Research article

Risk factors for hepatitis C virus infection among blood donors in southern Brazil: a case-control study

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Keywords: Hepatitis C, blood donors, intravenous drug abuse, blood transfusion, incarceration, risk factors

Abstract

Background: In Brazil, it is estimated that between 2.5 and 4.9% of the general population present anti-hepatitis C virus (HCV) antibodies, which corresponds to as many as 3.9 to 7.6 million chronic carriers. Chronic liver disease is associated with HCV infection in 20% to 58% of the Brazilian patients. The objective of this case-control study was to investigate the risk factors for presence of anti-HCV antibody in blood donors in southern Brazil.

Methods: One hundred and seventy eight blood donors with two positive ELISA results for anti-HCV were cases, and 356 controls tested negative. A standardized questionnaire was used to collect data concerning demographic and socioeconomic aspects, history of previous hepatitis infection, social and sexual behaviors, and number of donations. Variables were grouped into sets of hierarchical categories. Cases and controls were compared using logistic regression, odds ratios, and 95% confidence intervals. The statistical significance of the associations was assessed through likelihood ratio tests based on a P value < 0.05.

Results: The prevalence of anti-HCV among blood donors was 1.1%. Most of the donors were white and males. In the multivariate analysis, independent predictors of anti-HCV positivity were: intravenous drug use, blood transfusion >10 years earlier, having had two to four sexually transmitted diseases, incarceration, tattooing, sex with a hepatitis B or C virus carrier or with intravenous drug users.

Conclusion: Intravenous drug use, blood transfusion, and tattooing were the main risk factors for anti-HCV positivity among blood donors from southern Brazil, but sexual HCV transmission should also be considered.

Background

The worldwide prevalence of hepatitis C virus infection is estimated by the World Health Organization (WHO) to

be approximately 3% – corresponding to 170 million infected persons [1,2]. In Brazil, WHO estimates suggest that between 2.5 and 4.9% of the general population present anti-HCV antibodies [3]. This corresponds to as many as 3.9 to 7.6 million chronic carriers at risk for developing liver cirrhosis and/or liver cancer. Studies show that among Brazilian patients with chronic liver disease, 20 to 58% are anti-HCV positive [4], and that in southern Brazil 27% of the patients with hepatocellular carcinoma are anti-HCV positive [5].

While the search for an effective therapy [6] and vaccine [7] continues, prevention and control of HCV infection should be the goals of public health efforts. Preventive strategies aim to reduce the exposure to HCV should be based on blood testing of individuals, screening of blood and blood products, sterilization of reusable equipment, destruction of potentially contaminated disposable instruments, and promotion of barrier methods of contraception to prevent sexually transmitted diseases [2]. To increase the efficacy of these interventions, it is important that the main risk factors for HCV infection in different populations be known. Therefore, the present case-control study was designed to evaluate risk factors in a representative sample of blood donors in southern Brazil.

Methods

Study population and design

A sample of blood donors was randomly selected from eight blood centers in Porto Alegre, state of Rio Grande do Sul, Brazil, out of 11 centers available in the city. Three centers were not included due to the small number of donors. The number of blood donors sampled was proportional to the number of donors of each center. A standard predonation interview was conducted at each center to exclude donors potentialy ill or who reported previous hepatitis or jaundice, and drug addiction. Cases were donors with two positive enzyme-linked immunosorbent assay (EIA) results for anti-HCV carried out using the same manufacturers' kits. Controls were blood donors who tested negative for anti-HCV. Two frequency-matched controls were selected next to a case in the same center.

Blood tests

All blood samples were screened for anti-HCV, hepatitis B surface antigen, hepatitis B core antibody, HIV-1, human T-cell lymphotropic virus types 1 and 2, syphilis (Sexually Transmitted Disease Research Laboratory), *Trypanosoma cruzi* (etiologic agent of Chagas' disease) and alanine aminotransferase (ALT), using licensed assays. Serological testing for HCV was performed according to standard procedures at the eight blood centers. Initial screening was by second-generation EIA and, after January 1996, by third-generation EIA. The type, generation, and manufacturer of tests varied with blood center.

Data collection

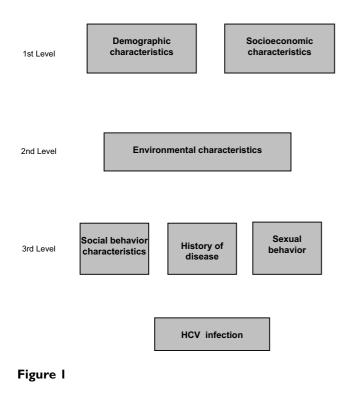
Data were collected between August 1995 and October 1996. We collected information concerning demographic (gender and age) and socioeconomic (education) aspects, parenteral exposure to blood or blood products, social and sexual behavior, occupational exposure, personal history of jaundice or hepatitis or history of these diseases in the donor's family, and signs and symptoms of human immunodeficiency virus infection. All patients agreed to participate and signed a consent form. Research assistants interviewed cases and controls on a private room at each center, using a standardized questionnaire. Because of the sensitive character of some questions, female or male interviewers were assigned according to the gender of the respondent. The pretested instrument, which was identical for cases and controls, included questions concerning detailed history of blood exposure on previous transfusion and occupational hazard (unintentional needle-stick injuries, for instance), intravenous drug use, tattooing, acupuncture, surgery, previous hospitalization and parenteral administration of drugs. Other questions included age at first intercourse, number of sexual partners (risk category was determined separately for men and women according to the median), history of sexually transmitted diseases -STD- (gonorrhea, syphilis, or an open sore), sex with partners who had been intravenous drug users or who had had hepatitis.

Sample size calculation and data analysis

The calculated sample size of 411 donors (137 cases and 274 controls) allowed detection of a 2.0 odds ratio (OR), with an 80% power at a 0.05 significance level (two tailed), and an exposure prevalence ranging from 15 to 50%. The sample size was increased in approximately 30% to allow assessment of less prevalent types of exposure and adjustment for confounding variables on multi-variate analyses.

Data entry was checked for consistency and accuracy using the Epi-Info software, version 6.04, and the analysis was conducted using the Statistical Package for the Social Sciences, version 8.0.

Cases and controls were compared using logistic regression analysis through odds ratios (OR) and 95% confidence intervals (95%CI). Variables were grouped into sets of hierarchical categories that guided the analysis [8]. The hierarchy assumes that each set of distal variables influences the level immediately below or the same level; however, the distal set is not affected by proximal determinants, since it is likely that the effect of this distal set will be mediated through the proximal determinants (Figure 1). According to this conceptual model, the socioeconomic level may determine all variables being studied, except for gender and age. The variables associated ($P \leq$



0.10) with presence of a positive anti-HCV test in the univariate analyses were selected to be part of the final model. Independent risk factors were evaluated after adjustment for confounding variables in the same set or in hierarchically superior sets. This approach enables quantification of the contribution of each level of adjustment, understanding of the model-building strategy, and interpretation of independent associations. Crude as well as adjusted odds ratios were presented according to the hierarchical framework. The presence of confounding bias was detected through the change in odds ratio before and after adjustment for confounding factors. The adjusted odds ratio did not result from the full model with all variables, but from the equation corresponding to the level in which each risk factor was first entered. This avoids the possibility that intermediate variables will remove some of the explanatory power of more distant determinants. The statistical significance of the associations was assessed through likelihood ratio tests based on a P value < 0.05. Results with a P value > 0.05 and <0.10 were interpreted as having a trend for association.

Results

Description of the sample

The overall prevalence of anti-HCV was 1.1% (95%IC = 0.3–1.7), corresponding to 711 repeatedly reactive EIA tests out of 66,414 blood donations in all centers in the city of Porto Alegre between August 1995 and October

1996. A representative sample of 178 anti-HCV positive cases and 356 controls were investigated.

Most of the donors were white (85%) and males (73%). These characteristics were equally distributed among cases and controls. Table 1 shows that the risk for HCV seropositivity increased in 30 to 59 year-old blood donors and schooling was inversely associated with HCV seropositivity. Donors who were illiterate or who had completed less than five years of school were approximately three times more likely to be anti-HCV positive when compared to college-educated donors. First-time donors had twice the risk for anti-HCV positivity.

Environmental exposures

Table 2 indicates that environmental exposure, such as having lived in a shelter or boarding school, was likely to increase the risk, but household contact with a relative with jaundice or hepatitis was not associated with HCV seropositivity. Donors who had been inmates in a prison or who had lived in juvenile detention centers presented an increase of approximately five times in the risk for HCV seropositivity. However, working in closed institutions, such as prisons, having been enrolled in the army, and occupational blood exposure did not increase the risk for a positive anti-HCV test.

Table I: Distribution of demographic and socioeconomic charac-
teristics of cases and controls

Variable	% cases (n = 178)	% controls (n = 356)	OR (95%CI)	
Age (years)				
Ĩ18–21	6.2	15.4	1.0	
22–29	21.3	26.7	2.3 (1.1–5.3)	
30–39	38.2	31.5	3.5 (1.7–7.8)	
40–59	34.3	26.4	3.8 (1.8–8.4)	
P value			0.003	
Schooling (years)				
12-15	13.5	27.5	1.0	
9-11	27.5	30.1	1.8 (1.0–3.4)	
5–8	40.4	30.9	2.7 (1.6-4.8)	
0-4	18.5	11.5	3.2 (1.7-6.6)	
P value			<0.0001	
Number of				
previous donations				
≥I	56.7	72.5	1.0	
None	43.3	27.5	2.0 (1.3-3.0)	
P value			<0.0001	

Variable	% cases (n = 178)	% controls (n = 356)	Unadjusted OR (95%CI)	Adjusted OR (95%CI)*
Lived in shelter/boarding school P value	11.2	6.7	I.8 (0.9–3.4) 0.08	1.9 (1.0–3.6) 0.07
Household contact with jaundice or hepatitis P value	18.5	14.0	I.3 (0.8–2.3) 0.2	I.6 (0.2–2.6) 0.08
Incarceration P value	11.8	2.0	6.7 (2.6–17.7) <0.0001	5. 2 (2.1–12.8) 0.0003
Enrolled in the army P value	24.7	27.5	0.8 (0.6–1.3) 0.5	0.9 (0.6–1.4) 0.6
Worked at detention center or prison P value	2.2	2.5	0.8 (0.2–3.2)	0.8 (0.2–2.7) 0.7
Occupational blood exposure P value	7.9	9;8	0.8 (0.4–1.6) 0.5	0.6 (0.3–1.3) 0.2

Table 2: Environmental risk factors for HCV seropositivity among blood donors from southern Brazil

*OR adjusted for age, schooling, living in a shelter/boarding school and incarceration.

Table 3: Previous morbidity as risk factor for HCV seropositivity test among blood donors in southern Brazil

Variable	% cases (n = 178)	% controls (n = 356)	Unadjusted OR (95%CI)	Adjusted OR (95%CI) [*]
Time since last blood transfusion (years)				
>10	9.6	2.5	4.2 (1.8–9.6)	9.9 (3.6–27.2)
≤ I0	19.0	24.4	0.9 (0.5-1.4)	0.8 (0.4–1.5)
P value			0.002	<0.00001
Time since hospital admission for clinical treatment (years)				
≤ I0	14.0	8.7	1.9 (1.1–3.3)	2.2 (1.0-4.6)
>10	17.4	11.8	1.7 (1.0–2.9)	1.9 (1.0-3.6)
Any time	1.1	0.3	4.7 (0.4–52.3)	2.9 (0.1–57.I)
P value			0.03	0.09
Time since parenteral drug treatment (years)				
≤ I0	24.2	31.2	0.8 (0.5–1.3)	0.6 (0.3–1.2)
>10	27.5	22.2	1.3 (0.8-2.0)	1.1 (0.6–2.0)
Any time	5.1	1.1	4.7 (1.4–15.9)	3.9 (1.0–15.3)
P value			0.02	0.05
Acupuncture therapy	5.6	4.2	1.4 (0.6–3.4)	2.6 (0.9-7.2)
P value			0.5	0.08
Previous jaundice	9.6	2.0	4.6 (1.8–11.9)	4.6 (1.9–11.3)**
P value			<0.0001	0.001

*OR adjusted for age, schooling, living in a shelter/boarding school, incarceration, tattooing, intravenous drug use, blood transfusion, personal history of previous jaundice, number of lifetime STDs, sexual intercourse with intravenous drug user. ** OR adjusted for age, schooling, living in a shelter/boarding school and incarceration.

Previous morbidity

Regarding history of morbidity (Table 3), the risk for HCV seropositivity was approximately 10 times higher in blood donors who had received blood transfusion more than 10 years before the study than in those not exposed to transfusion. The effect of this factor became more evident after adjustment for confounding factors. There was a trend for

association of positive anti-HCV test with history of previous parenteral treatment, hospital admission for clinical treatment, and acupuncture therapy. HCV seropositivity was also associated with personal history of jaundice, with an adjusted OR of 4.6.

Variable	% cases (n = 178)	% controls (n = 356)	Unadjusted OR (95%Cl)	Adjusted OR (95%CI) [*]
Social behavior				
Tattooing	15.2	4.2	4.1 (2.0-8.3)	4.4 (1.6–11.9)
P value			<0.001	0.004
Intravenous drug use	25.3	0.3	120.1 (17.7–2368.7)	105.2 (12.8-865.3)
P value			<0.000 I	<0.00001
Sexual behavior				
Age of the first sexual intercourse (years)				
≥ 18	24.9	31.1	1.0	1.0
15–17	43.5	44.7	1.2 (0.8–1.9)	0.7 (0.4–1.4)
<15	31.6	24.2	1.6 (1.0–2.7)	1.0 (0.5–1.7)
P value			0.1	0.6
Number of sexual partners (median)**				
Past ten years				
Male <5 / Female <2	57.1	61.5	1.0	1.0
Male \geq 6 / Female \geq 2	42.9	38.5	1.2 (0.8–1.7)	1.3 (0.8–2.2)
P value			0.3	0.3
Lifetime				
Male \leq 10 / Female \leq 2	46.3	56.7	1.0	1.0
Male \geq 11 / Female \geq 3	53.7	43.3	1.5 (1.1–2.2)	0.8 (0.5-1.3)
P value			0.02	0.4
Number of lifetime sexually transmitted diseases				
0	49.2	76.9	1.0	1.0
I	41.2	21.9	3.0 (1.9–4.5)	2.0 (1.1–3.5)
2-4	9.6	1.1	13.2 (4.0-47.7)	6.2 (1.5-25.3)
P value			<0.0001	0.006
Sexual orientation				
Heterosexual	84.2	95.1	1.0	1.0
Bisexual/homosexual	15.8	4.9	3.7 (1.9–7.3)	0.8 (0.3-2.2)
P value			<0.0001	0.7
Sexual intercourse with intravenous drug user	8.0	2.0	4.8 (1.8–13.3)	3.5 (1.2–9.8)
P value			0.001	0.02
Sexual intercourse with a partner with hepatitis B or C virus	4.5	1.7	2.8 (0.9-9.3)	3.7 (1.1–12.8)
P value			0.04	0.04

Table 4: Social and sexual behavior as risk factor for positive HCV test among blood donors from southern Brazil

*OR adjusted for age, schooling, living in a shelter/boarding school, incarceration, tattooing, intravenous drug use, blood transfusion, personal history of previous jaundice, number of lifetime STDs, sexual intercourse with intravenous drug user. ** 177 cases and 351 controls.

Social and sexual behavior

Table 4 shows the association of social behavior (tattooing and intravenous drug use) and sexual characteristics with anti-HCV seropositivity. Donors with a tattoo were approximately four times more likely to be anti-HCV positive than donors without tattoos. The rate of intravenous drug use was greater among cases (25.3%) in comparison with controls (0.3%). Intravenous drug use was the strongest independent risk factor for HCV seropositivity.

Cases were younger at their first sexual intercourse and had a higher number of lifetime partners than controls. However, these differences did not reach statistical significance after adjustment for confounding factors. Cases were more likely to have had one or more sexually transmitted diseases during life than controls. This resulted in an OR six times higher for presence of anti-HCV. Sexual intercourse with an injection drug user tripled the risk for HCV seropositivity. Of the 178 cases, 7.9% reported having had sexual intercourse with a partner with hepatitis (vs. 3.4% of the controls), which was associated with a 2.7 fold (95% CI 1.0–7.4) increase in the risk for anti-HCV positivity. This association was even more evident in individuals who had a sexual partner with hepatitis B or C. Sexual intercourse with a partner with hepatitis B or C resulted in a 3.7 fold increase in the independent risk for HCV seropositivity.

Discussion

This case-control study shows that first-time blood donors, as well as 30 to 59 year-old donors and less educated donors were at higher risk for HCV seropositivity. In addition, our results confirm that injection drug use, blood transfusion, incarceration, tattooing, history of sexually transmitted diseases, and sexual intercourse with injection drug users, partners with hepatitis, or carriers of HBV or HCV increase the risk for a positive anti-HCV test. In the study of risk factors associated with the presence of HCV antibodies, the potential for bias must be ruled out. In the present study, the sampling process enabled a representative sample of donors in the city of Porto Alegre to be detected and enrolled. The inclusion of center-matched control donors with a negative HCV test avoided bias related to differences in the recruitment policy and in the anti-HCV test employed in each center. Immunoblot tests and polymerase chain reaction (PCR) or transcriptionmediated amplification (TMA) were not adopted as a routine to test blood donors in Brazil. Even though the specificity of currently available EIAs for anti-HCV is high, the positive predictive value is lower in blood donors [9]. Accordingly, we avoided to label the cases as HCV infected, but anti-HCV seropositives.

An attempt was made to minimize recall bias by asking cases and controls to report information about the same period, and by using the same standardized questionnaire. Since most episodes of hepatitis C are free of symptoms, cases were as likely as controls to recall exposures potentially associated with HCV infection.

When interpreting the results of this study, it is meaningful to bear in mind that the eligible donors were in good health and did not represent the general population.

First-time blood donors had twice the risk for presenting a positive HCV test. This association is plausible, since first-time donors have never been screened [10,11]. The test-seeking behavior for infectious diseases is unlikely as the Brazilian public health care system offers alternative test sites. In addition, first-time blood donors are less likely to report exposure to risk factors for HCV infection. For instance, in the United States, the deferrable risk (defined as estimated prevalence rate for risk behaviors that would have been a basis for donation deferral if reported at the time of the donor screening among first-time donors was 1.6 times greater than among repeated donors [12]. In this study, 26% of all donors presented a history of jaundice, incarceration, injection drug use, and tattooing. These donors should have been excluded in the predonation interview, according to the Brazilian criteria for blood donation [13].

Concerning demographic variables, the increase in the risk for HCV seropositivity between 30 and 59 years was different to that described for donors in Australia [14] or the United States [15,16], where the infection rate declines in persons greater than 50 years old, and similar

that described in other region of our country [17]. This pattern of transmission suggests that the risk for HCV infection was greater in the distant past [18] and reached a decreased risk of infection along generations as consequence of improving sanitary and socioeconomic conditions over time.

Our results show an inverse and statistically significant association between positive HCV testing and schooling – the risk was greater among illiterate donors or donors with little schooling. These socioeconomic factors have been previously described as acting through risk factors such as illicit drug use [15] or unsafe injection practices [19].

In terms of environmental exposure, having been incarcerated in a juvenile detention center or prison was the main risk factor, independently associated with a five-fold increase in positive HCV testing. The association between a previous imprisonment and positive HCV testing is a proxy of the true risks. The risk for inmates to be HCV positive has been associated with injection drug use, tattooing and previous incarceration [20–26]. In addition, the possibility of person-to-person HCV transmission through exposure to contaminated blood cannot be dismissed [27].

Our study shows that exposure to blood was, and still is, the main risk factor for HCV infection. Blood transfusion more than 10 years ago was associated with positive HCV testing, and the association became even stronger after adjustment for confounding factors. The residual risk for transfusion-transmitted hepatitis C infection decreased after Brazilian blood centers adopted anti-HCV screening tests 10 years ago, but it is still higher than in developed countries [28]. As in other studies [10,11,17,29-33], we observed that illegal injection drug use is the dominant mode of HCV transmission, mostly due to sharing of needles or syringes [34]. Approximately 75% of Brazilian injection drug users are HCV seropositive [35], and 85% of them share needles [36]. In addition, other parenteral exposure, such as tattooing, was independently associated with HCV seropositivity. Tattooing still is not a regulated businesses and, in the past, most of the tattoing occurred at places where contamination were more likely. This potential risk for HCV transmission in blood donors has been previously reported [11,30]; presumably, non-sterile or non-disposable equipment is the source of transmission [37]. HCV seropositivity was also independently associated with personal history of jaundice; however, jaundice may be a marker of disease, rather than a risk factor. In this study, the prevalence of HCV infection was not associated with employment in a health-related occupation. Although occupational exposure can result in infection, [38,39] only 8.9% of blood donors were health care workers and 32% did not handle blood samples.

The importance of sexual activity in the transmission of HCV has not been well-established [37]. Nevertheless, studies with blood donors suggest an association between sexual transmission and HCV [10,11,17,31,40]. This study detected an independent association between sexual behavior and positive anti-HCV test. The results of the logistic regression model show that the number of sexually transmitted diseases during life and sexual intercourse with injection drug users and with HBV or HCV carriers were independent risk factors for anti-HCV positivity. Even though number of sexual partners and sexual orientation were associated in the bivariate analysis, they were not independent of other risk factors such as transfusion and injection drug use by the donor or his/her sexual partner. In South America, the prevalence of HCV infection in heterosexuals attending STD clinics and with no history of injection drug abuse (11.5%) is greater than in North America (6.0%) and Europe (1.9%) [37]. It is speculated that HCV transmission may be facilitated by sexually transmitted diseases [41,42]. However, there is a possibility that HCV seropositivity associated with sexual intercourse with injection drug users or HBV/ HCV carriers may result from actual sexual transmission of HCV - although it may also result from exposure to unreported parenteral risk factors or from sharing certain personal items, such as toothbrushes or razors, which can result in accidental exposure to the partner's blood [11,33,42].

In conclusion, this case-control study confirmed some independent risk factors for anti-HCV positive test described in other studies of blood donors. The hierarchical multivariate analysis identified age, schooling, incarceration, past transfusion, tattooing, intravenous drug use, number of sexually transmitted diseases, sexual intercourse with injection drug users and partners with hepatitis B or C as the main risk factors for HCV seropositivity in the present population of southern Brazilian donors. Some characteristics of sexual behavior were detected as independent risk factors, suggesting that sexual intercourse is an important source of transmission. The identification of groups at risk and the planning of interventions aimed at controlling HCV infection should take these findings into account.

Competing interests

None declared.

Authors' contributions

These authors contributed equally to this work. All authors read and approved the final manuscript.

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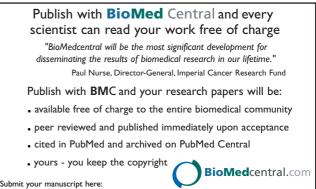
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