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Prevalence, risk factors and genotypes of hepatitis C virus infection among drug users, Central-Western Brazil

ABSTRACT

OBJECTIVE: To estimate prevalence of hepatitis C virus (HCV) infection and identify risk factors associated and circulating HCV genotypes and subtypes.

METHODS: Study conducted including 691 drug users attending 26 charitable, private and public drug treatment centers in Goiânia and Campo Grande, central-western Brazil, between 2005 and 2006. Sociodemographic characteristics and risk factors for HCV infection were collected during interviews. Blood samples were tested for HCV antibodies (anti-HCV). Positive samples were submitted to HCV RNA detection by PCR with primers complementary to 5' NC and NS5B regions of viral genome and genotyped by line probe assay (LiPA) and direct nucleotide sequencing followed by phylogenetic analysis. The prevalence and odds ratio were calculated with 95% confidence intervals. Risk factors were first estimated in the univariate analysis ($p<0.10$) and then analyzed by hierarchical logistic regression. Statistical significance was assessed at a 5% significance level.

RESULTS: The prevalence of anti-HCV was 6.9% (95% CI: 5.2–9.2). The multivariate analysis of risk factors revealed that age over 30 years and injecting drug use were associated with HCV infection. HCV RNA was detected in 85.4% (41/48) of anti-HCV-positive samples. Thirty-three samples were genotyped as genotype 1 by LiPA, subtypes 1a (63.4%) and 1b (17.1%), and 8 samples (19.5%) were genotype 3, subtype 3a. The phylogenetic analysis of the NS5B region showed that 17 (68%), 5 (20%), and 3 (12%) samples were subtypes 1a, 3a, and 1b, respectively.

CONCLUSIONS: The results show a high prevalence of HCV infection and predominance of subtype 1a among drug users in Brazil. In addition, injecting drug use was a major risk factor associated with HCV infection.

DESCRIPTORS: Hepatitis C. Hepatitis C Antibodies, diagnostic use. Hepacivirus, genetics. Substance Abuse, Intravenous. Needle Sharing. Risk Factors. Seroepidemiologic Studies.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health concern. It is estimated that 2.2% of the world's population, around 130 million people, are infected with HCV.[1] Acute hepatitis C is often asymptomatic but it is a major cause of morbidity and mortality as most cases progress to chronic disease and can lead to liver cirrhosis and hepatocellular carcinoma.[3]

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HCV genoma is characterized by high genetic variability. Six genotypes and multiple subtypes have been identified and they show different response to antiviral therapy as well as diverse geographical distribution. Genotypes 1, 2 and 3 are disseminated worldwide. Genotype 4 is more prevalent in North Africa and Middle East. HCV infections with genotypes 5 and 6 are commonly seen in South Africa and Asia, respectively.[24] In Brazil, studies have demonstrated that genotype 1 is the most prevalent, followed by genotype 3.[5,18]

Hepatitis C transmission most effectively occurs through the parenteral route, transfusion and/or exposure to blood and blood products, but can also be less effectively transmitted through sexual contact and from mother to child.[1] Drug users (DU) have an increased risk of HCV infection due to sharing of injecting materials and unsafe sex practices.[12,13,16,23]

There are few Brazilian studies investigating HCV infection in DU including both injecting and non-injecting drug users. Their HCV prevalence rates range from 5.8% to 36.2%.[2,7,10,19,23]

The present study is the first to investigate HCV infection in illicit DU with the objective of estimating the prevalence of and factors associated with HCV infection and identifying circulating HCV genotypes and subtypes.

METHODS

A cross-sectional study was conducted in a convenience sample drawn from patients attending all charitable, private and public drug treatment centers in the municipalities of Goiânia and Campo Grande, central-western Brazil, between August 2005 and July 2006. There were selected 851 users, 542 from 18 centers (ten to 70 patients per center) in Goiânia and 309 from eight centers (ten to 76 patients per center) in Campo Grande. All subjects were informed on the study objectives and procedures and invited to participate. The following inclusion criteria were applied: 18 years of age or more; injecting and/or non-injecting drug use; and enrollment in a drug treatment center. All patients agreed to participate in the study. Injecting drug users (IDU) were defined as those patients reporting intravenous drug use; and non-injecting drug users (NIDU) were defined as those patients who reported never using intravenous drugs and reported lifetime use of marijuana, cocaine (powder, crack, and "merla"), heroin, LSD, and ecstasy through other routes (sniffing, smoking, and ingestion). Of 851 users, 691 (81.2%) were eligible to participate in the study, of which were 102 IDU and 589 NIDU.

A standardized questionnaire based on the World Health Organization (WHO) recommendations[8] was applied to all subjects and data was collected on sociodemographic characteristics (age, sex, marital status, skin color, schooling, and family income) and potential risk factors for HCV infection (route of administration and length of time of drug use, prior blood transfusion/year of first transfusion and surgery, tattoos/piercing, acupuncture, condom use, number of sexual partners, IDU partner, history of sexually transmitted disease [STD] and prior imprisonment). Then 10 ml blood was drawn from each subject and sera were stored at –20°C.

All samples were tested for HCV antibodies (anti-HCV, Hepanostika Ultra, Biomedical, China) using enzyme-linked immunosorbent assay (ELISA). Positive samples for the marker anti-HCV were confirmed by immunoblot (Bioblot HCV, Biokit, Spain).

Anti-HCV positive samples were submitted to RNA extraction, reverse transcription, and polymerase chain reaction (PCR), with primers complementary to noncoding (NC) regions of the virus genoma, as described by Ginabreda et al[11] (1997). RNA HCV-positive samples were genotyped by line probe assay (LiPA – Versant HCV Genotype Assay, Innogenetics, Belgium).

The phylogenetic analysis of the NS5B region of the virus genoma was performed by confirming HCV genotypes and subtypes. cDNA samples obtained by reverse transcription were amplified by PCR with primers under the same conditions as described by Sandres-Sauné et al[22] (2003). PCR products were then purified using the QIAquick Gel Extraction kit (Qiagen, GmbH, Hilden, Germany) and submitted to direct nucleotide sequencing in both directions using the BigDye Terminator kit (version 3.1, Applied Biosystems, Foster City CA, USA) and analyzed in an ABI-3730 sequencer (Applied Biosystems). The sequences obtained containing 340 nucleotides (from position 8279 to 8619 of the virus genoma) were aligned using ClustalX program.[25] A phylogenetic tree was constructed with a neighbor-joining method using software Mega 2.1,[14] and the Kimura 2-parameter model. Reliability of clusters of the phylogenetic tree was assessed using the bootstrap test based on 1,000 pseudoreplicates. Genotypes and subtypes of HCV samples were identified by adding in the phylogenetic analysis reference sequences representative of the main HCV genotypes/subtypes available in Genbank (referred in the phylogenetic tree by subtype, followed by the number of access to Genbank).

Data analysis was carried out using Epi Info program version 2000. Prevalences and odds ratios (OR) were calculated with 95% confidence intervals (95% CI). Risk factors with $p< 0.10$ in the univariate analysis were then analyzed by hierarchical logistic regression using SPSS program version 11.0 to identify potential confounders. χ^2 test for association, χ^2 test for tendency and Fisher's exact test were also performed. A 5% significance level was set.

The study was approved by the Research Ethics Committee of *Hospital Materno Infantil* (Mother-Child Hospital) (Protocol No. 004/05) in Goiânia. All subjects signed a free informed consent.

RESULTS

Mean age of the sample studied was 28.3 years (SD= 9.0), 88.3% were males, 64.3% single, 59.5% white, 70.2% reported up to eight years of schooling and 46.5% reported family income between two and five monthly minimum wages. Most used two or more non-injecting drugs. The main drug consumed were marijuana (82.9%), powder cocaine (59.6%), "merla" cocaine (52.8%), and crack (37.9%).

Of 691 drug users studied, anti-HCV marker was detected in 49 (7.1%) blood samples. Of these, 48 were positive by immunoblot, showing a 6.9% prevalence of anti-HCV (95% CI: 5.2;9.2) (Table 1).

RNA virus was detected in 85.4% (41/48) of anti-HCV samples reactive by PCR with primers complementary to the 5' NC region. All were genotyped by LiPA. Thirty-three samples were genotype 1, 63.4% subtype 1a, and 17.1% subtype 1b. The remaining samples (19.5%) were genotype 3, and subtype 3a (Table 1). Of 41 RNA HCV-positive samples for the 5' NC region, 25 (60.1%) were amplified and genotyped in the NS5B region. Seventeen (68%) were identified as subtype 1a, five (20%) as subtype 3a, and three (12%) as subtype 1b (Figure).

The univariate analysis showed that HCV infection was significantly associated to age, marital status, family income, route of administration and length of time of drug use, prior blood transfusion and surgery, IDU partner, and history of STD (Table 2). The multiple

Table 1. Prevalence of hepatitis C virus infection among illicit drug users. Municipalities of Goiânia and Campo Grande, Central-western Brazil, 2005–2006

Marker	Positive		95% CI
	n	%	
Anti-HCV			
ELISA	49	7.1	5.3;9.3
Immunoblot	48	6.9	5.2;9.2
Subtypes			
1a	26	63.4	46.9;77.4
1b	7	17.1	7.7;32.6
3a	8	19.5	9.4;35.4

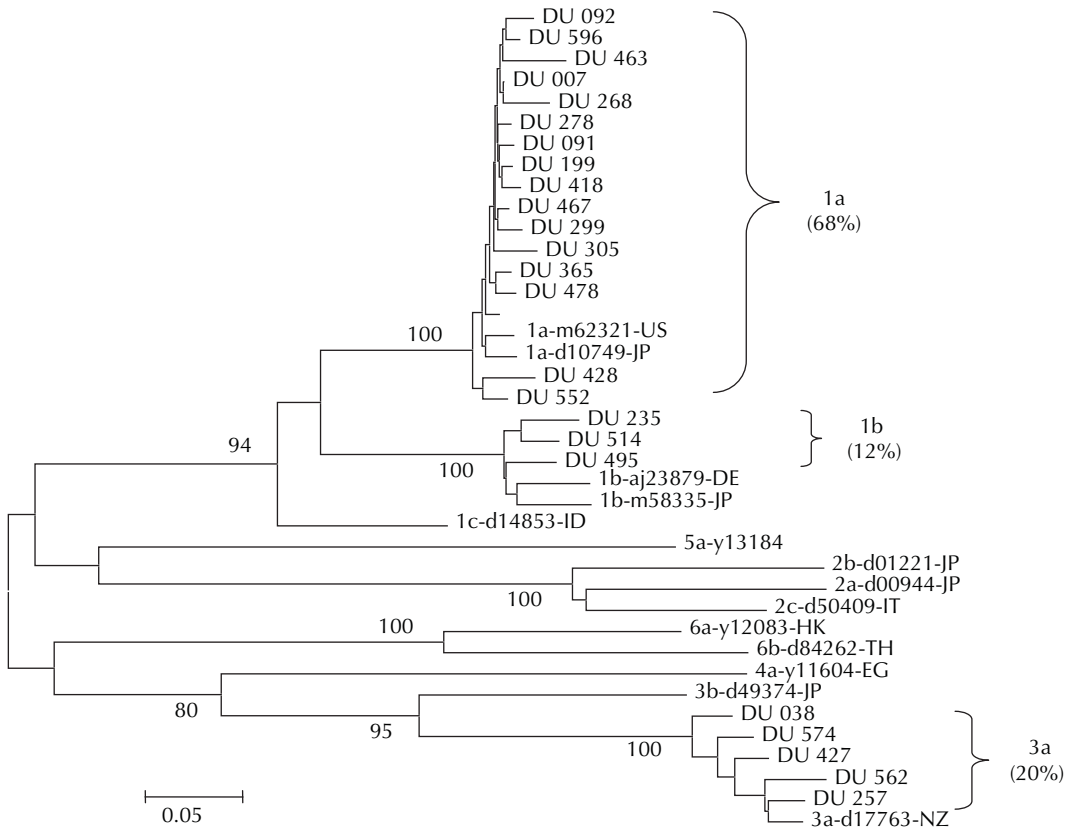

Figure. Phylogenetic tree of the NS5B region, including isolates of drug users and GenBank sequences of genotypes 1 to 6 represented by the main subtypes of hepatitis C virus. Municipalities of Goiânia and Campo Grande, Central-western Brazil, 2005–2006

Table 2. Risk factors associated to hepatitis C virus infection among illicit drug users. Municipalities of Goiânia and Campo Grande, Central-western Brazil, 2005–2006

Risk factor	Anti-HCV Positive/ Total[a]	(%)	Odds ratio (95% CI)	p
Age (years)				
≤30	4/452	0.9	1.0	
>30	44/239	18.4	25.3 (8.9;71.3)	0.000
Sex				
Female	3/81	3.7	1.0	
Male	45/610	7.4	2.1 (0.6;6.8)	0.222
Marital status				
Single	20/444	4.5	1.0	
Married/living with a partner	17/161	10.5	2.5 (1.2;5.1)	0.012
Separated/ widowed	11/86	12.8	3.1 (1.3;7.2)	0.000
Skin color				
White	34/411	8.3	1.0	
Non-white	14/280	5.0	0.6 (0.3;1.1)	0.131
Schooling (years of study)				
≤8	27/485	5.6	1.0	
9–12	17/173	9.8	1.9 (0.9;3.6)	0.084
>12	4/33	12.1	2.3 (0.6;7.7)	0.122
Family income (minimum wage)				
≤1	16/282	5.7	1.0	
2–5	22/302	7.3	1.3 (0.6;2.7)	0.491
≥5	9/65	13.8	2.7 (1.0;6.8)	0.043
Route of drug use				
Non-injecting	16/589	2.7	1.0	
Injecting	32/102	31.4	16.4 (8.5;31.3)	0.000
Length of time of drug use (years)				
≤10	5/330	1.5	1.0	
>10	43/350	12.3	9.1 (3.5;23.2)	0.000
Prior transfusion				
No	32/617	5.2	1.0	
Yes	14/63	22.2	5.2 (2.6;10.4)	0.000
Prior surgery				
No	20/406	4.9	1.0	
Yes	27/283	9.5	2.0 (1.1;3.7)	0.018
Tattoos/Piercing				
No	27/411	6.6	1.0	
Yes	21/280	7.5	1.1 (0.6;2.2)	0.751
Acupuncture				
No	44/652	6.7	1.0	
Yes	4/29	13.8	2.2 (0.7;6.6)	0.280
Condom use				
Regular	8/145	5.5	1.0	
Irregular/never	40/546	7.3	1.3 (0.6;3.0)	0.560
Sexual partners				
≤10	18/332	5.3	1.0	
>10	30/331	9.1	1.7 (0.9;3.2)	0.070
IDU partner				
No	28/488	5.7	1.0	
Yes	16/101	15.8	3.1 (1.6;6.0)	0.000
History of STD				
No	18/459	3.9	1.0	
Yes	30/205	14.6	4.2 (2.3;7.7)	0.000
Prior imprisonment				
No	12/250	4.8	1.0	
Yes	36/437	8.2	1.8 (0.9;3.5)	0.089

[a] The denominator refers to the number of users who did not answer this question
IDU: Injecting drug user
STD: Sexually transmitted disease

logistic regression analysis revealed that age over 30 years (OR= 16.0; 95% CI: 3.9;66.0) and injecting drug use (OR= 18.0; 95% CI: 6.9;47.1) were independently associated to HCV infection (Table 3).

DISCUSSION

The present study has limitations that should be considered in the interpretation of results. The number of DU and their general characteristics in the cities of Goiânia and Campo Grande are not actually known since drug use is an illicit activity. For that reason, HCV infection was only investigated among those IDU and NIDU enrolled in charitable, private, and public drug treatment centers during the study period. On the other hand, given the scarcity of epidemiological data about HCV infection among DU in the cities studied, our results provide background information for formulating policies and strategies for reducing risk and damage associated to the use of illicit drugs.

The prevalence of HCV infection found (6.9%, 95% CI: 5.2;9.2) was higher than that reported in non-drug user blood donors (1.4%, Martins et al,[17] 1994) in this Brazilian region. Based on other studies investigating illicit drug users in Brazil, the prevalence here estimated was similar to that reported by Bastos et al[2] (2000) in the city of Rio de Janeiro (5.8%, 95% CI: 1.7;7.1) and by Carvalho et al[7] (2003) in the city of São Paulo (6%, 95% CI: 2.2;14.1). But it was lower that

Table 3. Multivariate analysis of risk factors associated to hepatitis C virus infection among illicit drug users. Municipalities of Goiânia and Campo Grande, Central-western Brazil, 2005–2006

Risk factors	Estimated risk (95% CI) Non-adjusted	Adjusted[a]	p
Age (years)			
≤30	1.0	1.0	
>30	25.3 (8.9;71.3)	16.0 (3.9;66.0)	0.000
Marital status			
Single	1.0	1.0	
Married/living with a partner	2.5 (1.2;5.1)	1.5 (0.5;3.9)	0.436
Separated/ widowed	3.1 (1.3;7.2)	0.4 (0.1;1.4)	0.172
Schooling (years of study)			
≤8	1.0	1.0	
9–12	1.9 (0.9;3.6)	1.2 (0.2;6.6)	0.862
>12	2.3 (0.6;7.7)	0.7 (0.1;4.1)	0.670
Family income (minimum wage)			
≤1	1.0	1.0	
2–5	1.3 (0.6;2.7)	0.5 (0.1;1.5)	0.205
≥5	2.7 (1.0;6.8)	0.3 (0.8;1.3)	0.117
Route of drug use			
Non-injecting	1.0	1.0	
Injecting	16.4 (8.5;31.3)	18.0 (6.9;47.1)	0.000
Length of time of drug use (years)			
≤10	1.0	1.0	
>10	9.1 (3.5;23.2)	1.6 (0.4;5.9)	0.468
Prior transfusion			
No	1.0	1.0	
Yes	5.2 (2.6;10.4)	3.1 (1.0;9.9)	0.056
Prior surgery			
No	1.0	1.0	
Yes	2.0 (1.1;3.7)	1.0 (0.4;2.5)	0.939
Sexual partners			
≤10	1.0	1.0	
>10	1.7 (0.9;3.2)	2.0 (0.8;4.7)	0.130
IDU partner			
No	1.0	1.0	
Yes	3.1 (1.6;6.0)	0.6 (0.2;1.8)	0.374
History of STD			
No	1.0	1.0	
Yes	4.2 (2.3;7.7)	1.2 (0.5;2.8)	0.673
Prior imprisonment			
No	1.0	1.0	
Yes	1.8 (0.9;3.5)	1.8 (0.7;4.7)	0.229

[a] Estimated risk adjusted for age, gender, marital status, schooling, family income, route of administration and length of time of drug use, prior transfusion and surgery, number of sexual partners, IDU (injecting drug user) partner, history of sexually transmitted disease (STD) and prior imprisonment.

those found in the cities of Porto Alegre (25%; 95% CI: 15.1;38.1), Belém (16%, 95% CI: 11.3;2.0) and Santos (36.2%, 95% CI: 31.9;40.4).[10,19,23] This inconsistency can be explained by the greater proportion of IDU in the samples of the latter studies compared to the former ones (22%–27% versus 10%–15%).

The investigation of HCV RNA reveals those individuals who are HCV carriers, especially in high-risk groups such as drug users. In the present study, of 48 subjects with anti-HCV positive, 41 were HCV RNA-positive, showing a high rate of viremia (85.4%). This finding shows that drug users are thus major HCV carriers and potential transmitters, which is corroborated by other studies.[10,13]

All 41 samples HCV-RNA positive were genotyped by LiPA, and genotype 1 was found to be the most prevalent, followed by genotype 3. Similar results were demonstrated in IDU in the city of Rio de Janeiro,[20] and these same genotypes are the most prevalent in Brazil,[5] as well in central-western region.[18] Subtypes 1a (63.4%), followed by 3a (19.5%) and 1b (17.1%) were the most predominant. The 5' NC region, as it is the most preserved in the virus genoma, could be amplified by PCR in a large number of samples genotyped. That is why the 5' NC region is often investigated for HCV detection and genotyping, especially for the clinical purpose of choosing the best therapeutic regimen for viral hepatitis C.[9]

A similar distribution was seen for subtypes 1a, 3a, and 1b in the phylogenetic analysis of the NS5B region (68%, 20%, and 12%, respectively). There is no inconsistency between genotypes and subtypes of 25 samples tested by both methods. Although both methods are as effective for genotyping, inconsistent results between HCV subtypes have been reported in other studies. Some authors[9,15,22] believe that the NS5B region is more adequate for subtyping that the 5' NC region, which is too preserved and cannot differentiate some subtypes, especially 1a and 1b. Hence, the phylogenetic analysis of the NS5B region is more adequate to identify HCV in epidemiological studies and was used to confirm HCV genotypes and subtypes in the sample studied.

In the present study, age over 30 years was associated to HCV infection (OR=16.0; 95% CI: 3.9;66.0), which is consistent with other studies investigating illicit drug users.[12,16,21] This association can be explained by higher risk of virus exposure during their lifetime.

IDU subjects were more likely to have HCV infection (OR= 18.0, 95% CI: 6.9;47.1) compared to NIDU. In addition, HCV prevalence was nearly 12-fold greater in IDU than NIDU (31.4% versus 2.7%, Table 2). Higher risk for hepatitis C infection in IDU compared to NIDU also was reported by several authors,[2,7,10,13,23] which corroborates the finding of efficient HCV transmission through parenteral route.

There was seen a tendency for the association between prior blood transfusion and HCV infection. This association has been reported especially in blood transfusions before the implementation in Brazil of anti-HCV screening in blood banks in November 1993.[4,6] In fact, in the present study, among 14 anti-HCV positive subjects who had prior blood transfusion, most of them took place prior to this date (data not shown), stressing the importance of anti-HCV screening in blood banks in Brazil.

It was also observed an association between HCV infection and anti-HBc (χ^2= 20.2; p<0.05; data not shown), a marker of HBV exposure. This finding with potential clinical and epidemiological relevance corroborates the findings of other authors investigating drug users,[21,23] and suggests common transmission forms of HBV and HCV in DU.

In conclusion, the present study evidenced high prevalence of HCV infection and subtype 1a among illicit drug users. It was also found that injecting drug use is a major risk factor for HCV infection. This data, associated to the progress to chronic infection, low response of genotype 1 to antiviral therapy and unavailability of a hepatitis C vaccine, reinforces the need for effective programs for prevention and control of hepatitis C among illicit drug users.

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