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HIV/HCV coinfection at an university hospital in Recife, Brazil

ABSTRACT

OBJECTIVE: To estimate the prevalence of hepatitis C virus (HCV) infection and risks factors associated with coinfection in HIV-positive individuals.

METHODS: A cross-sectional descriptive study was conducted with 343 HIV patients attended at a university hospital in Recife, Northeastern Brazil, from March to December 2003. A standardized questionnaire about risk factors was administered. Serum samples were analyzed for anti-HCV antibodies using enzyme-linked immunosorbent assay (ELISA), HCV-RNA using reverse transcription-polymerase chain reaction (RT-PCR), and genotyping using the ABI 377 (PE Biosystems®). Univariate and multivariate analyses and multiple logistic regression were performed.

RESULTS: HCV prevalence was 4.1% (14/343) using ELISA and 3.2% (11/343) using RT-PCR. The most common genotypes were 1b (45%), 3 (33%) and 1a (22%). Co-infection was higher among those aged 30 to 39 years, and predominantly in males (64.3 %). In the multiple logistic regression, the variable blood transfusion was the single remaining risk factor for HCV (OR=4.28; 95% CI 1.44;12.73).

CONCLUSIONS: The prevalence of HIV/HCV coinfection was low. Blood transfusion was a risk factor and HCV genotype 1b was the most frequently found.

DESCRIPTORS: HIV Infections, epidemiology. Hepatitis C, epidemiology. Risk Factors. Seroepidemiologic Studies. Cross-Sectional Studies.

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INTRODUCTION

Coinfection by the hepatitis C virus (HCV) in people who are carriers of the human immunodeficiency virus (HIV) is often observed due to the similar transmission route, particularly in relation to the parenteral route.²³ It is estimated that approximately 30% of all HIV-positive individuals are coinfecting with HCV in the United States and Europe.²¹

In Brazil, prevalence depends on the geographic area, ranging from 8.9% to 54%.^{9,16,22,27} Therefore, the interaction of these viruses raises concern and constitutes one of the most important public health problems to be faced by health professionals and authorities worldwide.

Coinfection is higher in patients who acquired HIV through injecting drug use or blood transfusions than in patients who were contaminated through sexual intercourse.²⁶

The highly active antiretroviral therapy (HAART) has been used over the last years and led to an improvement in the survival of HIV-infected patients. On the other hand, there is a high risk of hepatotoxicity in individuals coinfecting with HCV because of the use of antiretroviral drugs.¹⁹

The progression of the disease caused by HCV among coinfecting individuals is usually more aggressive and presents a high level of viremia. There is also a greater risk of the association of HCV with hepatic cirrhosis and/or hepatocarcinoma.⁸ The prevalence of cirrhosis is three times higher in patients with HIV/HCV coinfection than in patients exclusively infected with HCV.¹⁷

Studies have shown that risk factors such as illicit injecting drug use, blood transfusions and sex with infected partners are important in determining coinfection determinants.^{1,13-15}

The objective of the present study was to estimate the prevalence of HCV and risk factors associated with coinfection among HIV-positive individuals.

METHODS

A cross-sectional analytical study was carried out. The sample size was calculated based on a 17.1% prevalence (Mendes Corrêa et al¹³ 2001), error of 4% and 95% confidence intervals (CI). A total of 345 individuals living with HIV or AIDS, from both sexes, attending a university hospital in the municipality of Recife, Northeastern Brazil, were invited to participate in the study between March and December, 2003. There were two refusals, thus totaling 343 participants.

The population attending this hospital was comprised of individuals from the state capital and from inner state areas. During the research period, around 700 people living with HIV/AIDS were attended per year.

The service had four consultation rooms and 15 beds for admissions.

The HIV load and the CD4 lymphocyte count of patients who had made a prior appointment were weekly checked in the hospital's clinical analyses laboratory. These patients were studied because they would have to draw blood for medical monitoring examinations. Those who agreed to participate in the research answered a standard questionnaire.

The analyzed and categorized variables were: age, gender, history of blood transfusion and/or derivatives (yes or no), number of transfusions (one, two to five, and more than five transfusions), period of hemotransfusion (before 1993, after 1993 and had never had one), tattoos (yes or no), injecting drug use (yes or no) and history of homosexual relationships (yes or no, and only considered for males).

Ten milliliters of blood were collected from each individual by venipuncture. Serum was obtained by centrifuging and stored at -20°C until serology was carried out.

The immunoenzymatic test (ELISA) was carried out on all the serum samples to check for anti-HCV, using commercial third generation kits (Hepatitis C anti-HCV Wiener® lab.) and following the manufacturer's instructions.

The polymerase chain reaction (RT-PCR) was carried out for all samples, regardless of the serological result. The method used was the nested PCR^{10,11} with some modifications. Quality control norms, proposed by Kwok & Higushi¹² (1989), were followed to avoid contamination of the rooms and consequently of the material and serums being handled.

The genomic fragments of HCV amplified through PCR technique were sequenced in ABI377 equipment (PE Biosystems®) using the "DNA sequencing kit Big Dye™ Terminator" from Applied Biosystems®, in accordance with the manufacturer's instructions.

The data for each patient were stored and analyzed using the EpiInfo (v.6.04) program for univariate analysis, calculation of means, frequency distribution, the prevalence ratio (PR) and 95% confidence intervals.

In the univariate analysis, the variables that had significance probability below 20% ($p < 0.20$) were analyzed in the multiple logistic regression model, using backward elimination.

Initially, the model was saturated with the inclusion of all the variables. Then, the statistical significance of the removal of each variable was tested using as the cut-off point $p > 0.10$. The program used for the multivariate analysis was SPSS PC (v. 8.0).

The study was approved by the Ethics Committee of the Health Sciences Center of the *Universidade Federal de Pernambuco* (Protocol 008/2003). All participants signed a free and informed consent statement.

RESULTS

The majority of the 343 participants in the survey were males (65%), aged between 30 and 39 years (43.4%).

Of all the patients surveyed, 25.3% (86) had received blood transfusions. Of these, 82.6% (71) had received a transfusion after 1993 and 53.5% (46) only one transfusion. As for the presence of tattoos, 13.1% (45) of the patients mentioned this item and 4.7% used illicit injecting drugs. Of the 223 men interviewed, 55.6% (124) reported homosexual experiences (Table 1).

The prevalence of coinfection based on the detection of anti-HCV was 4.1% (14) and 3.2% (11) when the RT-PCR technique was used. The most frequent genotypes were 1b (45%), 3 (33%) and 1a (22%).

There was a higher prevalence of males (9/14), representing 64.3% of all those coinfecting and aged between 35 and 39 years. However, there was no evidence of a statistically significant association in both cases (Table 2).

Table 3 shows the results of the association of the variables, according to positive results for anti-HCV obtained from the univariate analysis. Blood transfusions proved to be statistically significant ($p < 0.05$) for coinfection. With regard to the period when the transfusion was carried out, the risk for HCV infection was higher in patients who had received transfusion before 1993, with a prevalence ratio of 5.71. The risk of infection increased with the raise in the number of transfusions, but it was only significant in the six transfusions or more category. However, the confidence interval was very wide due to the small population in this particular category.

The variables with significance probability below 20% ($p < 0.20$) in the univariate analysis – age group, blood and/or hemoderivatives transfusions, period and number of transfusions, tattoos and injecting drug use – were then included in the multiple logistic regression model, with backward elimination. After multivariate analysis, only the blood transfusion variable remained as a risk factor for HCV (OR= 4.28; 95% CI: 1.44;12.73, $p = 0.0087$).

DISCUSSION

The prevalence of HIV/HCV coinfection found in our study was low (4.1%) when compared with the studies in the literature,^{6,15} possibly due to differences in the risk factors for acquiring HCV in different places.

Table 1. Characteristics of the HIV-positive patients. Municipality of Recife, Northeastern Brazil, 2003. (n=343)

Variable	n	%
Gender		
Male	223	65.0
Female	120	35.0
Age group (years)		
Under 30	61	17.8
30 to 39	149	43.4
Over 40	133	38.8
Blood transfusion		
Yes	86	25.3
No	257	74.7
Year of transfusion		
Before 1993	15	17.4
After 1993	71	82.6
Number of transfusions		
One	46	53.5
2 to 5	34	39.5
6 and more	06	7.0
Tattoos		
Yes	45	13.1
No	298	86.9
Use of injecting drugs		
Yes	16	4.7
No	327	95.3
Homosexual relationships		
Yes	124	55.6
No	99	44.4

There is evidence of an evolving pattern of the epidemiology of infection by HIV in various countries in the Americas, including Brazil. Substantial differences in case distribution according to risk factors have been found in the current decade, when compared with the 1980s. Initially, the epidemiology was predominant among male homosexuals, bisexuals, hemophiliacs or in those who had received blood transfusions.²⁴

However, there has been a significant increase in HIV transmission among users of injecting drugs, particularly cocaine and heroin, and in heterosexuals who have unprotected sexual practices. On the other hand, as HCV transmission occurs principally by parenteral route, HIV-positive individuals who are drug users have a four times higher risk of becoming infected with HCV.²⁴

In Northeastern Brazil, only 8.4% of the patients acquire HIV through injecting drug use, differently from the Southeastern and Southern regions, where the figures are 25.8% and 30.7%, respectively.²⁵

Table 2. Distribution of patients coinfecting with HIV, according to gender and age group. Municipality of Recife, Northeastern Brazil, 2003.

Variable	Anti-HCV				PR	95% CI	p
	Positive	%	Negative	%			
Gender							
Male	9	4.0	214	96.0	0.97	0.33;2.83	0.953
Female	5	4.2	115	95.8	1.00	–	–
Age group (years)							
Under 30	5	3.8	128	96.2	1.00	–	
30 to 39	9	6.0	140	94.0	1.61	0.55;4.67	0.544
Over 40	0	0.0	61	100.0		Not calculated	0.327

In the population studied, there was no statistically significant association between the use of injecting drugs and HCV, perhaps because of the reduced number of coinfecting patients who were illicit injecting drug users. In the Northeast, marijuana is the main drug used, unlike in other regions in Brazil and the world, where injecting drugs predominate. Consequently, HIV/HCV coinfection is higher in users of this type of drugs.

In the sample studied, there was no statistically significant association between age group and gender.

However, the most affected coinfecting patients belonged to the 30-39 years age group and were predominantly male, thereby corroborating other literature findings.^{6,15}

The performance of a blood and/or hemoderivative transfusion is a significant risk factor for HCV transmission. In addition, the period in which the first transfusion occurred is also relevant, since there was no screening for HCV in Brazilian blood banks before 1993.⁵

Table 3. Association of the variables related to HIV-infected patients, according to positive results for anti-HCV, after univariate analysis. Municipality of Recife, Northeastern Brazil, 2003.

Risk factor	Anti-HCV				PR	95% CI	p
	Positive	%	Negative	%			
Blood transfusion							
Yes	08	9.3	78	90.7	3.98	1.42;11.1	0.009
No	06	2.3	251	97.7	1.00	–	–
Year of transfusion							
Had never had one	06	2.3	251	97.7	1.00	–	–
Before 1993	02	12.5	13	87.5	5.71	1.26;25.9	0.065
After 1993	06	8.3	65	91.7	3.62	1.20;10.8	0.025
Number of transfusions							
Had never had one	06	2.3	251	97.7	1.00	–	–
One	03	6.5	43	93.5	2.79	0.72;10.7	0.141
2 to 5	03	8.8	31	91.2	3.78	0.99;14.4	0.074
6 and more	02	33.3	04	66.7	14.2	3.59;56.7	0.011
Tattoos							
Yes	04	8.9	41	91.1	2.65	0.87;8.09	0.096
No	10	3.4	288	96.6	1.00	–	–
Use of injecting drugs							
Yes	02	12.5	14	87.5	3.41	0.83;13.9	0.133
No	12	3.7	315	96.3	1.00	–	–
Homosexual relationships							
Yes	05	4.2	119	95.8	1.00	0.28;3.62	0.997
No	04	4.4	95	95.8	1.00	–	–

Studies show that the number of transfusions is an important factor for acquiring HCV, with indices as high as 30% for testing positive for anti-HCV in patients who have had multiple transfusions.¹⁵

In univariate analysis, three variables were indicated as risk factors: receiving a blood transfusion, the period and the number of transfusions. However, only receiving a blood transfusion remained associated with testing positive for HCV in the multiple logistic regression model, with a risk 4.28 times higher of an HIV-positive individual acquiring HCV. Therefore, the other variables were considered confounding factors.

No association was found between the presence of tattoos and testing positive for HCV, corroborating other studies.³

For the laboratory analysis of hepatitis C, in addition to serological methods to detect anti-HCV, molecular methods are also necessary. RT-PCR is a suitable method for detecting cases when there has not been serum-conversion yet, but the virus is circulating. It is also useful for monitoring response to treatment by the viral load.²⁸

It can be observed that all patients who were seronegative for HCV were also negative by RT-PCR. Considering that these are serious immunosuppressed patients,

in whom the immunological response may be altered, this result indicates that ELISA is a good diagnosis method for HCV infection. However, the immunological response to HCV may be detected within one to three months, thus making RT-PCR an important method for confirming the diagnosis.

Another important molecular method is genotyping, which is clinically significantly important, both from the prognosis and treatment points of view, since there are data indicating worse prognosis for the disease when genotype 1 is present. Therefore, genotyping is indicated as one of the parameters used for assessing prognosis and planning therapy for patients infected with HCV.⁴

The frequency of the HCV genotypes in patients coinfecting by HIV was similar to various studies carried out in Europe^{18,20} and Brazil,^{2,7} with a high frequency of genotypes 1 and 3.

The research data show a low prevalence of coinfection in Recife in comparison with other cities in Brazil and the world. On the other hand, the study provides evidence of the significance of hemotransfusions in the transmission of infection by HCV and the greater frequency of the 1b genotype of this virus.

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