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Hepatitis C Virus and Human T-cell Lymphotropic Virus Co-infection: A Epidemiological, Clinical and Laboratory Analysis

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Abstract

Background: Hepatitis C is the principal cause of chronic liver disease. In Brazil, statistics show that 2.5 million individuals are infected with HTLV-I, representing in absolute terms the largest number of individuals in the world infected by this virus. Data from the literature suggest that HTLV infection exerts a detrimental effect on cell immune response, exacerbates the progression of hepatitis C and negatively affects treatment response in HCV co-infected patients. The objective of this study is to describe epidemiological, clinical and laboratory characteristics of a group of patients co-infected with both hepatitis C virus (HCV) and human T-cell lymphotropic virus (HTLV) and another with HCV monoinfected patients.

Methods: Anti-HTLV I/II ELISA was performed in HCV-RNA-positive patients of Gaffrée and Guinle Teaching Hospital (HUGG) Outpatient Hepatology Department, followed by Western blot and/or PCR for confirmation. Groups of co and monoinfected individuals were submitted to epidemiological analysis and to clinical and laboratory evaluation: hematological, biochemical, HCV genotype, HCV viral load and liver fibrosis score. Statistical analysis was performed using the SPSS statistical software program, version 16.0. For comparison between the groups of study, p-values and their respective 95% confidence intervals were calculated using the Fisher´s exact test or Mann-Whitney´s test.

Results: The seroprevalence of HCV/HTLV co-infection was 7.4% (95%CI: 4.5-11.6%; n=16/215). A control group of 83 HCV monoinfected individuals was constituted. Most participants in the two groups were male, white and symptomless. Intravenous drug abuse was more common in co-infected than in monoinfected individuals (33.3% versus 10.8%; p=0.037). Advanced liver fibrosis (Metavir fibrosis score ≥ 2) was more common in monoinfected patients (59.3% versus 10%; p=0.005), as were elevated levels of ALT (p=0.030), AST (p=0.015) and GGT (p=0.055).

Conclusions: Contrary to the literature, this study found no clinical, biochemical or histological differences between HCV/HTLV coinfected patients compared to HCV monoinfected patients.

Keywords: Hepatitis C; HTLV; Co-infection; Epidemiological; Clinical; Laboratory analysis; Brazil

Introduction

Hepatitis C is the principal cause of chronic liver disease [1]. Over 180 million individuals worldwide are infected with the hepatitis C virus (HCV), the chronicity rate of this condition being over 70% [2]. After 20-30 years of chronic infection, 20% of individuals will develop cirrhosis, with an annual risk of developing hepatocellular carcinoma (HCC) of 3-7.8% [1].

Following a latency period of 20-30 years, around 5% of patients infected with HTLV type I (HTLV-I) may develop two severe diseases: adult T-cell leukemia/lymphoma (ATL) and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM-TSP) [3,4]. In Brazil, statistics show that 2.5 million individuals are infected with HTLV-I, representing in absolute terms the largest number of individuals in the world infected by this virus [3].

In a recent past, blood transfusions used to be an important form of HCV and HTLV transmission, as nowadays is the sharing of needles by intravenous drug abusers (IVDA). This epidemiological similarity facilitates dual transmission and coinfection by these viruses. Data from the literature suggest that HTLV infection exerts a detrimental effect on cell immune response, exacerbates the progression of hepatitis C and negatively affects treatment response in HCV coinfected patients [1,5,6].

The significant morbidity associated with these conditions, the risk of coexistence of these two viruses, the reports of an exacerbated progression of hepatitis C in the presence of HTLV co-infection and the scarcity of reports in the literature on this subject, led to the design of this protocol, the objective of which was to analyze the behavior of the epidemiological, clinical and laboratory variables associated with this co-infection.

Methods

This cross-sectional descriptive study was carried out at the Hepatology Department of the Gaffrée and Guinle Hospital, a University Teaching Hospital, which is part of the public health network of the city of Rio de Janeiro, Brazil and a referral centre for the treatment of hepatitis C. Between April 2007 and July 2008, male and female HCV-RNA-positive patients were systematically screened for HTLV co-infection using an anti-HTLV I/II immunoenzymatic assay (ELISA). Positives ELISA individuals were confirmed by Western Blot (WB) whenever was possible. HCV/HTLV co-infected individuals constituted the case group. The remaining HCV-RNA-positive patients who tested negative for HTLV were divided into two strata according to gender and then randomly selected to constitute the control group.

Inclusion criteria comprised:

1) Over 18 years of age

2) Detectable HCV-RNA and the presence of anti-HTLV antibodies (case group)

3) Detectable HCV-RNA and negative anti-HTLV I/II (control group)

Exclusion criteria consisted of:

1) Individuals with current or past history of chronic alcohol abuse (over 40 grams of ethanol/day for a period of at least 180 consecutive days)

2) Evidence of other liver disease (metabolic, autoimmune, neoplasic)

3) Positive anti-HIV test (ELISA)

4) Positive HBsAg

5) History of interferon treatment for hepatitis C prior to inclusion in the study

Eligible individuals were submitted to an epidemiological analysis, anamnesis and physical examination. Results of complete blood count, prothrombin time, ALT (alanine aminotransferase), AST (aspartate aminotransferase), total protein and albumin levels, total bilirubin and fractions, Gamma-glutamyl-transferase (GGT), fasting glucose level and total cholesterol performed within six months of the inclusion date of the individuals in the study were recorded, as well as HCV genotype, HCV viral load and liver fibrosis score.

Confirmation of HCV infection

HCV-RNA investigation, determination of HCV viral load and genotyping were performed using the following tests, respectively: Amplicor® HCV Test, version 2.0, for which the detection limit is 50 IU/mL; Cobas® Amplicor HCV Monitor Test, version 2.0, Roche, for which the detection range is 600-700 000 IU/mL, and Versant HCV genotype Assay / LiPA Bayer (region 5'NC of HCV).

Confirmation of HTLV infection

Investigation of anti-HTLV I/II antibodies by ELISA was performed using the Abbott I/II test, while the HTLV Blot 2.4 kit (Genelabs Diagnostics) was used for Western Blot.

Liver biopsy

Fragments of the liver were analyzed at the Anatomopathology Department of the Gaffrée and Guinle Teaching Hospital using the Metavir score for the classification of liver fibrosis [7].

Statistical analysis

Statistical analysis was performed using the SPSS statistical software program, version 16.0. For comparison between the groups of study, p-values and their respective 95% confidence intervals were calculated using the Fisher´s exact test or Mann-Whitney´s test.

Ethical approval

The protocol of this study was submitted to the Ethics Committee of the Gaffrée and Guinle Teaching Hospital and approved under reference number 05/2008. The subjects enrolled gave informed consent to this work.

Results

Among 215 individuals HCV-RNA positive screened, 16 patients were also found to be HTLV co-infected, resulting in a seroprevalence rate of HCV/HTLV co-infection of 7.4% (95%CI: 4.5-11.6%). One co-infected patient was excluded because he was also HIV-positive; therefore, the group of cases was finally composed of 15 HCV/HTLV co-infected patients. The presence of HTLV infection was confirmed by Western Blot (WB) in 13 of the co-infected patients, 9 (60%) of whom tested positive for HTLV-I and 4 (26.7%) for HTLV-II. The two remaining patients (13.3%) who tested positive for HTLV I/II were not submitted to confirmation of diagnosis due to a failure in the recruitment process. The control group was constituted by 83 patients with hepatitis C who tested negative for HTLV I/II. Due to the small

sample size of the case group (n=15), it was decided not to stratify patients according to HTLV type in the statistical analysis.

There were no statistically significant differences in demographic characteristics (gender, age and ethnic origins) between the two groups of this study (Table 1). The principal risk factors for the acquisition of the viruses in both groups was a history of a prior surgical procedure, blood transfusion and intravenous drug abusers (IVDA), with a statistically significant difference between the groups only with respect to the last factor (p=0.037). Three of five co-infected patients with positive history of IVDA were infected with type I, and in the remaining two, serological confirmation was not obtained. Only one patient reported a history of sexual contact with an HCV-infected partner and he belonged to the co-infected group. There wasn't history of acquisition of HTLV from an infected mother. Table 2 summarizes the epidemiological data.

No statistically significant difference was found between the two groups with respect to any of the clinical variables analyzed. The majority of the individuals included in this study were asymptomatic, clinical findings of hepatic disease being detected in only three (20%) of the co-infected patients and 11 (13.3%) of the controls. Arthralgia was the most common symptom, both in the case group (13.3%) and in the control (3.6%). Neither neurological abnormalities nor sphincterial disorders were found in any of the patients in this study.

Genotype 1 was predominant in both groups (85.7% versus 89.9%) followed by genotype 3 (14.3% versus 10.1%). The only one patient infected by genotype 2 belonged to the control group. No statistically significant difference was found between the two groups in this respect (p=0.643) (Table 3).

In the majority of patients, HCV viral load was >600 000 IU/mL, 70% of the coinfected patients and 64.4% of the controls being in this range. There was no statistically significant difference between the two groups (p=1.0) (Table 3).

Data on liver biopsies were available for 65.1% of the controls and 66.7% of the cases. The majority of the coinfected subjects were found to have stage 1 fibrosis (70%), and no cases of grade 3 or 4 were found. In the control group, grade 2 was more common (31.5%), while 9.3% of patients had grade 4 fibrosis. Fibrosis grade < 2 was found to be predominant in the co-infected patients (90%), while in the control group grade ≥ 2 predominated (59.3%) (p=0.005) (Table 3).

Higher levels of AST, ALT and Gamma-GT were present in control group, with statistically significant difference in all enzymes (Table 4). The majority of individuals of both groups has shown ratio among patients' value (P) and upper limit of normal values (N) of ALT (ALT P/N) abnormal (>1): 81.9% of monoinfected and 66.7% of co-infected (p=0.181). On the other hand, only in monoinfected group the majority of individuals (68.3% versus 35.7% of co-infected; p=0.033) has shown Gamma-GT P/N ratio abnormal (>1). Comparison between the two groups with respect to the other laboratory tests showed no statistically significant differences.

Discussion

Few studies have been carried out on HCV/HTLV co-infection, the majority being confined to areas of high endemicity for both infections in Japan. These papers have indicated an increase in HTLV seroprevalence in HCV-positive patients, exacerbated clinical progression of liver disease and poorer response to interferon treatment, as well as a greater likelihood that co-infected individuals will go on to develop hepatocellular carcinoma (HCC) [1,6,8-11].

In the present study, the seroprevalence rate of HCV/HTLV co-infection was 7.4% (95%CI: 4.5-11.6%), which is within the range reported in the reference literature (1.4-37.9%) [1,8,10,12-14]. The prevalence of HTLV infection was significantly higher in this study's HCV infected individuals when compared to the one estimated in blood donors candidates of the same geographical region (city of Rio de Janeiro – 0.47%) [3]. This finding emphasizes the association between these viruses [8,9].

Contrary to the usual pattern, the results of the present study revealed a predominance of males among the co-infected individuals despite the fact that HTLV is usually more prevalent

Table 1 Demographic variables (gender, age and ethnic origin) of patients with hepatitis C according to the study group: monoinfected or coinfected.

Demographics Variables	Group				Fisher's Exact Test
	Monoinfected HTLV negative negative) (n=83)		Coinfected HCV/HTLV (n=15)		
	n	(%)	n	(%)	p-value
Gender					
Male	54	(65.1)	10	(66.7)	1.000
Female	29	(34.9)	5	(33.3)	
Age					
18 to 49 years	35	(42.2)	7	(46.7)	0.782
50 to 80 years	48	(57.8)	8	(53.3)	
Ethnic origin					
White	65	(78.3)	9	(60.0)	0.189
Black	18	(21.7)	6	(40.0)	

Table 2 Analysis of the epidemiological variables according to the presence of coinfection.

Epidemiological Variables	Group				Fisher's Exact Test
	Monoinfected (HTLV negative) (n=83)		Coinfected HCV/HTLV (n=15)		
	n	(%)	n	(%)	p-value
Blood Transfusion					
No	39	(47.0)	8	(53.3)	0.781
Yes	44	(53.0)	7	(46.7)	
IVDA [a]					
No	74	(89.2)	10	(66.7)	0.037
Yes	9	(10.8)	5	(33.3)	
Tattoo					
No	78	(94.0)	14	(93.3)	1000
Yes	5	(6.0)	1	(6.7)	
Acupuncture					
No	79	(95.2)	12	(80.0)	0.070
Yes	4	(4.8)	3	(20.0)	
Prior Surgical Procedure					
No	32	(38.6)	4	(26.7)	0.562
Yes	51	(61.4)	11	(73.3)	
STD [b]					
No	76	(91.6)	12	(80.0)	0.179
Yes	7	(8.4)	3	(20.0)	
Homosexuality					
No	80	(96.4)	14	(93.3)	0.491
Yes	3	(3.6)	1	(6.7)	

a - IVDA = Intravenous Drug Abuse. b- STD = Sexually Transmitted Diseases.

Table 3 HCV infection's variables (PCR, genotype and hepatic fibrosis – Metavir Score) according to the study group: monoinfected or coinfected.

HCV Infection's Variables	Group				Fisher's Exact Test
	Monoinfected (HTLV negative)		Co-infected HCV/HTLV		
	n	(%)	n	(%)	p-value
PCR					
≤ 600.000 IU/mL	21	(35,6)	3	(30,0)	1,000
> 600.000 IU/mL	38	(64,4)	7	(70,0)	
Genotype					
1	71	(89,9)	12	(85,7)	0,643
3	8	(10,1)	2	(14,3)	
Hepatic Fibrosis - Metavir					
< 2	22	(40,7)	9	(90,0)	0,005
≥ 2	32	(59,3)	1	(10,0)	

among monoinfected women [14]. This distinct pattern was also found in studies conducted by Okayama et al. and Magalhães [11,8]. This pattern may be due to an HCV influence.

In Brazil, screening of HCV and HTLV infection in potential blood donors is mandatory since 1993 [15]. In this study, all but one individual related blood transfusion prior to this year. Thus, as expected, blood transfusion represented an important risk factor to acquisition of both viruses in this study. The majority of individuals with a positive history of IVDA in the present sample were infected by HTLV-I, despite reports in the literature of a greater association between this risk factor and HTLV-II infection [3]. The reduced number of individuals enrolled in this study has probably influenced this result. A history of prior surgery was the most prevalent factor found in this study and the importance

Table 4 Descriptive analysis and statistical comparison of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Gamma-GT (GGT) levels between monoinfected (HTLV-negative) and coinfected (HTLV-positive) patients.

Serum Enzymes	HTLV	Descriptive Statistic					Mann-Whitney's Test p-value
		Mean	Standard Deviation	Minimum	Median	Maximum	
AST-P [a]	Negative	63.0	50.3	12.0	47.0	358.0	0.015
	Positive	42.1	17.5	25.0	35.0	81.0	
AST-P/N [b]	Negative	1.66	1.28	0.26	1.24	8.95	0.013
	Positive	1.08	0.45	0.65	0.94	2.13	
ALT-P	Negative	76.3	48.0	10.0	65.0	247.0	0.030
	Positive	50.7	25.4	8.0	49.0	112.0	
ALT-P/N	Negative	1.88	1.21	0.20	1.64	6.18	0.046
	Positive	1.25	0.65	0.12	1.20	2.80	
GGT-P	Negative	77.0	52.0	11.0	57.5	270.0	0.055
	Positive	52.9	40.1	19.0	43.5	168.0	
GGT-P/N	Negative	1.69	1.25	0.28	1.30	6.75	0.099
	Positive	1.11	0.66	0.40	0.86	2.80	

a- P= patients' enzymes values. b- P/N ratio: ratio between patients' enzymes values (P) and the enzymes' upper limit of normal values (N).

attributed to this factor refers to the risk of contamination during surgical procedures through contaminated material, a not insignificant possibility in the precarious healthcare units of in developing countries, such as Brazil. History of sexually transmitted diseases (STD) was the fourth most common risk factor in both groups, fact that emphasizes the significance of the sexual route of transmission of HTLV and the increase in HCV sexual transmission in patients with STD [16].

In the present study, HCV co-infection did not appear to stimulate the onset of HTLV-related diseases. This fact may be linked to the low prevalence of diseases associated with this infection (HAM-TSP and ATL), to the small sample size of the group of co-infected patients (n=15) and to the fact that not exclusively patients with HTLV-I were selected.

In spite of the epidemiological and clinical similarity of all individuals of this study, the reasons for the greater proportion of patients with fibrosis grade 2 or higher in the control group remain unclear. In accordance with the reference literature, more advanced liver disease would have been expected in the co-infected group [1,6,10]. Okayama et al. suggested that a dysfunction in cytokine production by CD4+ T-lymphocytes and the reduction in cell immunity caused by HTLV would lead to an inability to eliminate HCV and contribute towards the development of cirrhosis followed by HCC [11]. Takeoka et al. suggested that the presence of antibodies against region 197 of the gp46 protein of the HTLV-I envelope (anti-gp46-197) in co-infected patients would be related to the severity of the HCV-associated liver disease [13]. The production of anti-gp46-197 would be triggered by HCV infection in patients previously infected by HTLV-I, which contrary to Okayama's impressions would increase immune system activity, thereby leading to exacerbation of liver disease [11]. Moreover, at least four individuals in the case group were infected with HTLV-II and the effect of this type of HTLV on liver disease has yet to be established.

AST, ALT and Gamma-GT levels were all higher in HCV-monoinfected patients in this study, just like in papers conducted by Milagres [17] and Kishihara et al. [6]. The immune deficiency, likely due to HTLV infection, may result in a lesser degree of local inflammation, less hepatocyte destruction and a lower release of these enzymes without, however, impeding the direct cytopathic effect of HCV and the development of fibrosis [18-20]. The similarity of the findings of these three studies may suggest a particular trend in the behavior of liver enzymes in HCV/HTLV co-infected individuals. Nevertheless, new studies with larger sample sizes are required to confirm this initial impression.

Data about HTLV infection and fibrosis score not completely available should be considered as limitations of this study, as well as the reduced absolute number of co-infected individuals included (n=15). It's reasonable to believe that another study with complete data of an enlarged number of co-infected individuals will bring more confident information about this condition. Moreover, at least four individuals in the case group

were infected with HTLV-II and the effect of this type of HTLV on liver disease has yet to be established.

Conclusion

In conclusion, contrary to reports in the literature, the HCV/HTLV co-infected patients in the present study did not present more severe clinical, biochemical or histological evidence of chronic liver disease compared to the HCV monoinfected patients.

Competing Interest

There are no competing interests.

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