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Profile of HCV genotypes and HIV-subtypes among HIV-coinfected patients in Southern Brazil

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ABSTRACT – **Background** – Hepatitis B and C virus (HBV and HCV) are the two most common infections among human immunodeficiency virus (HIV)-infected patients. **Objective** – To identify the frequency of HIV subtypes and HCV genotypes in HIV-coinfected patients. **Methods** – A cross-sectional and retrospective study was carried out into two reference centers in Southern Brazil between January 1, 2002 and June 30, 2016. The Abbott Real Time HCV Genotype II system was used for routine diagnostics to determine the HCV genotype based on dual-target real-time PCR. Proviral HIV-1 RNA was extracted from serum samples and fragments of the *pol* gene were generated by PCR. The HIV-1 PT and RT gene sequences were submitted to Maximum Likelihood Phylogenetic analysis by collecting reference sequences from the HIV-1 group M subtype of the Los Alamos database. **Results** – During the study period, 3340 patients with HIV were diagnosed at both referral centers, of which 4.97% (166/3340) had HBV and/or HCV coinfection. Seroprevalence of HIV-HBV, HIV-HCV and HIV-HBV-HCV was 37.4%, 58.4%, and 4.2%, respectively. HIV-HCV-coinfected patients had a lower median nadir CD4+ T-cell count when compared to HIV-HBV-coinfected patients (P=0.01). Among those coinfected with HCV, HCV-1 (HCV-1) and HCV-3 (HCV-3) genotypes were the most prevalent, being detected in 73.8% and 21.4%, respectively. Among the HCV-1 coinfected patients, 79.3% and 20.1% had subtypes 1a and 1b, respectively. HIV subtype B was the most prevalent in HIV-coinfected patients. There was no significant difference regarding nadir CD4+ T-cell count and HIV viral load when compared to coinfected with HCV-1 with HCV-3, as well as those co-infected with HCV-1a with HCV-1b. **Conclusion** – In the present study, a higher frequency of subtype B of HIV and HCV-1 were found in HIV-coinfected patients. Further larger-scale and long-term studies are needed to better understand the effect of HCV genotypes in HIV-infected patients.

HEADINGS – HIV infections. Hepacivirus. Genotype. CD4 lymphocyte count.

INTRODUCTION

Globally, it has been estimated that 257, 71, and 36.7 million people are infected with chronic hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV), respectively[1,2]. HIV, HBV, and HCV share similar forms of transmission, and thus, it has been estimated that 2.7 and 2.3 million people are living with HIV-HBV and HIV-HCV coinfections, respectively[1].

Infections by these viruses present a dynamic interaction, thereby amplifying each other and leading to increased morbidity in HIV coinfected patients[3]. Among HIV-HBV coinfected patients, liver-related complications were increased, and immune recovery is attenuated[4]. For HIV-HCV coinfections, several studies have shown effects of coinfection on the CD4+ T-cell count[5-8]. However, despite evidence of the impact of HIV-HCV coinfection on CD4+ T-cell counts, it is unclear whether this effect was related to the different HCV genotypes.

HCV is currently classified in seven genotypes (HCV-1 to HCV-7) and multiple subtypes according to their genetic sequence[9]. Globally, the occurrence of the HCV genotype is variable. HCV-1 is predominant in Australia, Europe, Latin America, and North America (53%–71% of all cases), whereas HCV-3 occurs predominantly in Asian countries (40% of all infections)[10]. In Brazil, there is a predominance of HCV-1 (64.9%), followed by HCV-3 (30.2%)[11]. However, there is little information about the frequency of HCV genotypes among HIV-coinfected populations in Brazil.

Among HIV-coinfected patients, the presence of a hepatotropic virus can lead to an overload of the host's immune system, which may lead to the emergence of specific HIV variants[12]. However, there is little information on the genetic variability of HIV in HBV- and/or HCV-coinfected patients. Thus, we investigated the frequency of HIV subtypes and HCV genotypes/subtypes, and their relationship to the nadir CD4+ T-cell count among HIV-coinfected patients.

METHODS

Study area

This study was carried out into two reference centers for the diagnosis, treatment, and follow-up of patients with HIV and viral hepatitis: Specialized Center for Infectious and Parasitic Diseases (*Centro Especializado de Doenças Infecto Parasitárias*) in Cascavel city, and the Specialized Service of Sexually Transmitted Infections (*Serviço Especializado de Infecções Sexualmente Transmissíveis*) in Maringá city, both in Paraná State, Southern Brazil. These two centers serve 25 and 30 municipalities, respectively, with a total population of 1,189,062[13], and are part of Unified Health System (*Sistema Único de Saúde*).

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Study design

We developed a cross-sectional and retrospective study between April and December 2017. HIV-coinfected patients and who tested positive for HBV and/or HCV between January 1, 2002 and June 30, 2016 were included in the study. However, for HIV subtyping, only HIV-coinfected patients who had HIV viral loads between January 2014 and December 2016, regardless of whether they were treatment-naive or not were included. For calculations of the nadir CD4+ T-cell count and HIV viral load, only HIV-infected patients with a positive test for HBV or HCV at baseline were considered. Sociodemographic information of the enrolled participants were collected by trained nurses and physicians, using a structured and pre-tested questionnaire. The following variables were collected: date of birth, sex, ethnicity, use of antiretroviral drugs, level of educational attainment, sexual preference, fibrosis levels, time duration of HIV infection diagnosis and coinfection, use of injected drugs, and region. The classification of liver biopsies followed the METAVIR scale[14].

Laboratory measurements

HIV infection status was based on positive test results from two peripheral blood samples, performed using an HIV Enzyme-linked Immunosorbent Assay (Abbott Diagnostics, Chicago, USA) and confirmed by western blotting (Bio-Rad, Marnes La Coquette, France). HBsAg, anti-HBs, anti-HBc, and anti-HCV were tested by commercially available enzyme immunoassay (Abbott Diagnostics). Positive results for anti-HCV were confirmed by amplification of HCV RNA using Reverse Transcription Polymerase Chain Reaction (RT-PCR) by COBAS Ampliprep/Cobas TaqMan48 real-time RT-PCR (Roche Diagnostics, Pleasanton, USA), as described elsewhere[15]. Anti-HIV, anti-HCV, HBV markers, and HCV-RNA data were entered into the patients' medical records and subsequently collected.

The CD4+ T-cell count was performed by flow cytometry (BD Trucount™ Tubes) using the FACSCalibur apparatus (Becton-Dickinson, New Jersey, USA). HIV viral load was determined by PCR using Abbott Real Time HIV-1 (Abbott Diagnostics), and the results were presented as base 10 logarithms. The minimum detection value for the HIV viral load was 50 copies/mL. Data of CD4 cell count and HIV viral load were stored and subsequently obtained for tabulation of data from the national network of the Sistema de Controle de Exames Laboratoriais (SISCEL; the Laboratory Test Control System), at the virology laboratory of the State University of Maringá. All information from SISCEL is stored using data encryption in its central database, which is located in the Department of Chronic Diseases and Sexually Transmitted Infections.

The Abbott Real Time HCV Genotype II system (Abbott Diagnostics) was used for routine diagnostics to determine the HCV genotype based on dual-target real-time PCR: a 5' UTR target region was used to discriminate between HCV genotypes, and the NS5B gene was the target for 1a and 1b subtyping, using previously described methods[16]. The viral genotype was determined after phylogenetic analysis of the sequences obtained, along with established GenBank reference sequences[17].

Proviral HIV-1 RNA was extracted from serum samples using the QIAamp Viral RNA mini Kit® (Qiagen, Hilden, Germany) following the manufacturer's instructions, and the cDNA was sequentially obtained using the Superscript III RT-PCR kit (Invitrogen, CA). Fragments of the pol gene were generated by PCR according to a previously reported[18,19]. Strict laboratory precautions were taken to avoid cross contamination. The genes for protease (PR) and transcriptase (RT) were amplified, and the purified PCR products were sequenced with the ABI Prism® BigDye™ Terminator version 3.1 cycle sequencing kit ready reaction (Applied Biosystems, Foster City, CA) following the manufacturer's instructions in the automatic sequencer AB 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA).

The HIV-1 PT and RT gene sequences were concatenated and submitted to Maximum Likelihood Phylogenetic analysis by collecting reference sequences from the HIV-1 group M subtype of the Los Alamos database (https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html) and BioAfrica (http://www.bioafrica.net/rega-genotype/html/subtypinghiv.html). In addition, the HIV mutation profile associated with resistance to antiretroviral therapy (ART) was analyzed by subjecting the sequences obtained from PT and RT to the Stanford University HIV Drug Resistance Database (https://hivdb.stanford.edu/hivdb/by-sequences/).

Statistical analysis

Pearson's chi-square test or Fisher's exact test were adopted for categorical variables, and the Mann–Whitney test was used in terms of quantitative variables. The level of significance was set at $P<0.05$. Statistical analysis was performed using Stata (version 12.0)[20]. This study was reviewed and approved by the Research Ethical Committee of University Center of Assis Gurgacz Foundation (Report n. 1.397.212 of 28/01/2016). The norms for ethical research were followed by the researchers according to the requirements of the country, guaranteeing total confidentiality, and anonymity of the data.

RESULTS

During the study period, 3340 patients with HIV were diagnosed at both referral centers, of which 4.97% (166/3340) had HBV and/or HCV coinfection. Among them, 37.4% (62/166) were HIV-HBV, 58.4% (97/166) were HIV-HCV, and 4.2% (7/166) were HIV-HBV-HCV coinfected. Among all HIV-coinfected patients, 63.3% (105/166) were men (median age: 42 years, IQR 42–53 years) and 36.7% (61/166) were women (median age: 45 years, IQR 37–53 years).

Regarding HBV serological markers, 39.0% (30/77) and 37.5% (30/80) of HIV-HCV patients were positive for anti-HBc and anti-HBs, respectively; and 14.3%% (11/77) were positive for both anti-HBc and anti-HBs. The presence of HBeAg was detected in 50% (10/20) of HBV-coinfected patients. With regard to the risk factors for HCV coinfection acquisition, 31.6% (36/104) patients were illicit drug users, 21.2% (22/104) of which were people who inject drugs (PWID).

Of all patients, 91.6% (151/166) of HIV-coinfected patients had laboratory tests of CD4+ T-cell counts and HIV viral load determined, with a median nadir CD4+ T-cell count of 327 cells/mm^3 (IQR 225-494), and $\log_{10}$ HIV viral load of 4.5 (IQR 4-6) at the time of HIV diagnosis. In addition, there were no significant differences between HIV-HBV and HIV-HCV coinfection patients for median log HIV viral load, sex, ethnicity, education level, sexual preference, and number of sexual partners in last 12 months. However, compared to HIV-HBV patients, HIV-HCV patients had a lower median nadir CD4+ T-cell count, higher numbers of Illicit user drugs and PWID, and longer duration of HIV diagnosis and

coinfection compared to the HBV-coinfected patients, all with statistical significance (TABLE 1).

Of all the HCV-coinfected patients (HIV-HCV and HIV-HBV-HCV), 40.4% (42/104) had liver biopsies. Of these, 26.2% (11/42) presented some type of hepatic alteration: 9.1% (1/11) A1F0, 18.2% (2/11) A1F1, 9.1% (1/11) A2F1, 18.2% (2/11) A2F2, 18.2% (2/11) A2F3, 9.1% (1/11) A2F4, 9.1% (1/11) A3F2, and 9.1% (1/11) A3F3.

Of all HCV-coinfected patients, 76.9% (80/104) were submitted to HCV genotyping. Of these, 81.3% (65/80) had minimal viral load that allowed genotyping of HCV. HCV-1, 2, 3, and 4 were detected in 73.8% (48/65), 1.6% (1/65), 21.4% (14/65), and 1.6% (1/65) of HIV-infected patients, respectively; 1.6% (1/65) of patients were infected by both HCV-1 and 2. In relation to HCV-1, 79.3% (23/29) and 20.1% (6/29) of patients had subtypes 1a and 1b, respectively.

Patients coinfected with HIV-HCV-1b had lower nadir CD4+ T-cell counts (median, 111 cells/mm^3; IQR: 107–424) when compared to those with HIV-HCV-1a (median, 309 cells/mm^3; IQR, 197–528), but without significant difference (P=0.25). Patients coinfected with HIV-HCV-1a had a higher median log HIV viral load (median, 5.5; IQR, 4–6) compared to those with HIV-HCV-1b (median, 4.5; IQR, 3.5–5.5), but also with no statistical difference (P=0.87). There was also no significant difference in HCV-RNA levels between the genotypes of HCV-coinfected patients for HIV-HCV-1a (median, 6; IQR, 5–6) and HIV-HCV-1b (median, 6; IQR, 5–6), but also with no statistical difference (P=0.35). HIV-HCV-1 patients had a higher median nadir CD4+ T-cell count and lower levels of HIV viral load when compared to those with HIV-HCV-3, but both had no significant difference (P=0.47). Regarding the other characteristics, there were also no significant differences between HCV-1 and 3 (TABLE 2).

Among the all HIV-coinfected patients, 23.5% (39/166) were subjected to HIV subtyping. Of these, 43.6% (17/39) HIV-coinfected patients had sufficient HIV viral load for subtyping. Subtype B was found in 47.1% (8/17) of the HIV-coinfected patients, subtype C in 11.8% (2/17) of the HIV-coinfected patients, and subtype F in 17.6% (3/17) of the of the HIV-coinfected patients. The other strains (23.5%) presented recombinant structure and were classified as BC (11.7%), BDF1 (5.9%) and circulating recombinant forms (CRFs) 29-BF (5,9%). When patients were grouped by type of coinfection, those that were HBV-coinfected had the following HIV subtypes: 66.7% (4/6) were of the HIV subtype B, 16.7% (1/6) of the subtype F, and 16.7% (1/6) unique recombinant forms (URF) type 31-BC. However, the group of HCV-coinfected patients showed the following profile: 40% (4/10) were of subtype B, 10% (1/10) was of subtype C, 20% (2/10) were of subtype F1, 20% (2/10) were URFs (31-BC and BDF1), and 10% (1/10) was CRF (29-BF). One patient with triple infection (HIV-HBV-HCV) had subtype C.

There was no significant difference (P=0.82) in the nadir CD4+ T-cell count between subtype B (median, 424 cell/mm^3; IQR, 242–608) and non-B groups (median, 325 cell/mm^3; IQR, 242–826) of HIV. Correspondingly, there was also no significant difference (P=0.85) in the $\log_{10}$ HIV viral load levels between subtype B (median, 4; IQR, 3–6) and non-B (median, 3.5; IQR, 3–4). In the ART resistance analysis, 29.4% (5/17) HIV-coinfected patients showed resistance-related mutations, with 40% (2/5) nucleoside reverse transcriptase inhibitors (NRTIs) resistance; 20% (1/5) presented mutations related to non-NRTI resistance; 10% (1/5) presented mutations related to resistance to NRTI and non-NRTI; and 10% (1/5) for NRTIs, non-NRTI, and protease inhibitors were found.

TABLE 1. Characteristics of the study patients and association with HIV-HBV and HIV-HCV coinfection.

Variables	HIV-HBV n (%)	HIV-HCV n (%)	*P*-value
n	62 (100.0)	97 (100.0)	
Sex			
Male	37 (59.7)	62 (63.9)	0.59
Female	25 (40.3)	35 (36.1)	
Ethnicity			
White	42 (67.7)	72 (77.4)	
Black	4 (6.5)	3 (3.2)	0.36
Brown	16 (25.8)	18 (19.4)	
Education			
≤ 8 years	42 (68.8)	63 (67.7)	0.88
> 8 years	19 (31.2)	30 (32.3)	
Sexual preference			
Heterosexual	47 (78.3)	71 (85.5)	0.26
Homosexual/Bisexual	13 (21.7)	12 (14.5)	
Time diagnostic HIV (years)			
≤ 5	33 (53.2)	26 (26.8)	<0.001
> 5	29 (47.8)	71 (73.2)	
Time diagnostic HBV or HCV (years)			
≤ 5	38 (61.3)	32 (33.0)	<0.001
> 5	24 (38.7)	65 (67.0)	
Number of sexual partners in last 12 months			
≤ 1	30 (57.7)	38 (57.6)	
2-5	3 (5.8)	5 (7.6)	0.92
> 5	19 (36.5)	23 (34.8)	
Illicit drugs user			
Yes	8 (12.9)	30 (30.9)	0.01
No	54 (87.1)	67 (69.1)	
People who inject drugs			
Yes	2 (3.2)	17 (17.5)	0.01
No	60 (96.8)	80 (82.5)	
Reference center			
10th Regional Health	37 (59.7)	49 (50.5)	0.26
15th Regional Health	25 (40.3)	48 (49.5)	
Age (median; IQR)	45 (38-54)	47 (41-53)	0.17
Nadir CD4+ T-cell (median/mm3; IQR)	390 (287-559)	307 (197-443)	0.01
HIV viral load (log10 median; IQR)	5 (4-6)	5 (4-6)	0.96

n: number of patients; IQR: interquartile range; HIV: human immunodeficiency virus; HBV: hepatitis B virus; HCV: hepatitis C virus.

TABLE 2. Characteristics of HCV-coinfected patients and association with HCV-1 and 3.

Variables	Genotype 1 n (%)	Genotype 3 n (%)	*P*-value
n	48 (100.0)	14 (100.0)	
Sex			
Male	28 (58.3)	10 (71.4)	0.38
Female	20 (41.7)	4 (28.6)	
Ethnicity			
White	38 (82.6)	10 (71.4)	
Black	2 (4.3)	1 (7.2)	0.66
Brown	6 (13.1)	3 (21.4)	
Education			
≤ 8 years	29 (63.1)	8 (57.1)	0.69
> 8 years	17 (36.9)	6 (42.9)	
Sexual preference			
Heterosexual	33 (82.5)	9 (75.0)	0.56
Homosexual/Bisexual	7 (17.5)	3 (25.0)	
Time diagnostic HIV (years)			
≤ 5	9 (18.8)	4 (28.6)	0.43
> 5	39 (81.2)	10 (71.4)	
Time diagnosis HCV (years)			
≤ 5	11 (22.9)	5 (35.7)	0.34
> 5	37 (77.1)	9 (64.3)	
Number of sexual partners in last 12 months			
≤ 1	19 (55.9)	2 (20.0)	
2-5	3 (8.8)	2 (20.0)	0.13
> 5	12 (35.3)	6 (60.0)	
Illicit drugs user			
Yes	15 (31.3)	4 (28.6)	0.85
No	33 (68.7)	10 (71.4)	
People who inject drugs			
Yes	10 (20.4)	1 (7.1)	0.24
No	38 (79.6)	13 (92.9)	
Reference center			
10th Regional Health	14 (29.2)	5 (35.7)	0.64
15th Regional Health	34 (70.8)	9 (64.3)	
Age (median; IQR)	50 (42.5-54.5)	49 (45-54)	0.88
Nadir CD4+ T-cell (median/mm^3; IQR)	304 (190-424)	234 (129-317)	0.29
HIV viral load (log median; IQR)	5.5 (4.2-6)	6 (5.5-6)	0.73
HCV viral load (log median; IQR)	6.2 (5.9-6.5)	6.2 (5.7-6.3)	0.70

n: number of patients; IQR: interquartile range; HIV: human immunodeficiency virus; HCV: hepatitis C virus. Pearson's chi-square test for comparison between groups.

DISCUSSION

The genetic diversity of HCV has been linked to treatment responses. Direct-acting antivirals (DAAs) have been successfully used in the treatment of HCV, all of which have been strictly correlated with the genotype[10]. In 2015, the Brazilian Ministry of Health incorporated the first DAAs for the treatment of hepatitis C. The current guidelines in Brazil recommend that HIV-HCV-coinfected patients must be treated using the approach that non–HIV-infected individuals follow because the efficacy of the currently licensed DAA regimens does not appear to differ between HCV-monoinfected and coinfected individuals[21]. The treatment for hepatitis C and coinfections currently indicated in Brazil has two new therapeutic options that joins the scheme comprising the association of sofosbuvir/daclatasvir, pan-genotypic already offered since 2015. In addition to these options, the Brazilian Ministry of Health maintained the indications for ledipasvir/sofosbuvir (genotype 1), elbasvir/pibrentasvir (genotypes 1 and 4), ribavirin and alfapeginterferon (for some pediatric situations)[21]. Treatment schedules are defined based on genotype and sub genotype of HCV, and knowledge of HCV genotypes helps to predict therapeutic responses and determines the durations of drug treatments[22].

The geographic distribution of HCV genotypes is complex; the epidemic subtypes – especially, 1a, 1b, 2a, and 3a – are widely distributed around the world and account for a significant share of HCV cases[23]. In this study, HCV-1 presented the highest frequency (73.8%), followed by HCV-3 (21.4%) among HIV-coinfected patients. The frequency of HCV genotypes reported here was similar to that found by other authors among HIV-coinfected and HCV-monoinfected patients in Brazil[24-26] as well as in Greece[27], Northern and Central Asia, and in Central and Western Europe[23]. We did not detect HCV-5 or 6, which was in agreement with other studies[28-30]. More studies are needed to add data on the genetic diversity of HCV among HIV-infected patients and may provide important evidence for the understanding of the origin and spread of HCV infections in Southern Brazil.

The high frequency of HCV-1 has been associated with the transmission of HCV by PWID, which is responsible for increasing the risk of acquiring HCV[31,32]. In our study, PWID were the most infected with HCV-1 among the HIV-coinfected patients. These results suggest that HCV-1 was introduced into PWID and spread among HIV-infected patients. Considering that the administrative route of the drug was the only difference between PWID and non-PWID, these data emphasize the importance of not sharing needles to prevent the spread of HCV-1 among HIV-infected patients. However, the origins of HCV-1 have not yet been specified and further investigations are required.

HIV is characterized by high genetic diversity and extensive heterogeneity. This characteristic is due to multiple factors, including multiple human populations, high rates of viral evolution/recombination, selective pressure of the host immune system and/or antiretroviral therapy[12,33]. Although subtype B has been showing a declining trend in sexual transmission, it still accounts for a large proportion. In the United States and Western Europe, HIV-1 subtype B is the most common variant. In Brazil, HIV subtypes C, F1, and recombinants BC and BF are generally observed at low frequencies as previously described[34]. However, there have been a few studies showing the genetic variability of HIV-1 among patients with HIV-hepatitis coinfections. A study

in HIV-coinfected patients in Brazil reported that HIV-1 subtype C and B were present in 47.4% and 31.6% of HIV-coinfected patients, respectively[35]; another Brazilian study reported that HIV-1 subtype B was the most prevalent[12]. Our study corroborates the results of these studies. In addition, the results of the present study were in accordance with previous investigations, which report that B recombinant and CRFs of HIV-1 circulate among HIV-coinfected patients[12,35]. The detailed dynamics of HIV subtypes viral propagation in the study region is not well understood, although two phylogeographic studies have demonstrated possible routes for the origin and spread of subtype C in the Southern region of the country[36,37].

Studies on HIV-HBV coinfection have focused on the screening of HBsAg as a marker of HBV infections. However, information on other markers remains unclear[38]. In Brazil, studies among HIV-infected individuals have found positive rates for anti-HBc ranging from 38.6% to 55.1%[39-42]. These differences likely reflect the percentage of risk factors in the groups studied and the pattern of endemicity of HBV in different regions. Our study reported that 39.0% of HIV-HCV-coinfected people had anti-HBc reactivity. Anti-HBc alone in the HIV population can be interpreted as a marker of occult hepatitis B[43], a phenomenon that may be caused by either a resolved HBV infection[44], or loss of anti-HBs over time[45]; other studies have reinforced these findings[43,46]. In addition, in our study, we found a high frequency of patients with HBeAg (50%) among HIV-HBV-coinfected patients. The AIDS-related immunosuppression increases the frequency of reactivation of HBV, with the recurrence of HBeAg and reversion to the immunoactive phase among HIV-HBV-coinfected patients[47].

Assessing the severity and predicting the course of HIV infections with both CD4 + T cell counts and HIV viral load levels are essential for estimating the severity of HIV-related immunodeficiency. The present study showed a lack of statistical association between HCV-1 and HCV-3 for nadir CD4+ T-cell count and HIV viral loads. Few studies have systematically investigated the CD4+ T-cell count among HCV genotypes in HIV-infected patients. In a study carried out in Brazil, no significant differences were observed in the CD4+ T-cell count for HCV genotypes 1 and 3[48]; our data corroborate this finding. Our study was also in line with data already published in the EuroSIDA study[49]. The authors of this study report that they found no significant differences in the CD4+ T-cell count and HIV viral load in relation to the HCV genotype.

HCV infection could impact the course of HIV infections via chronic immune activation and cytokine production in coinfected individuals[50,51], which may result in diminished CD4+ T-cell counts[52]. In our study, patients with HIV-HCV had a significantly lower median nadir CD4+ cell count when compared with those with HIV-HBV; this result is supported by the biology of the coinfection[52]. Thus, HCV in HIV patients can cause damage to their immune system, which could subsequently increase viral replication of HIV and HCV, further contributing to an impaired immune system and consequently lower CD4+ T-cell count[53,54]. However, caution should be taken in the interpretation of CD4+ T-cell counts since it may not truly reflect the immunological status of HIV-infected patients. Factors such as medications, advanced liver disease, splenomegaly, and viral infections such as Epstein Barr Virus, cytomegalovirus, and HTLV-1, as well as bacterial infections such as tuberculosis may cause the absolute CD4+ T-cell count to decrease[55]. In addition, the lymphoid tissue fibrosis and liver fibrosis contributes to CD4+ T-cell depletion[56-58], and a low CD4+ T-cell count was associated with advanced and major liver disease histological index[59]. In this sense, our reports showed that patients HCV coinfected and with Fibrosis F3 had a higher median nadir T CD4 + when compared to individuals HCV coinfected with Fibrosis F4. However, due to the low amount individuals, we cannot position ourselves on this finding.

Our study presents some limitations. First, convenience sampling for HIV-1 subtyping among HIV-coinfected patients was the main limitation of our study. A more systematic sampling, spanning all coinfected patients, would certainly provide a more accurate picture for the diversity of HIV-1 in our region. Secondly, because of a few patients with HCV-3, the power of the study may be insufficient to compare HCV-1 and 3 in HIV-infected patients who may be masked by potential confounding factors and to allow for more precise conclusions. Thirdly, information on epidemiology was collected from questionnaires administered during individual interviews, all of which may not have been completed with the same level of detail, potentially generating biases.

CONCLUSION

HCV-1 was the most prevalent among HIV-HCV-coinfected patients, followed by HCV-3, as well as subtype B of HIV-1 among HIV-coinfected patients. Significantly lower values for nadir CD4+ T-cell counts in the HIV-HCV group were found when compared to those with HIV-HBV. There was no statistically significant association between nadir CD4+ T-cell counts and HIV viral load among HCV-1 and HCV-3 among HIV-infected patients. Prospective studies should be conducted to better understand the effect of HCV genotypes in HIV-infected patients.

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Authors' contribution

Silva CM and Bertolini DA conceived the study; Silva CM and Peder LD designed the study; Silva CM, Teixeira JJV and Bertolini DA analyzed and interpreted the data; Silva CM and Thomazella MV collected and processed the samples and interpreted the results; Teixeira JJV and Bertolini DA revised the paper. All authors contributed to and read and approved the final manuscript.

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RESUMO – **Contexto** – Os vírus das hepatites B e C (VHB e VHC) são os causadores das duas infecções mais comuns entre os pacientes infectados pelo vírus da imunodeficiência humana (HIV). **Objetivo** – Identificar a frequência dos subtipos do HIV e genótipos de VHC em pacientes coinfectados com HIV. **Métodos** – Estudo transversal e retrospectivo realizado em dois centros de referência do Sul do Brasil, entre 1º de janeiro de 2002 e 30 de junho de 2016. O sistema Abbott Real Time HCV Genótipo II foi utilizado para diagnósticos de rotina para determinar o genótipo do HCV com base na PCR em tempo real de duplo alvo. O RNA viral do HIV-1 foi extraído de amostras de soro e fragmentos do gene *pol* foram obtidos por PCR. As sequências do gene PT e RT do HIV-1 foram submetidas à análise filogenética por máxima verossimilhança através da coleta de sequências de referência do subtipo M do grupo HIV-1 da base de dados Los Alamos. **Resultados** – Durante o período do estudo, 3340 pacientes foram diagnosticados com HIV em ambos os centros de referência, dos quais 4,97% (166/3340) possuíam coinfecção com HBV e/ou HCV. A soroprevalência de HIV-HBV, HIV-HCV e HIV-HBV-HCV foi de 37,4%, 58,4% e 4,2%, respectivamente. Pacientes HIV-VHC possuíam menor nadir de células T CD4+ quando comparados aos pacientes HIV-VHB (*P*=0,01). Entre os pacientes HIV-VHC, os genótipos VHC-1 e VHC-3 foram os mais prevalentes, sendo encontrados em 73,8% e 21,4%, respectivamente. Entre os coinfectados com VHC-1, 79,3% e 20,1% tinham subtipos 1a e 1b, respectivamente. O subtipo B do HIV foi o mais prevalente em pacientes coinfectados. Não houve diferença significativa em relação nadir de células T CD4+ e carga viral do HIV quando comparadas os coinfectados com o VHC-1 com o VHC-3, assim como, os coinfectados com HCV-1a quando comparados com o HCV-1b. **Conclusão** – No presente estudo, uma maior frequência do subtipo B do HIV e do VHC-1 foram encontrados em pacientes coinfectados com HIV. Outros estudos em larga escala e a longo prazo são necessários para entender melhor o efeito dos genótipos do HCV em pacientes infectados pelo HIV.

DESCRITORES – Infecções por HIV. Hepacivirus. Genótipo. Contagem de linfócito CD4.

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