Prevalence and genotypes of hepatitis C virus among injecting drug users from Salvador-BA, Brazil

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Hepatitis C virus (HCV) is the major infectious disease agent among injecting drug users (IDUs), with seroprevalence ranging from 50-90%. In this paper, serological and virological parameters were investigated among 194 IDUs, 94 ex-IDUs and 95 non-IDUs that were sampled by the “snowball” technique in three localities renowned for both intense drug use and trafficking activities in Salvador, Brazil. The majority of the participants were male, but sex and mean age differed significantly between IDUs/ex-IDUs and non-IDUs (p < 0.05). Anti-HCV screening revealed that 35.6%, 29.8% and 5.3% of samples from IDUs, ex-IDUs and non-IDUs, respectively, were seropositive. HCV-RNA detection confirmed that the prevalence of infection was 29.4%, 21.3% and 5.3% for IDUs, ex-IDUs and non-IDUs, respectively. Genotyping analysis among IDUs/ex-IDUs determined that 76.9% were infected with genotype 1, 18.5% with genotype 3 and 4.6% with a mixed genotype; this result differed significantly from non-IDUs, where genotype 3 was the most frequent (60%), followed by genotype 1 (20%) and a mixed genotype (20%). We report a significantly higher prevalence of HCV infection in IDUs/ex-IDUs compared to the control group (p < 0.001). Although the sample size of our study was small, the differences in HCV genotype distribution reported herein for IDUs/ex-IDUs and non-IDUs warrant further investigation.

Key words: HCV - injecting drug users - HCV genotypes - prevalence - Brazil

It is estimated that 130 million people worldwide are infected with HCV (Flaviviridae family, Hepacivirus genus and hepatitis C virus species), with an additional 3-4 million new infections occurring each year (The Global Burden of Hepatitis 2004, Alter 2007). Injecting drug users (IDUs) are considered to be the main risk group for HCV infection and act as a reservoir for this blood-borne virus. The seroprevalence of HCV varies between 31% to as high as 98% in different parts of the world (Memon & Memon 2002). Unsurprisingly, the use of injecting drugs correlates strongly with HCV infection in Brazil (Carvalho et al. 1996, Oliveira et al. 1999a, Bastos et al. 2000). Risk factors such as polydrug use and the sharing of needles, syringes and equipment for drug injection are common among IDUs. In addition, there is little awareness of HCV and the associated risk of infection within this group (Vidal-Trecan et al. 2000). In the last decade, a declining prevalence of HCV infection has been described in IDUs in different countries, including Brazil (Lopes et al. 2009, Novais et al. 2009, Oliveira et al. 2009).

HCV is classified into six different genotypes, each consisting of different subtypes. The different genotypes are known to differ in their distribution, depending on both geographic region and mode of transmission (Zein 2000). Of the different HCV genotypes, 1 and 3 are the predominant types among blood donors and other groups at risk for transfusion-transmitted infection, such as haemophiliacs and haemodialysis patients in Salvador, Brazil and IDUs in Europe (Bourliere et al. 2002, Silva et al. 2005, 2006). However, the HCV genotype distribution found in these groups may differ from the genotype distribution seen in the general population. (Guadagnino et al. 1997, Zarife et al. 2006). HCV is one of the few microorganisms for which genotyping, besides providing epidemiological information, also provides information with regard to treatment. Treatment is more frequently successful in cases of infection with genotype 2 or 3 than in cases of infection with other genotypes (Francois et al. 2009). From a public health perspective, it is important to know how HCV spreads in the community and how IDUs contribute to the transmission of this infection.

The data from several previous studies have highlighted the need to prevent the spread of HCV and other blood-borne infections among IDUs. In the present paper, we investigated the prevalence of HCV infection among IDUs, ex-IDUs and non-IDUs and the incidence of their respective HCV genotypes.

PATIENTS, MATERIALS AND METHODS

Study population and data collection - This investigation utilises data collected between 2000-2001 from a Brazilian multi-centre study called the AjUDE-Brasil...
II Project. This study was designed to assess HIV infection among drug users (IDUs) and non-IDUs in the following six cities in Brazil: Salvador in the State of Bahia (BA), São José do Rio Preto in São Paulo (SP), Florianópolis and Itajai in Santa Catarina and Porto Alegre and Gravataí in Rio Grande do Sul (Caiaffa 2001, Caiaffa et al. 2006, Cardoso et al. 2006). Informed consent, individual questionnaires and blood samples were collected from all participants. IDUs and ex-IDUs were defined as individuals who had injected drugs in the last two months and those who had injected drugs in the last five years but not in the last two months, respectively. Non-IDUs were drug users that sniffed or smoked cocaine and were defined as potential IDUs. Due to the availability of samples only individuals residing in the city of Salvador, the state capital of BA and the third largest city in Brazil, were included in the present study. The study area comprised three localities that were renowned for both intense drug use and trafficking activities and had implemented the Needle Exchange Program of the Federal University of Bahia (Andrade et al. 2001a, b, MS 2001). All study groups were sampled using the snowball technique (Frankel & Frankel 1977, Biernacki & Waldorf 1981). The participants were classified into the following three groups: 194 IDUs, 94 ex-IDUs and 95 non-IDUs.

**Samples** - Serum samples were collected from each individual for a primary HIV and HTLV study in 2000 (Andrade et al. 2001a, b). Briefly, within 2 h after venipuncture, all samples were aliquoted and stored immediately at -70°C until use. For this study, we avoided RNA degradation by using only those aliquots that had not been thawed more than once prior to molecular testing.

**Serology** - Serological tests for anti-HCV detection were performed at the Bahia State Laboratory (LACEN/SESAB) using commercially available automated immunoassays (MEIA, Axym System, Abbott) according to the manufacturer’s instructions. HCV-RNA extraction was performed using the TRIzol LS reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer’s instructions. After precipitation and drying, HCV-RNA was immediately reverse transcribed into cDNA using random primers (Amersham Biosciences, Piscataway, NJ, USA).

**HCV-RNA detection and genotyping** - cDNA was detected by amplification of the 5’ untranslated region (5’ UTR) in nested PCR using primers 939, 209, 940 and 211, as described previously (Chan et al. 1992). The 251-bp (unlabelled) nested PCR product was analysed by electrophoresis using a 1.5% agarose gel in 1X Tris-borate (TBE) buffer and visualised by ethidium bromide staining and ultraviolet (UV) light. Positive samples were genotyped by restriction fragment length polymorphism analysis according to a previously published method (Davidson et al. 1995). Briefly, restriction enzyme digests were carried out for 4-16 h at 37°C in the presence of 10 units each of (i) Rsal and HaeIII and (ii) HinfI and Mval. Digestion products were analysed by electrophoresis in 4% Metaphor agarose gel (BMA, ME, USA) in 1X TBE buffer containing 0.5 µg/mL ethidium bromide) and were visualised under UV light. Previously characterised genotypes were used as positive controls for genotypes 1, 2 and 3. Genotypes were determined according to the Simmonds classification (Simmonds et al. 1993). Samples containing no or undetectable levels of HCV-RNA were re-extracted at least once more in an independent experiment. Negative individuals were tested again after six months to avoid potential false negative results.

**Data analysis** - The data were analysed using Epi Info, Version 6.04 (Centres for Disease Control and Prevention, USA). Fisher’s exact test and the χ² test (Yates corrected) were used to compare frequencies between groups when appropriate. In all tests, p values less than

| TABLE |
|-------|
| Serological and molecular results from the subjects enrolled in this study, in Salvador, Bahia, Brazil, 2000 |

| Study groups | IDUs % (n) | ex-IDUs % (n) | non-IDUs % (n) |
|-------------|------------|--------------|----------------|
| Anti-HCV positivity | 35.6 (69/194) | 29.8 (28/94) | 5.3 (5/95) |
| HCV-RNA positivity<sup>a</sup> | 83.8 (57/68) | 76.9 (20/26) | 100 (5/5) |
| HCV confirmed infection (95% CI) | 29.4 (57/194) | 21.3 (20/94) | 5.3 (5/95) |
| 23.2-34.5 | 13.8-27.7 | 1.1-9.5 |
| Genotype<sup>a</sup> | | | |
| 1 | 76.5 (39/51) | 78.6 (11/14) | 20.0 (1/5) |
| 3 | 19.6 (10/51) | 14.3 (2/14) | 60.0 (3/5) |
| Mix | 3.9 (2/51) | 7.1 (1/14) | 20.0 (1/5) |

<sup>a</sup>: total varies according to the availability of samples; CI: confidence interval; IDUs: injecting drug users.
0.05 were considered statistically significant. Estimates for 95% confidence intervals (95% CI) of prevalence were calculated using a resampling algorithm with a bootstrap value of 15,000 (BioEstat 4.0, PA, Brazil).

RESULTS

The majority of participants were male, composing 93.8% (182/194) of IDUs, 89.4% (84/94) of ex-IDUs and 80% (76/95) of non-IDUs. The mean age (± standard deviation) was 26.6 ± 7.7 for IDUs, 27.8 ± 6.9 years for ex-IDUs and 23.7 ± 6.4 years for non-IDUs. Sex and mean age differed significantly between IDUs/ex-IDUs and non-IDUs (p < 0.05).

HCV seroprevalence was significantly higher in IDUs and ex-IDUs, reaching 35.6% (95% CI 29.9-41.8%) and 29.8% (95% CI 21.3-37.2%), respectively, compared to 5.3% of non-IDUs (95% CI 1.1-9.5%; p < 0.05) (Table). Viraemia was detectable in the majority of the anti-HCV positive individuals in all three groups. The prevalence of HCV infection among IDUs/ex-IDUs was significantly higher compared to that of non-IDUs (p < 0.05). It was calculated to be 29.4% (95% CI 23.2-34.5%) among IDUs, 21.3% (95% CI 13.8-27.7%) among ex-IDUs and 5.3% (95% CI 1.1-9.5%) among non-IDUs.

HCV genotype distribution was similar between IDUs and ex-IDUs but different among non-IDUs (Table). Among IDUs and ex-IDUs, genotype 1 was the most prevalent genotype, identified in 76.5% (39/51) of IDUs and 78.6% (11/14) of ex-IDUs, followed by genotype 3, which was identified in 19.6% (10/51) of IDUs and 14.3% (2/14) of ex-IDUs. Mixed genotypes were detected in 3.9% (2/51) of IDUs and 7.1% (1/14) of ex-IDUs. Among non-IDUs, genotype 3 was the most prevalent genotype, identified in 60% (3/5) of the individuals, followed by genotype 1 and mixed genotype, both with 20% (1/5). None of the samples contained either genotype 2 or any other genotype. There was a significant difference in the HCV genotype distribution between IDUs/ex-IDUs and non-IDUs (p < 0.05). Some samples could not be genotyped due to low quantity of available serum.

DISCUSSION

The snowball sampling used in this project is a common technique used in the assessment of hidden populations where existing study subjects recruit future subjects from among their acquaintances. The present paper is not intended to be inferential because the samples were collected in a non-random way. However, the comparison of the HCV positivity and HCV genotype distribution between the groups of IDUs/ex-IDUs and non-IDUs seems to be consistent with a history of drug use and a common source of infection, respectively.

It has been shown that HCV prevalence among different populations is higher between the ages of 24-35 (Oliveira et al. 1999b, Bastos et al. 2000, Kapadia et al. 2002). The mean age of IDUs and ex-IDUs enrolled in the present study was 26.6 ± 7.7 and 27.8 ± 6.9 years, respectively. However, needle sharing among individuals belonging to either of these groups led to a five-fold increase in the transmission of HCV compared to non-IDUs. A previous study performed in Salvador and Rio de Janeiro showed that the first shot typically occurs at around 15 years of age (Andrade et al. 2001a, b, Oliveira et al. 2006). Therefore, IDUs are important reservoirs for HCV because they are infected at a much earlier age and have a higher risk of HIV co-infection or other parenterally transmitted viruses, which make HCV more difficult to eliminate.

This study confirms that HCV seroprevalence is significantly higher among IDUs and ex-IDUs (35.6% and 29.8%, respectively) compared to non-IDUs (5.3%). However, this was lower than that reported for studies of IDUs in the cities of Santos (SP) and Rio de Janeiro and for a previous study in Salvador (BA) (74%, 69.8% and 77%, respectively) (Carvalho et al. 1996, Carvalho et al. 1999, MS 2001). The lower seroprevalence observed in our study may be due to the introduction of a harm reduction program in Salvador by the Federal University of Bahia that began four years earlier in the same areas where individuals were recruited. This program resulted in a reduction of needle sharing from 40-17% (Andrade et al. 2001a, b). In countries such as England and Australia, the AIDS prevention programs and harm reduction programs resulted in a concurrent decrease of HCV infection among IDUs (MacDonald et al. 2000, Hope et al. 2001). Of note, HCV seroprevalence in non-IDUs was higher than that observed for the general population of Salvador (5.3% vs. 1.5%) (Zarife et al. 2006). Because the majority of the non-IDUs recruited for this study were cocaine drug users, this difference may be attributable to the sharing of contaminated implements such as straws that are commonly used to inhale this drug (McMahon et al. 2004).

HCV-RNA was detected in more than 70% of the seropositive individuals; this represents a final HCV prevalence of infection of 29.4% among IDUs, which is less than that of haemophiliacs (32.6%) (Silva et al. 2006) and chronic hepatitis outpatients from the same locality (> 50%, unpublished observations). IDUs are currently the main source of infection for persons with hepatitis C (Alter & Sreeff 2000, Farci & Purcell 2000). It is well known that such individuals tend to develop a chronic infection, ultimately leading to chronic hepatitis. Many of these individuals are not clinically ill and are therefore not aware of their infection, leading to activities that contribute to an increase in the incidence of HCV (Vidal-Trecan et al. 2000). Such activities include needle/syringe/equipment sharing, tattooing and body/ear piercing.

HCV genotype distribution varies geographically. Genotypes 1, 2 and 3 are mostly cosmopolitan, while genotypes 4 and 5 are predominant in Africa and genotype 6 is found in Asia (Simmonds et al. 1993). Several studies in Brazil have reported that HCV genotype 1 is more prevalent than genotype 3 among patients who had received a blood transfusion (Silva et al. 2005, 2006). Two population-based studies, one conducted in Italy and another in Brazil, identified a possible switch in the prevalence of these genotypes in the general population, indicating that specific risk factors may play a role in HCV transmission (Guadagnino et al. 1997, Zarife et al. 2006). Our study is the first report on the HCV geno-
type distribution among IDUs in Salvador. The most prevalent genotype among IDUs and ex-IDUs was genotype 1, followed by genotype 3 and mixed infection of genotypes 1 and 3. Of note, genotype 3 was the most predominant among non-IDUs, similar to a previous report for the general population of Salvador (Zarife et al. 2006). Considering that injection of cocaine is the only difference among IDUs/ex-IDUs and non-IDUs, these data emphasise the importance of needle sharing to the acquisition of genotype 1. However, the number of samples that tested positive for HCV among the non-IDUs was small; therefore, further studies will be required to confirm the frequency of genotype 3 in this group.

Finally, we demonstrated a decrease in the seroprevalence of HCV among IDUs from 77-35.6%; however, this result does not eliminate the need for public health interventions focusing on the transmission of HCV in this group (Carvalho et al. 1999, MS 2001). As IDUs are at high risk for parenteral infection, this group could be a better sentinel population for parenteral infection than blood donor candidates for molecular epidemiology purposes. The difference in the HCV genotype distribution observed among IDUs and ex-IDUs when compared to the general population could be attributed to the study design because the non-random sampling intrinsic to the design of snowball sampling may result in an over or under-representation of a given genotype with regard to its actual distribution in the population of IDUs living in the communities under analysis.

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