

BRIEF COMMUNICATION

DISTRIBUTION OF HEPATITIS C VIRUS GENOTYPES AMONG BLOOD DONORS FROM MID-WEST REGION OF BRAZIL

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SUMMARY

In order to investigate the hepatitis C virus (HCV) genotypes in mid-west region of Brazil, 250 anti-HCV positive blood donors were studied. Among them, the anti-HCV serological status was confirmed in 205 (82%). HCV RNA was detected in 165 samples, which were genotyped. HCV types 1, 2 and 3 were found in 67.9%, 3% and 29.1% of the donors, respectively. In Goiás state, subtype 1a (50%) was the most prevalent, followed by subtypes 3a (30.9%) and 1b (16.7%). In Mato Grosso state, subtype 1a was also predominant (41%), followed by subtypes 1b (29.5%) and 3a (25%). In Mato Grosso do Sul state, subtypes 1a and 1b were detected equally (36.8%), followed by 3a (21.1%). Subtype 2b was rare (2.4%, 4.5% and 5.3%, respectively). In Distrito Federal, subtype 3a (39%) was more frequent than 1a (31.7%) and the remaining (29.3%) belonged to subtype 1b.

KEYWORDS: Hepatitis C virus; Genotypes; Blood donors; Brazil.

INTRODUCTION

Hepatitis C virus (HCV) is a well-known agent of chronic infection, including cirrhosis and hepatocellular carcinoma, with an estimated 170-200 million carriers around the world¹. HCV has a positive-sense, single-stranded RNA genome that presents a high mutation rate (1.44 x 10⁻³ substitutions/site/year). HCV strains isolates worldwide have been classified into six different genotypes (1 to 6), each comprising multiple subtypes (designated a, b, c, etc)¹¹. HCV genotypes can be determined directly by molecular typing or indirectly by serological typing. Current genotyping methods include amplification of defined regions of genome, such as 5' non-coding (NC), core, envelope 1 and non-structural (NS) 5B, by polymerase chain reaction (PCR) followed by nucleotide sequencing or hybridization with type-specific probes or digestion with restriction enzymes and restriction fragment length polymorphism analysis or reamplification with type-specific primers. Indirect HCV serological typing is based on the detection of genotype-specific antibodies directed to epitopes encoded by NS4 or core regions of the genome. Although this has some advantages, such as the simplicity of the assay (ELISA) and low risk of contamination, it is not able to identify HCV subtypes¹⁵.

The genotype determination is a relevant predictive parameter of response to antiviral treatment since genotype 1 is associated with a

lower sustained virologic response (40 - 45%) compared to genotypes 2 and 3, whose sustained virologic response is 70 - 80% with peginterferon and ribavirin combination therapy for 48 and 24 weeks, respectively¹. In addition, the various genotypes have distinct geographical distributions. Although HCV genotypes 1, 2 and 3 appear to have a worldwide distribution, their relative prevalence varies from one geographic area to another. HCV genotype 4 is found in the Middle East and North Africa; and genotypes 5 and 6 in South Africa and Asia, respectively^{11,15}.

In Brazil, a continental country, previous studies were carried out in Rio de Janeiro, São Paulo (southeastern region), Porto Alegre (south region), Natal, Salvador and Recife (northeast region)^{2,3,6,8-10,13}. Given the limited amount of data concerning HCV genotypes in mid-west region⁷, we decided to investigate the distribution of HCV genotypes in blood donors from the four states of Mid-West region: Mato Grosso, Mato Grosso do Sul, Goiás and Distrito Federal.

MATERIALS AND METHODS

A total of 250 anti-HCV positive serum samples from volunteer blood donors were obtained in the States of Mato Grosso (n = 65), Mato Grosso do Sul (n = 63), Goiás (n = 65) and Distrito Federal (n = 57) between March 2001 and July 2002. The studied donors ranged in age

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from 18 to 57 years old (average 29.5 years). The majority of them (75%) were men. The study was approved by the Ethical Committee of the Federal University of Goiás. Anti-HCV positive samples by ELISA were retested by line immunoassay (INNO-LIA HCV Ab III, Innogenetics) and were also submitted to RNA extraction, reverse transcription, and nested PCR with primers complementary to the conserved area of the 5' NC region of HCV, essentially as described previously⁵. Positive samples were genotyped by line probe assay (INNO-LiPA HCV III, Innogenetics NV, Ghent, Belgium). Chi-square test or Fisher's exact test were used to analyze the HCV genotype data.

RESULTS AND DISCUSSION

The anti-HCV-ELISA serological status was confirmed with the anti-HCV-LIA antibody assay in 205 (82%) of the 250 blood donors studied, while the remaining was negative (16%) or indeterminate (2%). The HCV RNA positivity was 80.5% among anti-HCV-positive individuals. Figure 1 shows the distribution of HCV genotypes among 165 HCV-RNA-positive blood donors. In Goiás (n = 42), subtype 1a (50%) was the most prevalent, followed by subtypes 3a (30.9%) and 1b (16.7%). This genotype distribution, with subtype 1a predominance, was similar to that found (41%) in Mato Grosso (n = 44), however, there, subtype 1b (29.5%) was more frequent than 3a (25%). In Mato Grosso do Sul (n = 38), subtypes 1a and 1b were detected equally (36.8%), followed by 3a (21.1%). Subtype 2b was rare (2.4%, 4.5% and 5.3%, respectively). In Distrito Federal (n = 41), subtype 3a (39%) was found more frequently than 1a (31.7%) and the remaining (29.3%)

belonged to subtype 1b, however these differences were not statistically significant ($p > 0.05$).

In the present study, the use of the anti-HCV LIA 3.0 as a supplement test confirmed the occurrence of false positive results with anti-HCV ELISA 3.0 in 18% of the anti-HCV reactive samples in the screening test. Despite the incremental improvements in sensibility and specificity of the third generation assays, the proportion of positivity confirmation with HCV version 3.0 ELISA ranges 17 - 80% among low-risk populations such as blood donors^{4,14}. The finding of HCV RNA positivity in approximately 80% of the anti-HCV-positive blood donors was in agreement with that reported elsewhere¹².

In mid-west region, the proportions of blood donors with HCV types 1, 2 and 3 were 67.9%, 3% and 29.1%, respectively. These data were similar to those previously found in blood donors or non-blood donors in Brazil, where genotype 1 infection is predominant^{2,3,6-10,13}. This distribution was also similar to that detected in the United States and many European countries, where genotypes 1 (1a and 1b) and 3 (3a) have spread as a result of transmission through blood transfusion and needle-sharing between infected drug users over the past 30-70 years^{11,15}. In addition, HCV subtypes prevalence varies from one geographic region to another or even though in the same region^{7,9,10,15}. In Goiás and Mato Grosso, subtype 1a was dominant, and it was followed by subtypes 3a and 1b, respectively. In Mato Grosso do Sul, subtypes 1a and 1b were the most frequent, followed by 3a, while in Distrito Federal this subtype was the most common, followed by 1a and 1b. Regarding the characteristics of the studied population, there were no significant differences between gender and age of the blood

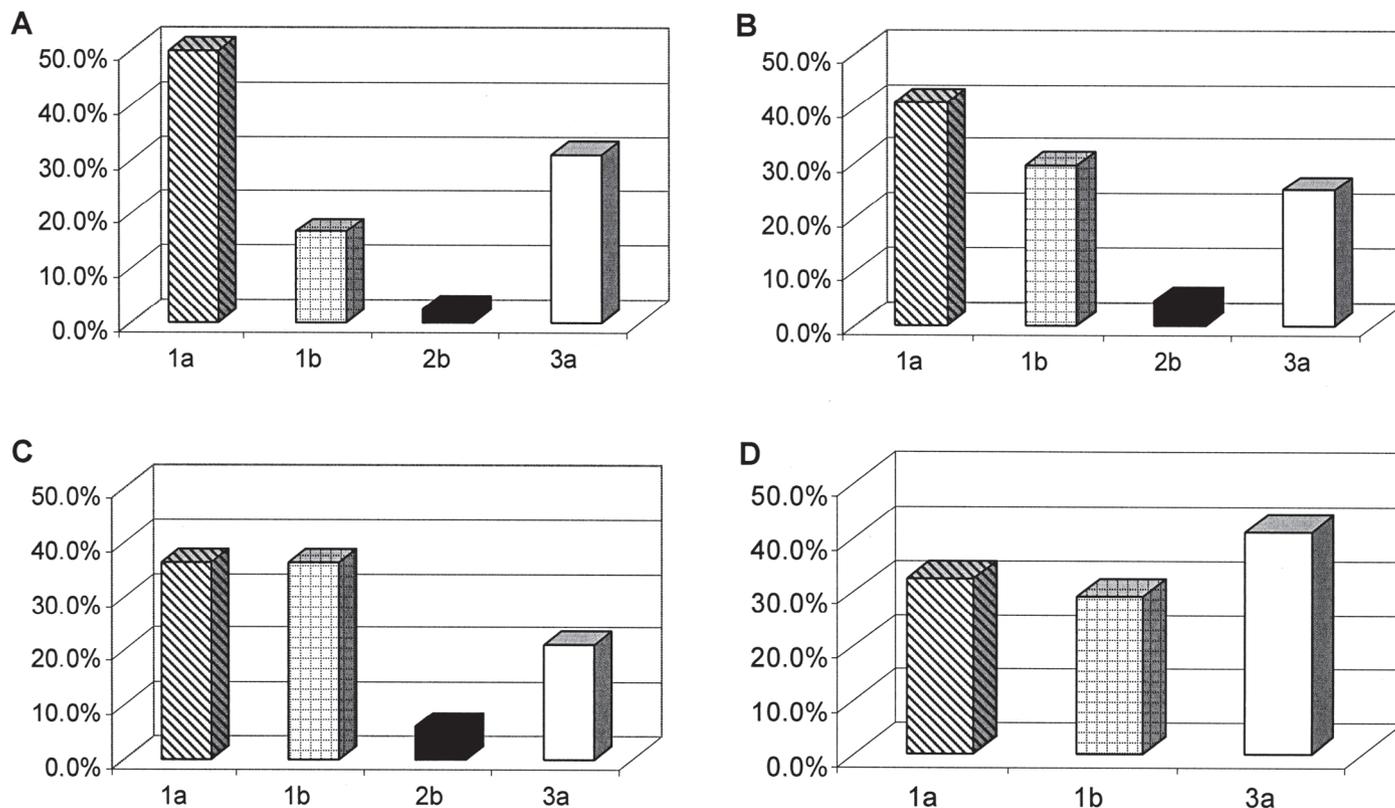


Fig. 1 - Hepatitis C genotypes in blood donors from Mid-west region of Brazil. A: Goiás state; B: Mato Grosso state; C: Mato Grosso do Sul state; D: Distrito Federal

donors infected with subtypes 1a, 1b and 3a (data not shown). In addition, risk factors such as injecting drug use or blood transfusion can influence HCV subtypes distribution^{11,15}, but they were not evaluated in this study.

Although the number of samples studied was small, these findings indicate variation in genotype circulation in mid-west region of Brazil. Further molecular studies with different HCV genomic regions are necessary to provide a better understanding of the dynamics of this infection in Central Brazil.

RESUMO

Distribuição dos genótipos do vírus da hepatite C em doadores de sangue na Região Centro-Oeste do Brasil

Com objetivo de determinar os genótipos do vírus da hepatite C (HCV) circulantes na Região Centro-Oeste do Brasil, 250 doadores de sangue anti-HCV positivos foram estudados. Dentre eles, a positividade para anti-HCV foi confirmada em 205 (82%). O RNA-HCV foi detectado em 165 amostras, as quais foram genotipadas. Os tipos 1, 2 e 3 do HCV foram encontrados em 67,9%, 3% e 29,1% dos doadores, respectivamente. No Estado de Goiás, o subtipo 1a (50%) foi o mais prevalente, seguido pelos subtipos 3a (30,9%) e 1b (16,7%). No Estado de Mato Grosso, o subtipo 1a (41%) foi também predominante, seguido pelos subtipos 1b (29,5%) e 3a (25%). No Estado de Mato Grosso do Sul, os subtipos 1a e 1b foram igualmente detectados (36,8%), seguidos por 3a (21,1%). O subtipo 2b foi raro (2,4%, 4,5% e 5,3%, respectivamente). No Distrito Federal, o subtipo 3a (39%) foi mais freqüente que 1a (31,7%), sendo o restante (29,3%) identificado como subtipo 1b.

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