural history of HCV-induced liver disease in coinfected patients. To describe the clinical, epidemiological, and histopathological aspects of HTLV-1/HCV coinfection in Brazil. A cross-sectional study with 23 patients coinfected with HCV/HTLV. The control groups consisted of 21 patients monoinfected with HCV and 20 patients monoinfected with HTLV-1. The cytokine profiles (Th1 and Th2 cell responses), clinical, laboratory features, and histopathological aspects were examined. The control group for cytokine analysis validation consisted of patients monoinfected with HTLV, and a fourth group consisted of healthy blood donors. No anthropometric differences present between the three infected groups. We observed higher serum concentrations of IFN-γ in patients coinfected with HCV/HTLV-1 than those in HCV monoinfected patients. The HCV/HTLV-1 coinfected group also exhibited a higher degree of liver steatosis than the HCV monoinfected patients. Results suggest that HCV/HTLV-1 coinfection may result in a different pattern of HCV infection due to the immunologic disorders likely associated with HTLV-1, but there is no clear evidence of the HTLV role in the natural history of HCV infection. ***J. Med. Virol. 88:1967–1972, 2016***.

KEY WORDS: cytokines; epidemiology; hepatitis C virus; T-lymphotropic virus type 1; HCV

INTRODUCTION

Chronic hepatitis C virus (HCV) infection affects more than 170 million people worldwide [Poynard et al., 2003]. Approximately, 20% of HCV carriers develop cirrhosis, and of these, 3–8% progress to hepatocellular carcinoma (HCC) [Seeff, 1997]. Several host-related risk factors are associated with more severe disease progression, such as gender, metabolic syndrome, age, and race. In addition, virological variables are also related to the severity of liver disease, including the HCV genotype and viral load [Poynard et al., 1997].

In addition, coinfection has also emerged as an important factor in morbidity related to severe progression of liver fibrosis. The deleterious effects of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) on fibrosis progression have been well documented [Sulkowski et al., 2000]. In contrast, the impact of HCV and human T-lymphotropic virus type 1 (HTLV-1) coinfection on the natural history of HCV is largely unknown. HCV/HTLV-1 coinfection has been described in some geographic areas, likely due to overlapping mechanisms of transmission. Because HTLV-1 causes immunological disorders in humans, it is postulated that HCV/HTLV-1 coinfection may increase the rate of disease progression and produce

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an increased risk of HCC and a poorer response to antiviral therapy [Tachibana et al., 1988; Stuver et al., 1996; Kishihara et al., 2001].

HTLV-1 is implicated in immunoproliferative disorders, such as adult T-cell leukemia/lymphoma; nevertheless, no studies have reported the relationship between liver fibrosis progression and hepatic carcinogenesis in patients coinfected with HCV and HTLV-I [Takatsuki et al., 1977; Poiesz et al., 1980].

African-Brazilians comprise more than 80% of the population of Salvador, the capital city of the state of Bahia. As expected, a high prevalence of HTLV-1 infection has been reported in this area. In addition, the HCV prevalence is 1.5% of the population [Okuda, 1992; Bittencourt, 2006]. Therefore, this region provides a unique opportunity to study the interaction between HTLV-1 and HCV infection, which is the main objective of this study.

MATERIALS AND METHODS

From March 2009 to December 2013, a cross-sectional study was performed on 44 patients selected from a referral center for liver disease of the Federal University Hospital of Bahia. This sample included 23 patients who were coinfected with HCV and HTLV-1 (group 1) and 21 patients who were infected with HCV alone (group 2). These two groups were matched by age (±5 years) and gender. To obtain control subjects for the validation of the serum cytokine tests, we also selected 20 patients infected with HTLV-I alone who did not manifest the disease (group 3). In addition, we included a fourth group composed of healthy volunteer blood donors to validate the immunological tests (group 4).

The subjects in group 3 were selected from the database of the Immunopathology Department at the Pharmacy School of the Federal University of Bahia, and the subjects in group 4 were selected from the database of the Hospital Blood Transfusion Center.

The assessment consisted of a clinical and epidemiological questionnaire as well as clinical and laboratory analyses (biochemical liver tests and autoantibody and cytokine assay profiles).

For patients who had previously undergone a liver biopsy, a histopathological review was performed by a single experienced liver pathologist who was not aware of the serologic status of the patient.

The immunologic profile included the serum concentrations of Type-1 T helper (Th1) cell cytokines (interferon [IFN]-γ and interleukin [IL]-2) for all of the 44 HCV patients participating in the study (groups 1 and 2). The Human Basic Kit, Flow Cytomox-BMS8420FF (Bioscience and Bender Medsystems, Wien, Austria), was used. For external validation, the cytokine results were compared with the results of groups 3 and 4.

The Type-2 T helper (Th2) cell profile (IL-4, IL-5, and IL-6) was assessed in 10 patients from group 1, and eight patients from group 2 who were randomly selected. To evaluate the cytokine levels, we used the Human Basic Kit, Flow Cytomox-BMS8420FF (Bioscience, Miami, FL).

The autoimmunity profiles of groups 1 and 2 were investigated by determining the levels of antinuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA), anti-mitochondrial antibodies (AMA), anti-TPO antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), rheumatoid factor, cryoglobulins, anti-SSA antibodies, anti-SSB antibodies, haptoglobin, anti-liver/kidney microsome antibodies (anti-LKM-I), anti-beta-2-glycoprotein antibodies (A, G, M), anti-cardiolipin IgG antibodies, IgA, and IgM (Orgentec, Mainz, Germany).

The presence of cryoglobulinemia was determined by the cryoprecipitation of samples in tubes for seven days at 4°C. The cryoprecipitates obtained were dissolved via incubation at 37°C for 30 min to detect the presence of cryoglobulins. Indirect immunofluorescence of HEp-2 cells was performed to investigate anti-nuclear antibodies. Anti-smooth-muscle, anti-mitochondrial, and anti-LKM1 antibodies were investigated by indirect immunofluorescence using histological sections of rat kidney, liver, and stomach.

The IgA, IgG, and IgM isotypes of anti-beta-2-glycoprotein I and anti-cardiolipin were measured by indirect ELISAs using commercial kits (Orgentec). The aminotransferase levels were determined in an automated dry-chemistry biochemical analyzer (Johnson and Johnson, Brunswick, NJ), and the ferritin levels were determined with a chemiluminescence immunoassay (Access two, Beckman-Coulter, VI).

The HCV viral loads in the group of patients coinfected with HCV and HTLV-1 and in the group of patients infected with HCV alone were evaluated using the COBAS TaqMan® HCV Quantitative Test. The results are expressed as IU/ml.

Statistical Analysis

The data were processed with Version 16.0 of the Statistical Package for the Social Sciences program (SPSS Inc., Chicago, IL, Release 16.0.2, 2008). Categorical variables are presented as absolute and relative frequencies; continuous variables are presented as measures of central tendency and dispersion (mean ± standard deviation). The normality of the distribution was assessed with the Kolmogorov–Smirnov test (K–S test). Continuous variables were compared using a *t*-test for independent samples. The relationship between categorical variables was determined by Pearson's chi-squared test (χ^2) and/or Fisher's exact test. The analyses were bilateral (two-tailed), and a value of $P \leq 0.05$ was considered statistically significant. The program Prism® Version 5.03 (GraphPad) was used for the analysis of cytokines. Pearson's and d'Agostino's tests were used to analyze continuous variables. The means and medians were evaluated with Student's unpaired

t-test and the Mann–Whitney nonparametric test, respectively. The medians from three or more groups were compared with the Kruskal–Wallis test, and correlation analysis was performed with Spearman's test.

RESULTS

The anthropometric assessment of patients showed that groups 1 (HCV/HTLV coinfected patients) and 2 (HCV monoinfected patients) had similar characteristics. The average ages of patients in groups 1 and 2 were 48.1 ± 6.0 and 49.3 ± 9.7 years (Table I), respectively.

Regarding the risk factors for parenterally transmitted viruses, patients in group 1 were more likely to have been intravenous drug users (IVDU) and/or have used inhaled illegal drugs than patients in group 2 (34.8% vs. 14.3%). In contrast, patients in group 2 reported a higher frequency of having used intravenous vitamin complexes and non-disposable glass syringes (85% vs. 77.3%). However, this variable was not statistically significant (Table II).

Concerning the symptoms, among the patients in group 1, 43.5% reported fatigue vs. 27.8% in group 2 ($P = 0.35$). Arthralgia was reported by 43.5% of the patients in group 1 and by 33% of patients in group 2 ($P = 0.54$). The laboratory analysis showed higher mean transaminase levels in group 1. The AST level was 71.0 ± 63.0 IU in group 1 versus 62.6 ± 41.7 IU in group 2 ($P = 0.64$).

The mean ALT level in group 1 was 67.5 ± 92.2 IU versus 64.6 ± 58.8 IU in group 2, but the difference was not statistically significant. All of the other laboratory parameters assessed were similar for both groups. The only difference was found in the assessment of total proteins; the average level of globulins in group one was 3.6 ± 0.7 versus 3.0 ± 0.6 in group 2 ($P = 0.01$) (Table III).

Genotype 1 prevailed in both groups, accounting for 78.2% of the patients (18/23) in group 1 and 90.4% of the patients in group 2 (19/21). Genotypes 2 and 3 were present in 4.3% of the patients in group 1 and no patient from group 2. The HCV viral load was higher in group 1 than in group 2.

Liver biopsies were reviewed in 16 patients from group 1 and in 19 patients from group 2. We found no differences regarding the necroinflammatory activity, fibrosis stage, and iron overload between the two groups; steatosis was present in 87% of the patients in group 1 versus 42.9% in group 2 ($P = 0.01$) (Table IV).

It was observed that 81.3% of the patients in group 1 and 63.2% in group 2 had mild or moderate stages

TABLE I. Demographic Characteristics of 21 Monoinfected HCV Patients and 23 Patients Coinfected with HCV and HTLV-1

	Coinfected HCV + HTLV1		Monoinfected HCV		
Variables	N	Mean ± SD	N	Mean ± SD	*P*-value
Age (years)	23	48.1 ± 6.0	21	49.3 ± 9.7	0.63
Weight (kg)	20	67.5 ± 15.6	16	69.5 ± 12.1	0.69
Height (cm)	19	165.2 ± 11.0	15	163.5 ± 11.5	0.67
Waist circumference (cm)	7	87.1 ± 7.6	6	95.1 ± 10.0	0.13
BMI (kg/m^2)	19	24.3 ± 4.7	16	26.3 ± 4.4	0.22

BMI, body mass index; N, number of patients evaluated; SD, standard deviation.

TABLE II. Comparison Between the Clinical Variables of 23 Patients Coinfected with HCV and HTLV-1 and 21 Patients Monoinfected With HCV

	Coinfected HCV + HTLV1	Monoinfected HCV	
Variables	N (%)	N (%)	X^2
Vaccinated against hepatitis B (Yes)	13 (59.1)	15 (71.4)	0.67
Blood or blood-product transfusion (Yes)	9 (40.9)	7 (33.3)	0.61
Tattoo (Yes)	8 (36.4)	2 (10.0)	0.07
Piercing (No)	22 (100.0)	19 (100.0)	–
Acupuncture (No)	22 (100.0)	18 (100.0)	–
Inhaled drugs (Yes)	9 (40.9)	4 (19.0)	0.19
Sharing of needles (Yes)	5 (22.7)	2 (10.5)	0.42
Prior use of glass syringes (Yes)	17 (77.3)	17 (85.0)	0.75
Prior use of intravenous vitamins (Yes)	8 (36.4)	12 (63.2)	0.49
Sexually transmitted disease (Yes)	8 (36.4)	7 (35.0)	0.93
History of surgery (Yes)	15 (68.2)	13 (61.9)	0.67
Dialysis (No)	21 (100.0)	21 (100.0)	–
Sharing of razor or toothbrush (No)	19 (86.4)	21 (100.0)	0.23

N, number of patients evaluated; X^2 = Fisher's exact test.

TABLE III. Biochemical Characteristics of 21 Patients Monoinfected With HCV and 23 Patients Coinfected With HCV and HTLV-1

	Coinfected HCV + HTLV1		Monoinfected HCV		
Variables	N	Mean ± SD	N	Mean ± SD	*P*-value
AST-Aspartate aminotransferase (mg/dl)	21	71.0 ± 63.0	17	62.6 ± 41.7	0.64
ALT-Alanine aminotransferase (mg/dl)	21	67.5 ± 92.3	17	64.6 ± 58.8	0.91
GGT-Gamma-transpeptidase (mg/dl)	20	137.8 ± 145.9	16	68.2 ± 64.9	0.67
Total bilirubin (units)	21	0.8 ± 0.5	16	0.8 ± 0.4	0.88
Direct bilirubin (units)	21	0.3 ± 0.2	16	0.3 ± 0.3	0.35
Serum iron (units)	20	120.1 ± 46.8	11	102.2 ± 33.7	0.27
Total protein (units)	19	7.8 ± 0.7	16	7.1 ± 0.6	< 0.01
Albumin (units)	19	4.1 ± 0.4	16	4.1 ± 0.5	0.98
Globulin (units)	19	3.6 ± 0.7	16	3.0 ± 0.6	< 0.01
Alkaline phosphatase (units)	19	80.2 ± 23.1	13	76.1 ± 22.8	0.62
Platelets (units)	19	210,052.6 ± 59,625.2	19	185,421.1 ± 67,559.3	0.24
Prothrombin time (%)	6	93.0 ± 14.4	2	87.6 ± 17.5	0.68
Triiodothyronine – T3 (units)	20	146.5 ± 25.9	11	152.3 ± 34.3	0.60
Free thyroxine – T4 (units)	21	0.9 ± 0.2	15	0.9 ± 0.1	0.91
TSH-Thyroid-stimulating hormone (units)	21	2.3 ± 2.0	14	1.5 ± 0.6	0.10
Haptoglobin (units)	21	117.5 ± 79.4	17	88.4 ± 80.4	0.32

N, number of patients evaluated; SD, standard deviation; *P*-value = Student's T test.

TABLE IV. Comparison of Histological Variables Between 23 Patients Coinfected with HCV and HTLV-1 and 21 Patients Monoinfected With HCV.Table IV Comparison of Histological Variables Between 23 Patients Coinfected With HCV and HTLV-1 and 21 Patients Monoinfected With HCV

	Coinfected HCV + HTLV1	Monoinfected HCV	
Variables	N (%)	N (%)	X^2
Iron overload in liver biopsy (No)	12 (85.7)	13 (76.5)	0.66
Steatosis in liver biopsy (No)	3 (13.0)	12 (57.1)	<0.01
Inflammatory activity in the biopsy (Mild/Moderate/Severe) (Yes)	16 (69.6)	18 (85.7)	0.29

X^2 = Fisher's exact test.

of fibrosis (F1-F2) according to their METAVIR score. More advanced fibrosis stages (F3-F4) were found in 18.8% of the patients in group 1 and in 36.8% in group 2. Among these patients, cirrhosis was diagnosed in only one patient from group 1 (4.3%) and in four patients from group 2 (19%).

The assessment of non-organ-specific autoantibodies was similar in both groups. In addition, the serum levels of IFN-γ in groups 4 and 2 were similar, with a median of 1.02 pg/ml in group 4 versus 1.42 pg/ml in group 2 ($P > 0.05$). In group 3 (HTLV monoinfected), we observed higher levels of IFN-γ than in the other groups (6.22 pg/ml) ($P < 0.001$). The level of this cytokine in group 2 was 2.85 pg/ml, which was higher than that found in groups 2 and 4 but less than that in group 3 (monoinfected by HTLV-1). The IFN-γ levels in groups 1 and 3 were not significantly different ($P > 0.05$).

The patients in group 1 had higher serum levels of IFN-γ than those in group 2 ($P < 0.01$). The comparison of IFN-γ levels among the four groups revealed a difference in the immunologic profile ($P < 0.0001$), mainly between groups 1 and 2 ($P < 0.01$) (Fig. 1).

The median levels of this cytokine were 6.37 pg/ml in group 1 and 8.71 pg/ml in group 3, which were significantly different ($P < 0.05$) but not statistically significant compared to the other groups (Fig. 2).

No differences in the Th2 serum cytokine responses were found between groups 1 and 2 (IL-04, IL-05, and IL-6).

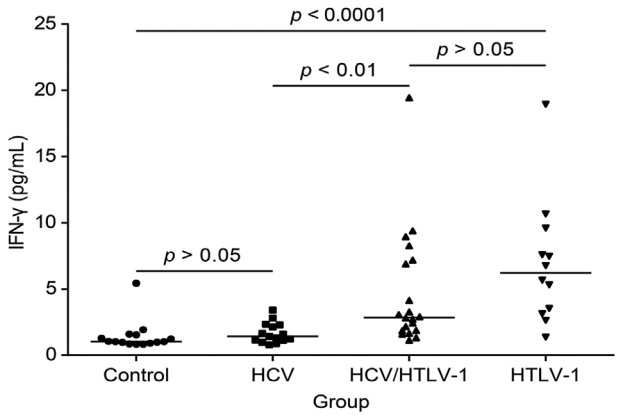

Fig. 1. Serum levels of IFN-γ in healthy patients (control), patients chronically monoinfected with HCV (HCV), patients coinfected with HCV and HTLV-1 (HCV/HTLV-1), and patients monoinfected with HTLV-1 (HTLV-1). The medians (control = 1.02 pg/ml, HCV = 1.42 pg/ml, HCV/HTLV–1 = 2.85 pg/ml, and HTLV–1 = 6.22 pg/ml) are represented by horizontal bars.

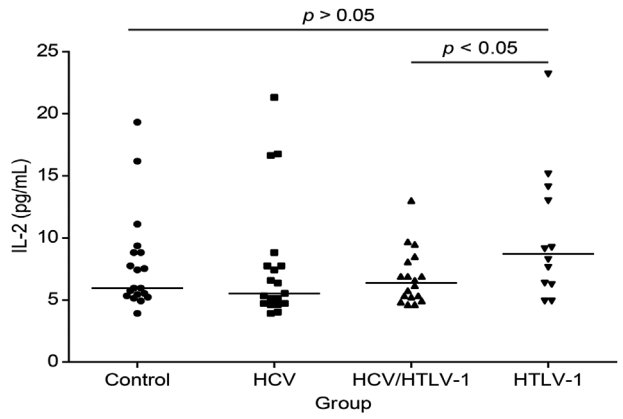

Fig. 2. Serum levels of IL-2 in healthy patients (control), patients chronically monoinfected with HCV (HCV), patients coinfected with HCV and HTLV-1 (HCV/HTLV-1), and patients monoinfected with HTLV-1 (HTLV-1). The medians (control = 5.96 pg/ml, HCV = 5.54 pg/ml, HCV/HTLV–1 = 6.37 pg/ml, and HTLV–1 = 8.71 pg/ml) are represented by horizontal bars.

DISCUSSION

No particular risk factor could be identified for HTLV/HCV coinfected patients in Brazil. The use of intravenous drugs did not seem to be an important risk factor for these parenterally transmitted viruses; however, the use of unsafe injections for licit medicines in the past was likely responsible for most cases of HCV transmission in many parts of the country during the 1970s and 80s [Paraná et al., 1999; Cardoso et al., 2009; Milagres et al., 2009].

Concerning the virological variables, the HCV viral load was similar between both infected groups (1 and 2), although a slightly higher viral load was observed in the coinfected group ($P > 0.05$). Therefore, the presumed immunologic depression caused by HTLV does not appear to affect HCV replication.

The liver fibrosis stage and necroinflammatory score were similar among groups 1 and 2. In both groups, we found a predominance of mild to intermediate stage liver fibrosis. Remarkably, steatosis was more frequent in group 1 ($P < 0.05$) regardless of the anthropometric variables. In these cases of coinfection, the pathogenesis of steatosis is difficult to explain. Clearly, it is not associated with genotype 3 because only a few cases of this genotype were present in both of the HCV-infected groups. Metabolic syndrome does not explain this finding either. It is possible that a putative effect of HTLV on the immunologic cytokine profile could provoke steatosis by an unknown mechanism, but this hypothesis needs to be validated.

It was difficult to compare our immunological analysis with those from other authors because few papers have been published on this topic. Nevertheless, other authors suggest that hepatocyte damage caused by the hepatitis C virus is known to be triggered by the innate immune and Th1 cell responses. The progression from a milder disease to an advanced liver disease is related to the balance in the serum levels of the Th1 and Th2 responses [Cacciarreli et al., 1996; Poynard et al., 1997; Murata et al., 2002]. HTLV-1 infection reinforces the predominant Th1 response. This mechanism would, therefore, have two functions: (1) to restrict viral replication and (2) to cause more immune-mediated liver damage, but this theoretical mechanism is not supported by our study.

IL-2 is produced by Th1 cells. It induces activated T- and B-cells proliferation, which stimulates cytotoxicity resulting in the death of the infected cell [Carvalho et al., 2001; Porto et al., 2002]. However, we hypothesize that in coinfected individuals, there is a balance in the immunologic profile of the Th1 and Th2 responses that likely decreases hepatocyte injury and, consequently, fibrosis progression.

The B-cell proliferation induced by HTLV could explain the higher globulin level observed in coinfected patients. It has been well documented that HTLV induces polyclonal proliferation [Tygstrup, 1990]. Another study conducted in a Brazilian center found a similar result [Cardoso et al., 2009].

In our study, when the serum IFN-γ profile was analyzed, we observed that patients in groups 1 and 3, both formed by HTLV carriers, had higher levels of this cytokine than patients in groups 2 and 4, who were not infected with HTLV. Therefore, HTLV could be responsible for our findings.

In addition, during the evaluation of the serum levels of IL-2, we observed that patients in group 1 had lower serum levels than those in the other groups (groups 2, 3, and 4). Therefore, the Th1 response induced by HTLV-1 infection seems to be less intense when it is associated with HCV infection. This information reinforces the balance between the Th2 immunological pattern induced by HCV and the Th1 pattern induced by HTLV as a method of modulation of the pathogenesis of HCV.

Other studies that have evaluated specific aspects of HCV/HTLV-1 coinfection have suggested that patients who did not respond to antiviral treatment had a higher probability of developing HCC [Asou et al., 1986; Kishihara et al., 2001]. Therefore, carcinogenesis in HTLV/HCV coinfected patients may be a valid concern.

The poorer response to antiviral treatment and disease accompanied by a milder and more prolonged progression could be related to the development of immunoproliferative disorders in coinfected patients, which has been reported in some studies and reinforces the immunomodulation and immunostimulation role of HTLV [Cacciarreli et al., 1996; Carvalho et al., 2001].

In summary, HCV/HTLV coinfection is an intriguing research field, and studies can reveal more information about the interplay of HCV and the host immune system.

Our study suggests that HCV/HTLV coinfection could result in a slower progression of liver disease.

Nevertheless, further studies are needed to characterize this putative slow progression of the disease in coinfected patients as well as the higher likelihood of immunoproliferative disease incidence due to proliferative B-cell stimulation. The risk for HCC and the poorer response to antiviral therapy in HCV/HTLV coinfected patients are also concerns that are not clearly defined.

Although this paper was not designed to determine the antiviral treatment response or natural history of HCV/HTLV-I coinfection, our findings do not provide evidence of a more aggressive disease among coinfected patients. Because most of the patients were African-Brazilians, we expected to find more severe disease among this group because this result has been described by other authors [Sterling et al., 2004].

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