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Short Communication

Prevalence of hepatitis C virus genotypes in the State of Pará, Brazil

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Abstract

Introduction: This study reports the genotype prevalence of hepatitis C virus (HCV) in Pará, Brazil. **Methods:** A retrospective cross-sectional study was conducted on 344 plasma samples sent to the Lacen-Pará for diagnostics by molecular techniques. **Results:** HCV genotypes identified in the different regions of Pará were 1b (47.7%), 3 (23.3%), 1a (18%), and 2 (4.4%). Genotype 1 occurred in 41.6% of men and 30.8% of women in the 18-86-year-old group. **Conclusions:** Genotype 1 is the most predominant in Pará, which reinforces the idea of its relationship with late-diagnosed chronic infection.

Keywords: Genotype. HCV. Brazil.

Hepatitis C virus (HCV) infection is a major public health problem in Brazil. HCV presents a variety of clinical aspects and disease progression ranging from acute hepatitis in asymptomatic carriers to the chronic form of the infection, which is accompanied by liver cirrhosis and hepatocellular carcinoma[1].

The prevalence of chronic HCV infection has been estimated at between 1.2 % and 1.7 % in the adult global population, suggesting that it affects 62 to 89 million people[2]. In Brazil, there is no precise data on the disease prevalence, but values of about 1 % to 3 % have been suggested by studies carried out in different regions[3].

HCV is a member of the Flaviviridae family and *Hepacivirus* genus. Its viral particle consists of an outer envelope (cell membrane containing E1 and E2 viral proteins), a viral core (p21), and a single-stranded ribonucleic acid (RNA) molecule containing approximately 9,500 nucleotides. The RNA molecule contains a single, long open reading frame encoding for a protein of approximately 3,000 amino acids. This protein is cleaved by viral and host proteases into at least 10 structural (core, E1, and E2) and non-structural (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins[4].

Molecular biology techniques have brought about important advances in the study of HCV and represent an extremely important resource for rapid and effective diagnostics. Among these techniques, HCV genotyping is an important tool for understanding the evolution and epidemiology of the virus and determining the prognosis and treatment time[5].

HCV is classified into seven major genotypes: 1 to 7. Each genotype is divided into subtypes, and there is variation in the prevalence of these genotypes among different continents and different regions of the same country[6]. Following the distribution pattern in the Americas, Brazil has a higher prevalence of genotype 1 (64.0%), followed by genotypes 3 (31.0%), 2 (4.0%), and finally 4 and 5 (both < 1.0%)[7].

When comparing different regions of Brazil, genotype 1 is more prevalent in the North, genotype 2 is more prevalent in the Central-West, especially in Mato Grosso State, and genotype 3 is more prevalent in the South region. Conversely, genotypes 4 and 5 are rarely found, with cases described mainly in São Paulo and only one case reported in Bahia[8,9].

Based on the scarcity of epidemiological data on infection in northern Brazil, especially in Pará, and the need to establish public health policies and strategies for hepatitis C prevention and control, this study aimed to determine the genotype prevalence of HCV in patients from different regions of Pará.

A retrospective, cross-sectional study was conducted using plasma samples from individuals with reactive anti-HCV serology [enzyme-linked immunosorbent assay (ELISA) and rapid immunochromatographic test] that were sent to the Central Laboratory of Pará State [*Laboratório Central de Saúde Pública do Estado do Pará* (LACEN-PA)] for molecular diagnosis

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between February 2011 and February 2013. All samples presented a medical request with the notification number of the suspect case. LACEN-PA performed molecular biology tests for hepatitis C diagnosis in samples from individuals visiting the centers for testing and counselling [*Centros de Testagem e Aconselhamento* (CTA)], primary health units and reference hospitals located in the capital City of Pará (Belém) and its metropolitan region and the state interior (Baixo Amazonas, Marajó, Southeast, and Northeast).

Plasma samples were collected in sterile tubes containing ethylenediaminetetraacetic acid and sent to the laboratory. Plasma was immediately separated, split into 2000-microliter aliquots, and frozen at -70°C prior to use. The presence of HCV RNA was confirmed by reverse transcriptase reaction followed by qualitative polymerase chain reaction (AMPLICOR®, Roche Molecular Systems, Brachburg, N.J). Subsequently, viral load was quantified using RT-PCR (AMPLICOR MONITOR®, Roche Molecular Systems, Brachburg, N.J), according to the manufacturer's specifications.

Genotypes were identified by analyzing the highly conserved 5' untranslated region with the Linear Array Hepatitis C Virus Genotyping Test (LiPA - Line Probe Assay – Roche Diagnostics), which allows the determination of six different genotypes and subtypes (1a, 1b, 2, 3, 4, 5, and 6), according to the manufacturer's guidelines.

Epidemiological data on age and gender were obtained from the Laboratory Management System [*Gerenciador de Ambiente de Laboratórios*(GAL)], whose function is to computerize the National System of Public Health Laboratories of the National Networks of Epidemiological and Environmental Health Surveillance Laboratories of the Brazilian Ministry of Health. All cases of positive anti-HCV serology confirmed by qualitative viral RNA analysis were included in the study. The following exclusion criteria were adopted: duplicate cases, date of birth equal to the date of first symptoms, or age under 18 years.

Statistical analyses were performed using the BioEstat 5.0 application[10] with a G-test of independence. Statistical significance level adopted was ≤ 0.05.

A total of 344 samples were analyzed. Of these, 280 were obtained from patients from the metropolitan area of Belém, 31 from Baixo Amazonas, 1 from Marajó, 17 from the Northeast region, and 26 from the Southeast region. No samples from the Southwest region were obtained. The genotypic pattern of HCV showed a high prevalence of genotype 1, followed by genotypes 3 and 2 (**Figure 1**).

An analysis of the genotype distribution revealed a predominance of genotype 1b (47.7%), followed by genotypes 3 (23.3%) and 1a (18.0%), with a smaller frequency of genotype 2 (4.4%). In addition, in some cases (6.7%), the subtype of genotype 1 could not be elucidated. The variation in the HCV genotypes among the regions of Pará was not significant ($p = 0.4092$) (**Table 1**).

In the confirmed cases of HCV infection, genotype 1 prevailed in both genders and in the age group between 18 and 86 years (average 63 years), with detection rates of 41.4% in men and 30.8 % in women (**Table 2**). However, when stratified by gender, most cases of genotype 1 among males occurred in individuals over 56 years of age [15.7% (54/143)], followed by genotype 3 [5.5% (19/42)]. For younger people (up to 28 years of age), the observed detection rates were similar between genders. Finally, no significant differences in the incidences of the three genotypes were found among male age groups ($p = 0.0716$), but a statistically significant difference was found among female age groups ($p = 0.0134$) (**Table 2**).

The different HCV genotypes correlate with different clinical syndromes of the disease associated with infection[1]. Molecular analysis methods capable of reliably determining the HCV genotype are essential for diagnostic and epidemiological studies, since research on hepatitis C viral diversity provides a better understanding of the origin and dynamics of viral infections[11].

Genetic variants of HCV are distributed world-wide, with genotypes 1, 2, and 3 found in all continents. This study revealed the genotypic frequency of HCV in the population of State of Pará, northern Brazil, which is similar to the global pattern[2]. These findings complement data from previous studies from this state covering limited groups of patients[12], such as blood donors and patients contaminated by blood transfusions, hemodialysis, or medical and hospital materials[13].

Generally, the distribution of the HCV genotypes found in Pará showed a predominance of three well-supported groups, 1 (72.4 %), 3 (23.3 %), and 2 (4.4 %), which is in agreement with previous studies[8]. This pattern is similar to the one found in western Europe and the United States, with a generally higher frequency of genotypes 1 and 3[6]. Interestingly, this study revealed that genotype 3 (45 %) is more prevalent in Baixo Amazonas than in other regions of Pará, suggesting that geographic proximity and migratory flow between this region and the state of Amazonas are responsible for introducing and spreading the HCV from the capital city of Amazonas, Manaus**.** The findings coincide with the studies[8], who exclusively found the genotype 3 in Manaus.

Although previous reports indicate that the different HCV genotypes vary geographically, the profile found in Pará did not show significant differences. The same pattern was identified in the different mesoregions of the state, corroborating studies from other states of northern Brazil, such as Acre and Amazonas[14,15].

Similarly to our observations for the state of Pará, subtype 1b is the most prevalent genotype in the largest part of Brazil, as well as in other countries, such as Colombia, Chile, Venezuela, or Argentina[3]. Based on these findings, we speculate that the genotypic profile of HCV in Pará relates with the arrival of European immigrants to Brazil, in a time when blood donation became more usual and tests to detect HCV were not available. Its subsequent transmission may have been sustained by different generations.

The high percentage of individuals infected with genotype 1 (1a/1b) reflects the probability of a large number of people not being evaluated. Further studies should be conducted to validate this finding.

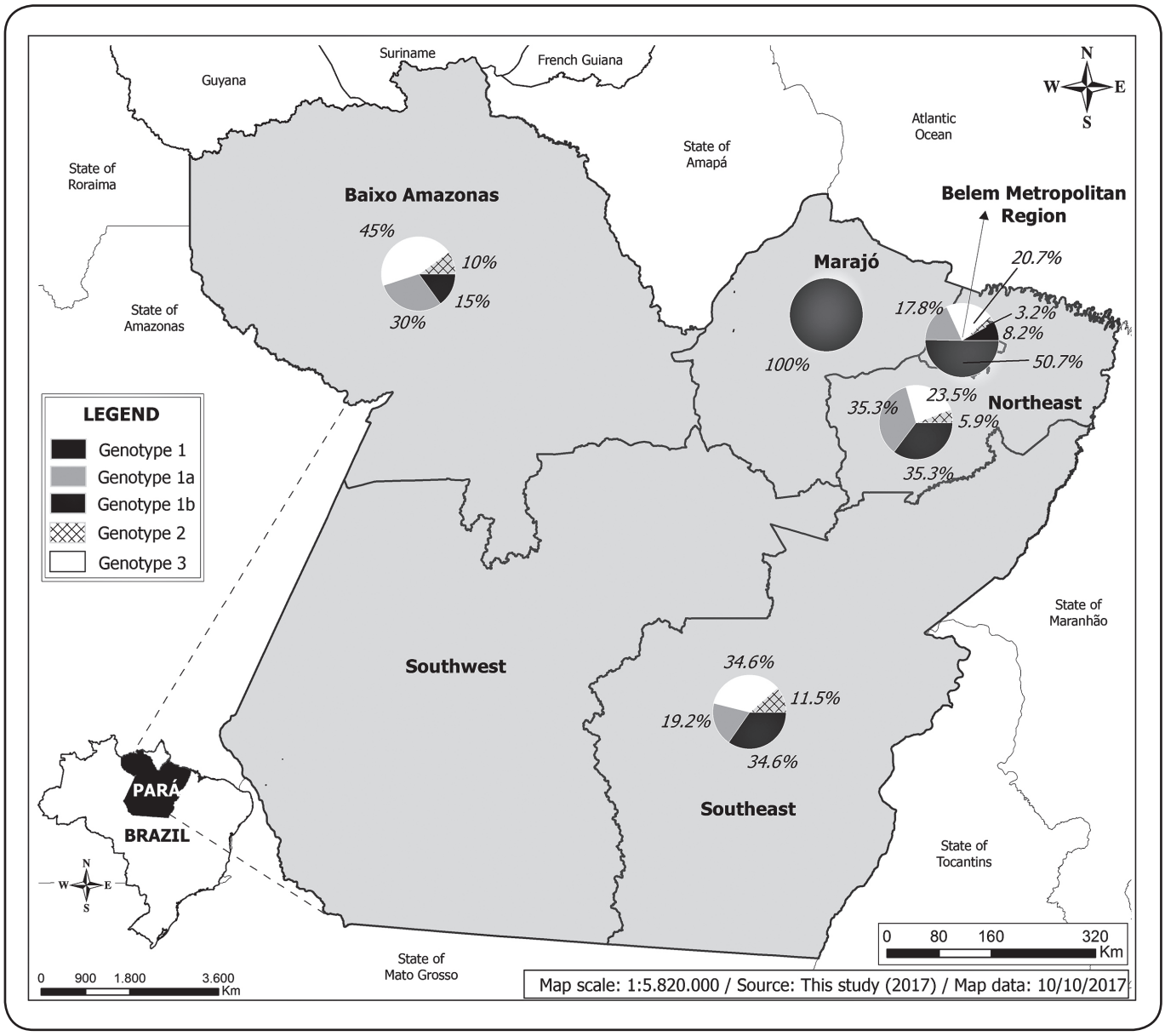

FIGURE 1: Distribution of HCV genotypes in the State of Pará, Northern Brazil, during the period ranging from February 2011 to February 2013. **HCV:** hepatitis C virus.

TABLE 1: Frequency of the different circulating HCV genotypes in the capital metropolitan region and interior region of State of Pará during the period ranging from February 2011 to February 2013.

HCV enotypes	Provenience											
	Metropolitan region of Belem		Northeast region		Southeast region		Marajó		Baixo Amazonas		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
1*	23	8.2	0	0.0	0	0.0	0	0.0	0	0.0	23	6.7
1a	48	17.1	6	35.3	5	19.2	0	0.0	3	30.0	62	18.0
1b	142	50.7	6	35.3	9	34.6	1	100.0	6	15.0	164	47.7
2	9	3.2	1	5.9	3	11.5	0	0.0	2	10.0	15	4.4
3	58	20.7	4	23.5	9	34.6	0	0.0	9	45.0	80	23.3
Total	**280**	**100.0**	**17**	**100.0**	**26**	**100.0**	**1**	**100.0**	**20**	**100.0**	**344**	**100.0**

HCV: hepatitis C virus.*1 comprises genotype 1 samples that could not be classified as 1a or 1b. G-test of Independence; p = 0.4092.

TABLE 2: Genotypic distribution of HCV by gender and age in the samples analyzed at LACEN-PA from February 2011 to February 2013.

Genotypes	Age group					
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
HCV	18-28	29-38	39-47	48-56	> 56	Total
Men*						
1	6 (1.7)	7 (2.0)	34 (9.8)	42 (12.2)	54 (15.7)	143 (41.4)
2	0 (0.0)	1 (0.3)	0 (0.0)	4 (1.2)	4 (1.2)	9 (2.7)
3	2 0.6	0 (0.0)	3 (0.9)	18 (5.2)	19 (5.5)	42 (12.2)
Women**						
1	7 (2.0)	10 (3.0)	22 (6.4)	27 (7.8)	40 (11.6)	106 (30.8)
2	0 (0.0)	0 (0.0)	3 (0.9)	3 (0.9)	0 (0.0)	6 (1.8)
3	2 (0.6)	5 (1.5)	9 (2.6)	13 (3.8)	9 (2.6)	38 (11.6)
Total	17 (4.9)	23 (6.8)	71 (20.6)	107 (31.1)	126 (36.6)	344 (100.0)

HCV: hepatitis C virus; **LACEN-PA:** *Laboratório Central de Saúde Pública do Estado do Pará.* *G-test of independence; p = 0.0716. **G-test of independence; p = 0.0134.

Corroborating other studies, most of hepatitis C cases in this study occurred in both genders and in the age group over 56 years, in which the rates of detection of genotype 1 reached 15.7% for men and 11.6% for women. These findings reinforce the relationship between late detection and the infection asymptomatic nature, and imply that many patients remain uninformed about their health status and their condition as possible transmitters of the disease[12].

Previous records have shown that advanced liver disease and hepatocellular carcinoma are more common in HCV patients with genotype 1[7]. Although this study clearly reflects the genotypic diversity of HCV in the population of the State of Pará, its results may reflect differences in the time of HCV acquisition, which in turn may influence the peak period of the viral load and viral complications, including cirrhosis and hepatocellular carcinoma. Therefore, further studies are needed to correlate the HCV genotypes, the severity of the disease in the study population, and the risk factors.

Social, behavioral and demographic factors (including international migrations) have been suggested to be more important than viral genetic variation in determining the regional prevalence of the different genotypes[8]. Therefore, the prevalence of HCV genotypes and the epidemiology of the disease in Pará justify the adaptation of prevention and treatment strategies that meet local needs, since the vast majority of these individuals do not have access to life-saving tests and treatments. Additionally, subsequent evaluations over time are necessary to assess the influence of the flow of immigrants from other regions and the progress of the National Program for the Prevention and Control of Viral Hepatitis, Brazilian Ministry of Health (*Programa Nacional para a Prevenção e o Controle das Hepatites Virais, Ministério da Saúde do Brasil*) in the interior of the state, with the implementation of new testing and counselling centers.

Finally, the finding of the predominance of genotype 1b in Pará reinforces the idea of its relationship with late-diagnosed chronic infection. Thus, further studies on the population of Pará are needed to determine possible consequences on disease dissemination and susceptibility to treatment with the new generation of direct-acting antivirals, which do not require interferon as an adjuvant. Moreover, these data are critical for predicting the clinical progression of and diagnosing HCV infection, as well as for the adoption of preventive measures, including vaccine development.

Ethical considerations

This research project was approved by the Ethics Committee on Human Research of the Department of Health Sciences, Federal University of Pará [*Universidade Federal do Pará* (UFPA); Opinion No. 1.725.969/2016].

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Conflict of interest

The authors declare that there is no conflict of interest.

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