

ANTI-HCV RELATED TO HCV PCR AND RISK FACTORS ANALYSIS IN A BLOOD DONOR POPULATION OF CENTRAL BRAZIL

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SUMMARY

Data concerning HCV infection in Central Brazil are rare. Upon testing 2,350 voluntary blood donors from this region, we found anti-HCV prevalence rates of 2.2% by a second generation ELISA and 1.4% after confirmation by a line immunoassay. Antibodies against core, NS4, and NS5 antigens of HCV were detected in 81.8%, 72.7%, and 57.5%, respectively, of the positive samples in the line immunoassay. HCV viremia was present in 76.6% of the anti-HCV-positive blood donors. A relation was observed between PCR positivity and serum reactivity in recognizing different HCV antigens in the line immunoassay. The majority of the positive donors had history of previous parenteral exposure. While the combination of ALT>50 IU/l and anti-HBc positivity do not appear to be good surrogate markers for HCV infection, the use of both ALT anti-HCV tests is indicated in the screening of Brazilian blood donors.

KEYWORDS: HCV; Anti-HCV; PCR; Risk factors; ALT; Anti-HBc; Blood donors.

INTRODUCTION

Following the isolation and characterization of the genome of hepatitis C virus (HCV) ⁴, studies have shown that HCV is responsible for the majority of cases of post-transfusion hepatitis in the world ¹¹. Chronic liver disease develops in more than half of the individuals with hepatitis C which many of these patients subsequently developing hepatocellular carcinoma ^{1, 7, 12}.

The prevalence of anti-HCV among blood donors has been found to differ with geographic location. For example, in Europe, higher prevalence rates (>1.0%) were found in Spain ⁷ and Italy ¹⁵ than in France ², and

lower rates (<0.5%) were observed in the United Kingdom ⁸ and Scandinavia ¹⁰.

In Brazil, a recent study in the blood donor population of Rio de Janeiro (Southeastern Region) revealed a high anti-HCV prevalence of 2.7% after confirmation testing ¹⁹. As data concerning HCV infection in other Brazilian regions are still rare, we sought to assess the prevalence of anti-HCV in a blood donor population of Central Brazil and compared these results with data using surrogate markers (ALT and anti-HBc) for non-A, non-B hepatitis (NANBH). In addition, we examined possible

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risk factors for HCV infection in the group of the positive donors and to evaluate the relation between an anti-HCV confirmatory assay and the detection of HCV RNA by the polymerase chain reaction (PCR).

SUBJECTS AND METHODS

Blood Donor Population

Between September 1992 and February 1993, we tested sera from 2,350 voluntary blood donors accepted for blood donation after clinical evaluation at the State Blood Bank in Goiânia, Central Brazil. This population ranged in age from 18 to 62 years (average age 31.8 years). The large majority of the donors (95.7%) were men. Seventy percent of donors earned less than US\$ 200 per month and had a low level of education.

All screened anti-HCV-positive donors were summoned for a clinical evaluation and interview. In addition, 50 (94.3%) donors returned a questionnaire exploring possible risk factors (blood transfusions, intravenous drug use, tattoos, acupuncture, injections with nondisposable needles and syringes, occupational or other exposure to blood, history of dental procedure with non-licensed dentists, sexual or household contacts with persons having hepatitis, number of partners and history of sexually transmitted diseases).

Serological Tests

Anti-HCV antibodies were detected by a second generation assay using a mixture of core, NS4 and NS5 antigens (Innotest HCV Ab, Innogenetics, Belgium). Positive samples were retested for confirmation with a line immunoassay using immunodominant epitopes of NS4, NS5, and HCV core (Inno-LIA HCV Ab, Innogenetics).

All donors were tested for HBsAg (Auszyme monoclonal, Abbott) and for anti-hepatitis B core antibody (anti-HBc; Uni-Form, Organon Teknika). A total of 1,233 sera were also tested for ALT levels by a colorimetric method¹⁶. Donated blood with ALT levels higher than 50 IU/l were discharged.

All tests were performed according to the manufacturer's instructions.

PCR

Nested PCR primers were used as described by STUYVER *et al.*¹⁸. They were complementary to the conserved areas of the 5'UR of the different HCV types. Viral RNA was extracted from 100 µl of sera,

essentially as described by CHOMCZYNSKI & SACCHI³. The RNA pellet was resuspended in 12 µl of diethyl-pyrocyanate-treated water. A 10-min denaturation step at 70°C was performed, after adding 2 µl of 150 ng/µl random primers (Pharmacia). First-strand cDNA synthesis was carried out in a 20-µl reaction mixture at 42°C in the presence of 25 units (U) human placental ribonuclease inhibitor (Amersham), 500 µM dATP, dCTP, dTTP and dGTP, 1 x avian myeloblastosis virus (AMV) buffer (Stratagene) and 2.5 U AMV reverse transcriptase (Stratagene). PCR was performed using the total cDNA synthesis product amplified during 40 cycles, each consisting of 30 sec at 95°C, 1 min at 55°C, and 1 min at 72°C in a total volume of 50 µl. The solution was adjusted to a final concentration of 200 µM dATP, dCTP, dTTP, and dGTP, 1 x Taq buffer (Stratagene), 0.2 µM of each primer, and 1 U Taq Polymerase (Stratagene). From the first round of amplification, 1 µl product was amplified again with the nested primers for 40 cycles in buffer of the same composition. The PCR products from both runs 1 and 2 were separated on agarose gel and visualized under U. V. light after staining with ethidium bromide.

Statistical Methods

The Chi-square test was used for statistical analysis.

RESULTS

Of the 2,350 tested blood donors, 53 (2.2%) were found to be anti-HCV-positive in the screening test (ELISA), and 33 subsequently confirmed positive by the Inno-LIA HCV Ab assay, resulting in an anti-HCV prevalence of 1.4%. This donor population showed prevalence rates of 0.8% and 14.7% for HBsAg and anti-HBc, respectively (Table 1).

TABLE 1

Prevalence rates of hepatitis C and B viral markers in blood donors, Central Brazil.

Viral markers	Positive	
	N°	%
Anti-HCV/ELISA	53	2.2
Anti-HCV/LIA	33	1.4
HBsAg	20	0.8
Anti-HBc	346	14.7
Total tested	2350	100.0

LIA: Inno-LIA HCV Ab, Innogenetics

Antibodies against core, NS4, and NS5 antigens of HCV were detected in 81.8%, 72.7%, and 57.5% of the positive samples, respectively. Sixty-two percent of ELISA-positive samples were confirmed by Inno-LIA HCV Ab test. All sera with a OD = >1.0 reacted positively in Inno-LIA, but only 23% were confirmed positive among those with an OD <1.0 (Table 2). All the lower OD positive samples reacted with one HCV antigen (core or NS4 or NS5) in Inno-LIA. One serum reacted only with the NS5 antigen. This sample was found to be HCV-RNA positive by nested PCR.

TABLE 2

Correlation between optical density and positivity by LIA in ELISA anti-HCV-positive samples from Central Brazil blood donors.

LIA	ELISA - OD		
	<1.0	1.0-2.0	>2.0
Core	2	1	4
NS4	3	1	0
NS5	1	0	0
Core/NS4	0	1	1
Core/NS5	0	0	2
NS4/NS5	0	0	1
Core/NS4/NS5	0	5	11
Negative	20	0	0
Total	26 (23)	8 (100)	19 (100)

LIA: Inno-LIA HCV Ab, Innogenetics

Values in parentheses are percentages of positive samples in Inno-LIA.

The correlation between HCV viremia and positivity by Inno-LIA is shown in Table 3. HCV RNA was detected in 76.6% (23/30) of the anti-HCV-positive donors (Inno-LIA HCV Ab). Among the PCR-positive samples, 15 (65.2%) were positive after the first run and 8 (34.8%) became detectable after the second run. Individuals with HCV viremia more often showed intense reactivity with the Inno-LIA HCV Ab assay. Among the 7 anti-HCV-positive donors who were PCR negative, 2 reacted to all three viral antigens and 5 reacted with core and/or NS4 antigens. Thirteen samples which were anti-HCV negative in Inno-LIA HCV Ab assay were also PCR negative.

When a combination of ALT > 50 IU/l and anti-HBc positivity was used as surrogate markers for HCV infection, only 3.0% of anti-HCV positive sera would have been eliminated. If elevated ALT alone or anti-HBc positivity had been used as a surrogate marker, 18.2% of the samples with elevated ALT levels or 27.3% of the anti-HBc-positive samples would have

been discharged among the anti-HCV-positive donated blood (Fig. 1). Only 2.4% in 1,233 blood donors were found to have elevated ALT levels (>50 IU/l). Anti-HCV prevalence rates of 20% and 1% occurred in donors with elevated and normal ALT levels, respectively ($p < 0.001$). Finally, 2.9% of the donors who had circulating anti-HBc antibodies were anti-HCV positive (data not shown).

Ninety-one percent of the donors who reacted positively with Inno-LIA HCV Ab were interviewed for possible risk factors. Among them, 70% had parenteral exposure to one or more factors: tattoos (23.3%), blood transfusion (13.3%), intravenous drug use (13.3%), occupational or other exposure to blood contact (13.3%), dental procedure with non-licensed dentists (10%), acupuncture (3.3%), Indian ritual scarification practices (3.3%). Among those cases not associated with parenteral exposure (30%), 6 (20%) had multiple partners and 10% did not mention any known source for infection. Only 10% (3/30) mentioned a previous history of hepatitis (data not shown).

DISCUSSION

In Central Brazilian blood donors, we found anti-HCV prevalence rates of 2.2% using a second generation ELISA, and 1.4% after confirmation by Inno-LIA. Although values are lower than those found among donors in Rio de Janeiro (Southeastern Region)¹⁹, they exceed anti-HCV prevalence rates in Southern Europe^{7, 15}. We also measured two serologic markers for HBV infection. The prevalence rates of HBsAg and anti-HBc were 0.8% and 14.7%, respectively, attesting to this

TABLE 3

Correlation between HCV viremia and positivity by LIA in ELISA anti-HCV-positive samples from Central Brazil blood donors.

LIA	PCR		
	Positive	Negative	N° tested
Core	4	2	6
NS4	2	2	4
NS5	1	0	1
Core/NS4	1	1	2
Core/NS5	1	0	1
NS4/NS5	1	0	1
Core/NS4/NS5	13	2	15
Negative	0	13	13
Total	23	20	43

LIA: Inno-LIA HCV Ab, Innogenetics

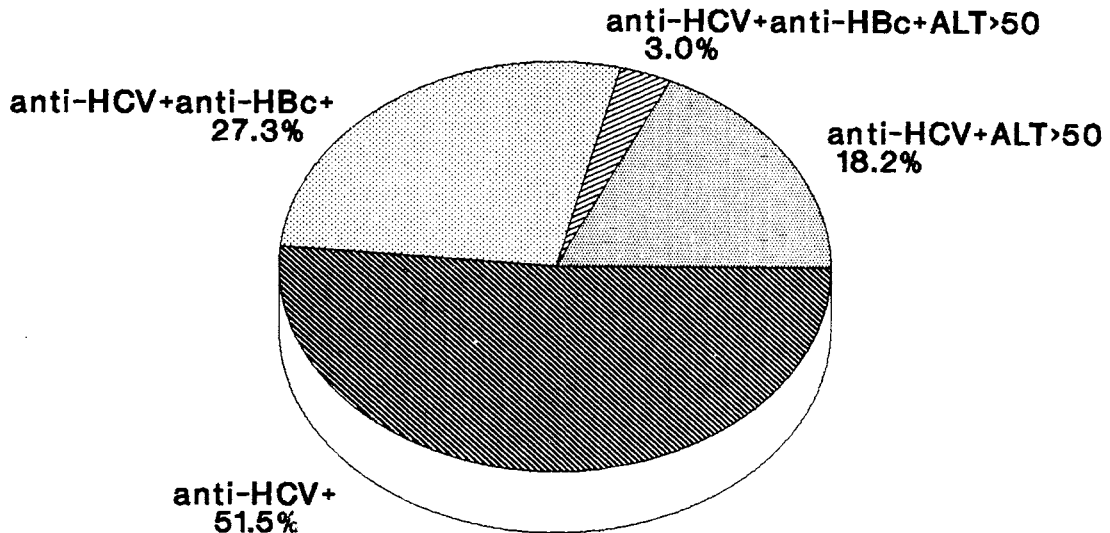


Fig. 1 - Correlation between anti-HCV positivity and surrogate markers for NANBH1. Central Brazil

region as an area of low to intermediate endemicity for HBV. Among the anti-HCV positive donors, none of them was HBsAg positive, but 27.3% had anti-HBc antibodies.

The present investigation did not reveal any significant trend of anti-HCV positivity in relation to age, although a higher anti-HCV prevalence (1.8%) was observed in donors between 31-40 years old (data not shown). There was no significant relation between anti-HCV prevalence and sex. Prevalence in male donors (1.4%) differed little from that found in pregnant women (0.9%) (submitted for publication).

Screening low-risk populations such as blood donors can give a substantial number of anti-HCV false-positive results, mainly when lower ELISA optical densities are obtained. We observed that 62% of ELISA positive samples were confirmed by Inno-LIA HCV Ab, and only 23% of the sera with an OD<1.0 were also positive in Inno-LIA HCV Ab.

HCV RNA was present in 76.6% of the anti-HCV-confirmed positive donors. HCV viremia was more often detected in the most reactive samples by Inno-LIA HCV Ab: among 15 sera which reacted with all antigens (core, NS4 and NS5), 13 (86.7%) were PCR positive. A substantial percentage (80%) of the donors with antibodies to the core antigens were HCV RNA positive, in agreement with others studies^{5, 14, 21}. How-

ever donors reactive to only one antigen (core or NS4 or NS5) by Inno-LIA HCV Ab may still be infectious. Seven blood donors who were LIA anti-HCV positive but PCR negative had lower ALT levels. These donors may have "recovered" from HCV infection.

In agreement with observations on blood donors in Rio de Janeiro¹⁹, our results indicate that elevated ALT values in combination with anti-HBc reactivity are not recommended as surrogate markers for HCV infection in Brazilian blood donors. If anti-HBc alone had been used as surrogate marker, then about 15% of the donated blood would be discharged of which only 2.9% would be positive for anti-HCV. By contrast, if elevated ALT levels (>50 IU/l) alone are considered, 2.4% of the donated blood would be excluded from transfusion and, among these, 20% would have been anti-HCV positive.

In Brazil, anti-HCV testing is gradually being introduced in the blood banks. We observed that the majority of anti-HCV-positive donors had previous exposure to one or more risk factors. Therefore, a better interview for risk factors before blood donation is necessary.

Unfortunately, due to the delay in the specific antibody anti-HCV appearance, the current tests might be negative in the early phase of HCV infection^{13, 20}. Therefore, ALT testing should be maintained in order

to identify donors in the "window" period of HCV infection, and for the indication of blood units infected with other NANBH viruses^{6,9,17}.

It is not known whether the RNA sequences detected by PCR are associated with infectious virus, but positive donors should be considered potentially infectious and be prevented from donating blood. Indeed, in the present study, the majority of HCV RNA positive donors had normal ALT levels (74%), and had no history of hepatitis (90%). Further studies are necessary to evaluate the clinical course of these apparently healthy carriers.

RESUMO

Anticorpo anti-HCV relacionado a detecção do HCV por PCR e análise de fatores de risco em uma população de doadores de sangue do Brasil Central

Ainda são raros os dados sobre a infecção pelo vírus da hepatite C (HCV) na região central do Brasil. Neste estudo, 2.350 doadores voluntários de sangue foram avaliados, resultando em prevalências para o anti-HCV de 2,2%, pelo ELISA de segunda geração, e de 1,4%, após o ensaio confirmatório "line immunoassay". Anticorpos contra os antígenos "core", NS4 e NS5 do HCV foram detectados em 81,8%, 72,7% e 57,5% das amostras positivas no "line immunoassay", respectivamente. A viremia do HCV foi observada em 76,6% dos doadores anti-HCV positivos. A positividade na reação em cadeia da polimerase (PCR) mostrou-se relacionada a reatividade aos diferentes antígenos do HCV no "line immunoassay". A maioria dos doadores positivos tiveram história prévia de exposição parenteral. A combinação de ALT > 50 UI/l e positividade ao anti-HBc parece não ser eficaz como marcadores indiretos para a infecção pelo HCV, entretanto a dosagem do ALT e a detecção de anti-HCV são indicadas na triagem de doadores de sangue brasileiros.

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