Hepatitis C virus and human T-lymphotropic virus coinfection: epidemiological, clinical, laboratory and histopathological features

Coinfecção vírus da hepatite C-vírus linfotrópico de células T humanas: aspectos epidemiológicos, clínicos, laboratoriais e histopatológicos

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ABSTRACT

Twenty-four hepatitis C virus patients coinfected with human T-lymphotropic virus type 1 were compared with six coinfected with HTLV-2 and 55 with HCV alone, regarding clinical, epidemiological, laboratory and histopathological data. Fischer's discriminant analysis was applied to define functions capable of differentiating between the study groups (HCV, HCV/HTLV-1 and HCV/HTLV-2). The discriminant accuracy was evaluated by cross-validation. Alcohol consumption, use of intravenous drugs or inhaled cocaine and sexual partnership with intravenous drug users were more frequent in the HCV/HTLV-2 group, whereas patients in the HCV group more often reported abdominal pain or a sexual partner with hepatitis. Coinfected patients presented higher platelet counts, but aminotransferase and gamma-glutamyl transpeptidase levels were higher among HCV-infected subjects. No significant difference between the groups was seen regarding liver histopathological findings. Through discriminant analysis, classification functions were defined, including sex, age group, intravenous drug use and sexual partner with hepatitis. Cross-validation revealed high discriminant accuracy for the HCV group.

Key-words: Hepatitis C virus. Human T-lymphotropic virus type 1. Human T-lymphotropic virus type 2. Epidemiological studies. Discriminant analysis.

RESUMO

Compararam-se 24 pacientes coinfectados pelos vírus da hepatite C/vírus linfotrópico de células T humanas do tipo 1 com 6 coinfectados por VHC/HTLV-2 e 55 infectados pelo VHC, no tocante a dados clínico-epidemiológicos, laboratoriais e histopatológicos. A análise discriminante de Fischer foi utilizada para definir funções capazes de diferenciar os grupos de estudo (VHC, VHC/HTLV-1 e VHC/HTLV-2). A acurácia discriminatória foi avaliada pelo por validação cruzada. O uso de álcool, drogas endovenosas, cocaína inalatória e a parceria sexual com UDEV foram mais freqüentes no grupo VHC/HTLV-2, enquanto queixa de dor abdominal e parceiro sexual com hepatite predominaram no grupo VHC. Os coinfectados apresentaram número maior de plaquetas, enquanto as aminotransferases e a gamaglutamiltranspeptidase foram mais altas no grupo VHC. Não houve diferença entre os grupos à análise histopatológica do fígado. Por análise discriminante definiram-se funções classificatórias, incluindo as variáveis sexo, faixa etária, uso de drogas endovenosas e parceiro sexual com hepatite, com acurácia discriminante alta para o grupo VHC.

Palavras-chaves: Vírus da hepatite C. Vírus linfotrópico de células T humanas do tipo 1. Vírus linfotrópico de células T humanas do tipo 2. Estudos epidemiológicos. Análise discriminante.

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Since hepatitis C virus (HCV) and human T-cell lymphotropic virus type 1 (HTLV-1) and type 2 (HTLV-2) share their transmission means, the occurrence of coinfections is expected in populations at higher risk of sexual or blood-borne viral acquisition. In fact, HCV/HTLV coinfections have been described as frequent among intravenous drug users (IDUs)[24], hemophiliacs[5] and individuals who report unsafe sexual practices[28].

However, the epidemiological and clinical features of HCV/HTLV coinfected individuals have been overlooked. In-depth investigation of these characteristics may reveal particularly

relevant information about the risk of viral acquisition, as well as about the pathogenetic impact of coinfection on HCV and/or HTLV-associated disease development. So far, virus-host interactions in coinfected individuals have been reported to be associated with dysfunctional cell immune response, which might impair HCV clearance and lead to accelerated progression of HCV liver disease[12 27 29].

To improve our understanding of HCV/HTLV-1 and HCV/HTLV-2 coinfections, in the present study we compared sociodemographic features, variables relating to exposure to these viral infections, clinical and laboratory abnormalities, and liver histological characteristics among three groups of patients: HCV-infected individuals and HCV infected individuals presenting coinfection with either HTLV-1 or HTLV-2.

MATERIAL AND METHODS

Patient selection. For this cross-sectional study, we selected patients who were seen at specialized outpatient services that provide counseling and follow-up care for HCV and HTLV patients at Hospital das Clinicas, School of Medicine, University of São Paulo, between January 1993 and August 2005. For the HCV/HTLV-1 and HCV/HTLV-2 groups, we enrolled adults who were diagnosed as HCV/HTLV-1 or HCV/HTLV-2 coinfected, respectively. The HCV group consisted of HCV-infected patients who presented neither HTLV-1 nor HTLV-2 infection. Patients with serological biomarkers indicating previous hepatitis B virus (HBV) infection, HIV coinfected individuals and transplant recipients were excluded.

The diagnosis of HCV infection was based on the seroreactivity of third-generation enzyme immunoassays (Murex Biotech, Baijing United Biomedical, and Embrabio, Brazil), followed by detection of HCV-RNA by means of nested RT-PCR amplification on a second plasma sample. The tests were performed in accordance with the manufacturers' instructions, or as previously described[15 16]. A serodiagnosis of HTLV infection was established whenever seroreactivity was demonstrated using the commercially available enzyme immunoassays (Organon Technika, USA; Embrabio, Brazil), and was confirmed using Western blot tests (WB 2.3; WB 2.4, Diagnostic Biotechnology, Singapore), following the manufacturers' instructions. Based on reactivity to type-specific recombinant peptides encoded by the *gag* and *env* regions of the HTLV proviral genome, the patients were discriminated as presenting either HTLV-1 or HTLV-2 infection, as recommended by the Brazilian Ministry of Health[23]. HTLV infection was further confirmed by nested PCR amplification of *tax* sequences of the proviral DNA from peripheral blood mononuclear cells (PBMC), as previously described[17 18 37].

For the histopathological evaluation, patients were eligible only if their liver biopsy specimen was considered suitable for grading and staging tissue abnormalities[8].

Study procedures. Patients were invited to take part in the survey at their regular medical follow-up visits to the outpatient clinic, between 2004 and 2006. Alternatively, those who did not attend the clinic during this period were contacted by telephone. If the patients agreed to participate, they were admitted to the study after giving their written consent. They then underwent a standardized interview with the research team, for epidemiological and clinical data to be obtained. In addition, the medical files were reviewed to collect laboratory and liver histopathological data. For analytical purposes, we used the results from hematological and liver function tests that had been performed less than 60 days before the liver biopsy procedure and before any therapeutic intervention for HCV liver disease. Throughout the data collection, confidentiality and patients' anonymity were ensured. The study protocol was approved by the institutional review board.

The variables of interest included:

- sociodemographic characteristics: gender, age, skin color, marital status, schooling and place of birth;
- habits: smoking and alcohol consumption;
- risk factors for acquisition of sexually-transmitted or blood-borne infections: history of intravenous drug use, history of inhaled cocaine use, history of blood transfusion, tattooing, acupuncture, sexual partnership with an IDU or with someone who reported hepatitis, and number of sexual partners in the year preceding admission to the study;
- clinical data: signs and symptoms of liver disease (jaundice, ascites, epistaxis, palmar erythema and spiders), malaise, abdominal pain, skin abnormalities, neurological symptoms (paresis and paresthesia), hepatomegaly or splenomegaly on clinical examination;
- results from laboratory tests: hemoglobin, hematocrit, white cell and platelet counts, serum liver enzyme concentrations (AST, ALT and GGT), alkaline phosphatase, serum bilirubin, albumin and gamma globulin concentrations and prothrombin time.

With regard to liver histopathological features, the data collection was based on a standardized review of all biopsy specimens. This was carried out by a single skilled professional, using Ishak's diagnostic criteria[19].

Statistical analysis. After setting up a database using the Microsoft Excel 2002 software, the patients in the different study groups (HCV/HTLV-1, HCV/HTLV-2 and HCV) were initially compared by means of bivariate analysis. The χ^2 test was used to compare the proportions of individuals with variables of interest among the different study groups and the Kruskal-Wallis tests was used to compare continuous variables[1 9]. Subsequently, Fischer's linear discriminant analysis was carried out to define classifying functions containing the variable set that together would best distinguish the patients in the three study groups[20]. Variables that exhibited p-values < 0.20 in bivariate analysis were chosen for multivariate analysis; age and gender were additionally included. The evaluation of the model's discriminating accuracy was then checked by cross-validation, using the leave-one-out technique[7 10]. In this procedure, each study subject was analyzed as external to the model and was classified again by applying his/her data to the new classifying functions that had been set up using the other (n-1) study subjects. The discriminating accuracy of the model was considered high when the cross-validation procedure yielded the right classification

in more than 75% of the cases[10]. The statistical analysis was performed using the SPSS version 13.0 software package, with a 5% significance level.

RESULTS

The study cohort comprised 85 patients, diagnosed as follows: 24 (28.3%) HCV/HTLV-1 coinfected individuals, 6 (7%) with HCV/HTLV-2 coinfection and 55 (64.7%) infected with HCV alone. Most (53%) patients were male and aged from 20 to 59 (mean age, 40). White (71.8%) and married (51.8%) subjects, with eight to ten years of schooling (45.9%), born in the State of São Paulo (71.8%), predominated (**Table 1**). With regard to smoking, no statistically significant difference was seen among the study groups. In contrast, reported alcohol consumption was significantly more frequent among the patients in the HCV/HTLV-2 group (p = 0.009).

Table 2 summarizes the exposure to sexually-transmitted or blood-borne infections among the patients in the different study groups. Reported intravenous drug use, inhaled cocaine use and sexual partnership with an IDU were significantly more frequent among the patients in the HCV/HTLV-2 group, compared with the other groups. Sexual partnership with an individual with hepatitis, however, was more often seen in the HCV group.

Regarding clinical complaints, abdominal pain was reported more frequently by the patients in the HCV group (25.5%, p = 0.024), compared with 16.7% of their counterparts in the HCV/HTLV-2 group and none of the patients with HCV/HTLV-1 coinfection. No significant differences were noticed among the

TABLE 1

Comparison of 24 HCV/HTLV-1 coinfected, six HCV/HTLV 2 coinfected and 55 HCV-infected patients with regard to sociodemographic features. Hospital das Clinicas, School of Medicine, University of São Paulo, 1993-2005.

	Group						
	HCV		HCV/HTLV-1		HCV/HTLV-2		
Variable	n	%	n	%	n	%	P
Gender							0.98
male	29	52.7	13	54.2	3	50.0	
female	26	47.3	11	45.8	3	50.0	
Age (years)							0.18
18 - 29	17	30.9	5	20.8	1	16.7	
30 - 39	9	16.4	6	25.0	4	66.6	
40 - 49	19	34.5	9	37.5	1	16.7	
≥ 50	10	18.2	4	16.7	-	-	
Color							0.50
white	39	70.9	17	70.8	5	83.3	
mulatto	13	23.6	3	12.5	1	16.7	
black	3	5.5	3	12.5	-	-	
yellow	-	-	1	4.2	-	-	
Marital status							0,59
married	30	54.5	10	41.6	4	66.6	
single	14	25.5	9	37.5	1	16.7	
divorced	11	20.0	4	16.7	1	16.7	
widowed	-	-	1	4.2	-	-	
Schooling (years)							0.56
≤ 7	19	34.5	6	25.0	1	16.7	
8 - 10	22	40.0	14	58.3	3	50.0	
≥ 11	14	25.5	4	16.7	2	33.3	
State of birth							0.08
São Paulo	35	63.6	21	87.5	5	83.3	
others	20	36.4	3	12.5	1	16.7	
Smoking*							0.179
no	38	70.4	12	50.0	3	50.0	
yes	16	29.6	12	50.0	3	50.0	
Alcohol consumption							0.009
no	40	72.7	12	50.0	1	16.7	
yes	15	27.3	12	50.0	5	83.3	

*data missing for one patient from the HCV group

HCV: hepatitis C virus, HTLV-1: human T-lymphotropic virus type 1, HTLV-2: human T-lymphotropic virus type 2.

TABLE 2

Comparison of 24 HCV/HTLV-1 coinfected, six HCV/HTLV 2 coinfected and 55 HCV-infected patients with regard to risk factors for virus acquisition. Hospital das Clinicas, School of Medicine, University of São Paulo, 1993-2005.

	Group						
	HCV		HCV/HTLV-1		HCV/HTLV-2		
Variable	n	%	n	%	n	%	P
Intravenous drug use							<0.001
no	53	96.4	20	83.3	2	33.3	
yes	2	3.6	4	16.7	4	66.7	
Inhaled cocaine use							0.022
no	41	74.5	21	87.5	2	33.3	
yes	14	25.5	3	12.5	4	66.7	
Sexual partnership with IDU*							<0.001
no	53	96.4	17	77.3	-	-	
yes	2	3.6	5	22.7	3	100.0	
Sexual partner with hepatitis**							0.001
no	50	90.9	16	94.1	4	100.0	
yes	5	9.1	1	5.9	-	-	
Recipient of blood transfusion							0.332
no	32	58.2	12	50.0	5	83.3	
yes	23	41.8	12	50.0	1	16.7	
Tattooing							0.061
no	51	92.7	23	95.8	4	66.7	
yes	4	7.3	1	4.2	2	33.3	
Acupuncture							0.349
no	48	87.3	23	95.8	6	100.0	
yes	7	12.7	1	4.2	-	-	
Number of sexual partners							
in the previous year***							0.224
1	50	92.6	19	79.2	5	83.3	
> 1	4	7.4	5	20.8	1	16.7	

*data missing for 7 and 2 patients from the HCV/HTLV-1 and HCV/HTLV-2 groups, respectively.

**data missing for 2 and 3 patients from the HCV/HTLV-1 and HCV/HTLV-2 groups, respectively.

***data missing for one patient from the HCV group

HCV: hepatitis C virus, HTLV-1: human T-lymphotropic virus type 1, HTLV-2: human T-lymphotropic virus type 2, IDU: intravenous drug user.

study groups regarding the frequency of signs and symptoms of liver disease (jaundice, ascites, epistaxis, palmar erythema, spiders, hepatomegaly or splenomegaly), reported malaise or skin and neurological abnormalities on clinical examination.

Laboratory investigations yielded significantly higher median platelet counts in HCV/HTLV-1 or HCV/HTLV-2 coinfected individuals, compared with patients in the HCV group ($p = 0.015$). Conversely, higher median serum AST, ALT and GGT concentrations were seen in HCV-infected patients ($p = 0.047$, $p = 0.011$ and $p < 0.001$, respectively).

Seventy-five patients (88.2% of the study cohort) were compared regarding their liver histopathological features. Although most of them exhibited abnormal findings in their liver biopsy specimens, no statistically significant differences were seen among the patients in the different study groups (**Table 3**).

TABLE 3

Comparison of 24 HCV/HTLV-1 coinfected, six HCV/HTLV 2 coinfected and 55 HCV-infected patients with regard to liver histopathological findings. Hospital das Clinicas, School of Medicine, University of São Paulo, 1993-2005.

	Group						
	HCV		HCV/HTLV-1		HCV/HTLV-2		
Variable	n	%	n	%	n	%	P
Fibrosis (score)							0.763
< 3	37	67.3	11	68.8	2	50.0	
≥ 3	18	32.7	5	31.2	2	50.0	
Necroinflammatory activity (score)							0.256
minimal (0 - 6)	20	36.4	4	25.0	-	-	
mild/moderate (7 -12)	35	63.6	12	75.0	4	100.0	
Portal inflammation							
present (score ≥ 1)	55	100.0	16	100.0	4	100.0	
Periportal or periseptal							
interface hepatitis							0.547
absent	18	32.7	3	18.8	1	25.0	
present	37	67.3	13	81.2	3	75.0	
Lobular necrosis							0.145
absent	1	1.8	2	12.5	-	-	
present	54	98.2	14	87.5	4	100	

After Fischer's discriminant analysis, the following discriminating functions were defined for each of the study groups:

y_{HCV} = - 6.558 + 0.605 (IDU) + 0.045 (sexual partnership with someone with reported hepatitis) + 4.976 (gender) + 1.485 (age group)

$y_{HCV/HTLV-1}$ = - 7.178 + 1.636 (IDU) + 1.437 (sexual partnership with someone with reported hepatitis) + 4.942 (gender) + 1.512 (age group)

$y_{HCV/HTLV-2}$ = - 9.685 + 9.265 (IDU) + 0.849 (sexual partnership with someone with reported hepatitis) + 6.303 (gender) + 0.487 (age group)

IDU (yes = 0, no = 1); sexual partnership with someone with reported hepatitis (yes = 0, no = 1); gender (male = 0, female = 1); age group (18 to 29 years = 0; 30 to 39 years = 1; 40 to 49 years = 2; 50 years or more = 3)

Cross-validation of the model using the leave-one-out technique showed that even though the discriminating functions were able to classify 67.1% of the patients in the study cohort correctly, their performance differed in each of the three study groups. The discriminating accuracy was high (87.3%) for the HCV group-defining function, but intermediate (66.7%) for the HCV/HTLV-2 function. In contrast, the HCV/HTLV-1 group function exhibited low (20.8%) discriminating accuracy.

DISCUSSION

HCV/HTLV coinfections are a matter of particular concern with regard to the clinical management of patients with infectious diseases, not only because of the epidemiological importance of these viral infections in Brazil, but also because of the possible pathogenetic interactions among them. However, the epidemiological, clinical and laboratory features of these coinfections have been overlooked so far, or alternatively, have been investigated without clear distinction of the HTLV type (HTLV-1 or HTLV-2) that is involved. By means of Fischer's discriminating analysis, our study investigated epidemiological, clinical and laboratory diagnostic tools that might better differentiate HCV/HTLV coinfected individuals from their HCV-infected counterparts.

Among the variables that were defined as useful for discriminating between coinfected individuals, self-reporting of intravenous drug use was particularly relevant. It was more often found among HCV/HTLV-2 coinfected patients, thus suggesting that this route was important for acquisition of this retrovirus among the study population, as previously reported in other epidemiological contexts. De la Fuente et al. found that HTLV-2 infection was strongly associated with injectable drug use among young Spanish addicts, as well as with HIV and HCV coinfections[13]. Likewise, in a prevalence study on HTLV infection among American women, HTLV-2 infection was shown to be significantly associated with injectable drug use, regardless of HIV coinfection[36]. Nonetheless, previous Brazilian studies presented conflicting data with regard to the association between intravenous drug use and HTLV-2 infection. Although some investigators have indicated an epidemiologic association[14], studies conducted more recently have highlighted the leading role of HTLV-1 among HTLV-infected drug addicts in Bahia[11]. Our results reinforce the idea that HTLV-2 infection is possibly spread by means of injectable drug use, and thus suggest that the way in which this retrovirus circulates may vary between different Brazilian geographic regions.

Regarding sexual activity, we found that the patients in the different study groups differed in their reports about having a sexual partner with hepatitis. Since the role of sexual transmission in the epidemiology of HCV infection is still controversial[34], we hypothesize that the routine counseling that was provided for individuals with HTLV, to encourage them to adopt safer sexual practices[6 23], may have accounted for the observed difference, to a certain extent. Sexual partnership with an IDU was significantly more frequent among the patients in the HCV/HTLV-2 group, thus suggesting that they were at greater risk of HTLV-2 acquisition through sexual means. However, even though HTLV-1 and

HTLV-2 have been acknowledged to be sexually-transmitted agents[26,35,38], recent evidence has suggested that transmission is not particularly associated with any of the HTLV types[30]. Alternatively, and speculatively, the increased risk of HTLV-2 acquisition among these individuals may have been due to non-reported IDU, as previously described in a serological survey of HCV coinfection among people living with HIV in Santos, Brazil[34].

Use of inhaled cocaine was significantly more often reported among the patients in the HCV/HTLV-2 group. Nevertheless, since there is no evidence that such practices add to the risk of HTLV-2 acquisition, and because no information was available about the sharing of instruments for inhalation[21], we believe that this might be a confounder. Based on reports in the literature that indicate that alcohol consumption is high among intravenous drug users[2], we suppose that this association may have contributed towards our finding of a higher frequency of alcohol consumption among HCV/HTLV-2 coinfected individuals from our cohort.

The chronic course of HCV infection, as well as the fact that HTLV infection is most often asymptomatic throughout life, may have impaired the statistical power of the clinical variables regarding their ability to differentiate patients in the different study groups, except for abdominal pain. This complaint was more often reported by patients in the HCV group. Although this symptom is frequently reported by HCV-infected individuals[22], pathogenetic mechanisms that might justify different frequencies of abdominal pain between HCV-infected and HCV/HTLV coinfected patients could not be identified.

Laboratory investigations demonstrated lower platelet counts among the HCV-infected patients, compared with the coinfected individuals. In contrast, although the aminotransferase and GGT levels were high in patients in all of the groups, they were significantly higher in the HCV group. This discrepancy might be due to HCV disease that was more advanced. Nevertheless, this was not supported by histopathological data. Alternatively, differences in platelet counts could be ascribed to HTLV-driven thrombocytogenesis, as hypothesized by Murphy et al[25] from an evaluation on hematological disorders among asymptomatic carriers of retroviral infection who were volunteer blood donors at different centers in the USA.

In our study, the analysis of liver histopathological abnormalities revealed higher frequencies of less severe fibrosis (score < 3) among the HCV (67.3%) and HCV/HTLV-1 (68.8%) individuals, compared with the HCV/HTLV-2 individuals (50%), although without statistical significance. Although length of time since HCV infection and age at the first biopsy procedure have been recognized as associated with progression of HCV-related fibrosis[3,31], these variables were not useful for clarifying differences in the histopathological data in our study. We found no significant age differences among the patients in the different study groups at the time when they underwent liver biopsy. Moreover, the cross-sectional design of our study precluded recognition of length of time with HCV infection and of the precise chronology of events among coinfected HCV/HTLV individuals. In contrast to these methodological constraints, Japanese longitudinal studies have provided evidence of accelerated HCV disease progression among HCV/HTLV-1 coinfected patients[4]. Follow-up of our patients, with inclusion of more HCV/HTLV-2 individuals, is thus warranted in order to clarify the impact of HTLV coinfection on the progression of HCV disease.

Another limitation of our study resulted from the fact that there were few HCV/HTLV coinfected individuals among the patients seen at the clinic, who were not also HBV or HIV-coinfected[32,33]. Since these viruses share their means of transmission, multiple coinfections are often identified among individuals at high risk of viral exposure.

Despite these limitations, we believe our study has improved the understanding of the epidemiological, clinical, laboratory and histopathological features of HCV/HTLV coinfection and has contributed towards better identification of distinctive features for discriminating between HCV-infected and HCV/HTLV coinfected individuals. Applying this information to the routine care of HCV-infected patients may help the healthcare team clarify when HTLV serological screening and counseling should be implemented for these individuals.

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