

Hepatitis C in the 1980s: case review of former non-A and non-B hepatitis from a hepatology service in the Brazilian Amazon

Hepatite C na década de 1980: resgate de casos das antigas hepatites "não A e não B" de um serviço de hepatologia na Amazônia, Brasil

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ABSTRACT

OBJECTIVE: To describe the frequency of *Hepatitis C virus* (HCV) infection in serum samples from patients diagnosed with "non-A and non-B" hepatitis attended at an Amazonian research institute between 1982 and 1988. **MATERIALS AND METHODS:** Descriptive, cross-sectional, and retrospective research including 396 serum samples preserved at -20 °C and tested for anti-HCV IgG antibodies by ELISA. The samples were from patients of both sexes, ranging from 1 month to 85 years old, from Belém and Ananindeua, Pará State, Brazil. For reactive anti-HCV samples, the presence of viral RNA was investigated, and sequencing and genotyping were conducted in those detected. **RESULTS:** Anti-HCV antibodies were detected in 10.9% (43/396) of the sera examined. HCV RNA was detected in 55.8% (24/43) of the reagent sera. For 33.3% (8/24) of these samples, it was possible to distinguish genotypes 1 (75.0%; 6/8) and 3 (25.0%; 2/8), with the confirmation of two subtypes (1b and 3a). **CONCLUSION:** Despite the limitations, due to the long storage time of the samples (three decades), it was possible to detect and characterize HCV in biobank sera, revealing the circulation of two genotypes and two viral subtypes at the time: genotype 1, detected in most genotyped samples and currently exhibiting greater drug resistance than other known genotypes; and genotype 3, less frequent in the study sample, associated with higher virulence. The analysis allowed the identification of HCV strains, favoring future studies to elucidate evolutionary aspects associated with the resistance and virulence of this pathogen.

Keywords: Hepatitis C Virus; Molecular Diagnosis; Genotypic Profile; Epidemiology.

RESUMO

OBJETIVO: Descrever a frequência de infecção pelo vírus da hepatite C (VHC) em amostras de soro de pacientes diagnosticados como hepatite "não-A e não-B", atendidos em um instituto de pesquisa da Amazônia, entre 1982 e 1988. **MATERIAIS E MÉTODOS:** Pesquisa descritiva, transversal e retrospectiva, incluindo 396 amostras de soro, preservadas a -20 °C e testadas para anticorpos IgG anti-VHC por ELISA. As amostras eram de pacientes de ambos os sexos, variando de 1 mês a 85 anos de idade, oriundos dos municípios de Belém e Ananindeua, Pará, Brasil. Para amostras anti-VHC reagentes, investigou-se a presença de RNA viral e, nos detectados, procedeu-se sequenciamento e genotipagem. **RESULTADOS:** Anticorpos anti-VHC foram detectados em 10,9% (43/396) dos soros examinados. O RNA do VHC foi detectado em 55,8% (24/43) dos soros reagentes. Para 33,3% (8/24) dessas amostras, foi possível a distinção dos genótipos 1 (75,0%; 6/8) e 3 (25,0%; 2/8), com a confirmação de dois subtipos (1b e 3a). **CONCLUSÃO:** Apesar das limitações, pelo longo tempo de armazenamento das amostras (três décadas), foi possível detectar e caracterizar o VHC em soros oriundos de biobanco, revelando a circulação de dois genótipos e dois subtipos virais à época: o genótipo 1, detectado na maioria das amostras genotipadas e, na atualidade, exibindo maior resistência a fármacos que os demais genótipos conhecidos; e o genótipo 3, menos frequente na amostra do estudo, associado a maior virulência. A análise permitiu identificar linhagens do VHC, favorecendo estudos futuros para elucidar aspectos evolutivos associados à resistência e à virulência desse patógeno.

Palavras-chave: Vírus da Hepatite C; Diagnóstico Molecular; Perfil Genotípico; Epidemiologia.

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INTRODUCTION

Hepatitis C virus (HCV) infection is still a serious public health problem in Brazil and worldwide¹. According to the European Association for the Study of the Liver (EASL), 71 million people are chronically infected with HCV around the world, and in the Americas, it is estimated to be 7 million². Of this number, only 25% were diagnosed^{2,3}. In Brazil between 1.4 and 1.7 million individuals may be infected with HCV⁴.

Viral hepatitis affects about 325 million people around the world, 95% of those infected are unaware of their disease and less than 1% has access to the treatment³. Hepatitis C is usually asymptomatic, with signs and symptoms occurring mainly in advanced stages of the disease⁴. Millions of people progress slowly to cirrhosis, decompensated liver disease and hepatocellular carcinoma, making this infection the most frequent cause of liver transplantation in the world^{1,2,4}. Only 10% of patients develop jaundice and less than 20% have more severe nonspecific symptoms, such as anorexia, nausea, vomiting, diarrhea, malaise, abdominal pain, and others⁵.

Transmission occurs by sharing personal items; syringes and needles for the use of injectable drugs⁴. The late diagnosis of hepatitis C contributes to the transmission of the disease and its high mortality^{1,3}.

Hepatitis C was first observed in blood donors in the 1980s and characterized as "non-A and non-B" hepatitis (HNANB)⁶. Initially referred to as "post-transfusion non-A, non-B hepatitis of parenteral transmission", the etiology of hepatitis C was discovered in 1989⁷. The agent of hepatitis C was classified in the family *Flaviviridae*, genus *Hepacivirus* and species *Hepacivirus C*, after the advent of molecular methods⁸.

Retrospective studies on the frequency of HCV infection are important sources of knowing the natural history of the disease⁹, but there are few studies in the Brazilian Amazon region about it. This parallel temporal situation also allows the comparison of the epidemiological profile of patients in two different scenarios, before and after the acquisition of diagnostic tests and treatment of that disease. This study aims to describe the prevalence of HCV infection in serum samples from patients diagnosed as HNANB, between 1982 and 1988, from the Legal Amazon.

MATERIALS AND METHODS

This descriptive, cross-sectional, retrospective study used 396 serological samples from the biobank of the Hepatology Section (SAHEP) of the Instituto Evandro Chagas (IEC), the Secretariat of Health Surveillance (SVS), the Ministry of Health (MS), stored at -20 °C for more than 30 years. These samples were collected from 1982 to 1988 of patients treated with clinical conditions compatible with acute HNANB and, with negative serological markers for hepatitis A and B, from a total of 2,223 consecutive cases treated in the Amazon Region. For the collection of clinical, demographic and laboratory data, the research was carried out in record books and in epidemiological records of the screened cases.

The 396 serum samples were tested for anti-HCV IgG antibodies by Elisa methods of fourth generation kits (DIA.PRO Diagnostic Bioprobes®, San Giovanni, Italy), following the procedures indicated by the manufacturers. The reduced volume of the samples made it impossible to use an automated device in serological diagnosis.

The final result of the samples was given in optical density by reading in spectrophotometer (BIOTEK EL800 Microplate Reader). The cut-off value of this test was calculated by adding the value 0.350 to the average absorbance of the negative controls. The results that exceeded the cut-off value plus 20% were considered reagents. The optical density values below the cut-off value minus 20% were considered non-reactive. Results with optical density up to 20% above or below the cut-off value were characterized as inconclusive.

The samples that presented inconclusive results were submitted to serological test for anti-HCV detection, using Elisa method of third generation kits (ORTHO HCV Version 3.0® ELISA Test System, New Jersey, USA). The interpretations of the research results were carried out by the spectrophotometer (Biotek EL800 Microplate Reader); for the determination of the cut-off values, the constant 0.600 was added to the value of the average absorbance of the negative controls.

Reagent samples for anti-HCV were selected for molecular detection and characterization of HCV. HCV-RNA was researched by RT-qPCR and RT-PCR. RNA extraction was processed in an automated manner (equipment m24sp/m2000rt – Abbott®), with final volume of 60 µL. After extraction, the amplification and detection of HCV-RNA were performed, using the Abbott RealTime HCV kit (Abbott®, USA), according to the manufacturer's instructions. HCV-RNA detection was carried out following TaqMan® systems and using specific Taqman® primers and probes. The Abbott RealTime HCV assay has a linearity of 12 to 100,000,000 IU/mL. The RNA of the samples extracted by automated method (equipment m24sp/m2000rt – Abbott®) was used for the synthesis of cDNA, made with random primers (IDT) under the following thermocycling conditions: 15 min at 25 °C, followed by 1 h to 37 °C and final incubation of 95 °C for 15 min.

The cDNA of the samples was used in RT-PCR assays of the NS5B and 5'UTR regions of HCV partially amplified, subsequent nucleotide sequence and phylogenetic analysis for virus genotyping. The amplification of the NS5B region was developed by heminested RT-PCR assays in order to produce a fragment of 382 base pairs. During the first-round PCR reaction, 5 µL of cDNA of each sample were added to the primers Pr3 (5'TATGAYACCCGCTGYTTTGACTC3') and Pr4 (5'GCNGARTAYCTVGTTCATAGTCTC3') and submitted to the thermocycling condition, as described by Morice et al.¹⁰ and Sandres-Sauné et al.¹¹. The second-round PCR reaction was performed with primers Pr3 and Pr5 (5'GCTAGTCATAGCCTCCGT3') under cycling conditions^{10,11}. For the 5'UTR region, nested RT-PCR was performed, aiming at amplifying a fragment of

230 base pairs (pb) of the gene encoding the 5'UTR region. 5 µL of cDNA in the PCR reaction and the primers PTC1 (5'CGTTAGTATGAGTGTTC3') and NCR2 (5'ATACTCGAGGTGCACGGTCTACGACCT3') were used under cycling conditions¹². The second PCR was carried out with the primers PTC3 (5'AGTGTCTGTCAGCCTCCAGG3') and NCR4 (5'CACTCTCGAGCACCTATCAGGCAGT3') under the same cycling conditions as the first PCR.

The detection of amplicons was performed in 1% agarose gel (Ultra Pure Agarose-Invitrogen, Spain), SYBR® Safe DNA Gel Stain (Invitrogen, USA) and molecular weight marker (50 bp DNA Ladder, Invitrogen, USA). The visualization and capture of the images were done with photodetection equipment (TFX-35M GIBCO BRL W Transluminador). Samples with amplification products close to the size expected, 230 bp for the 5'UTR region and 382 bp for the NS5B region were considered positive.

The amplified products were purified with the EXO/SAP-IT kit (GE Healthcare Bio-Sciences, Buckinghamshire, United Kingdom) and subsequently used the sense and antisense sequence with primers Pr3 and Pr5 for the region NS5B and PTC3 and NCR4 for the region 5'UTR, respectively, used with the kit BigDye® Terminator v3.1 Cycle Sequencing (Applied Biosystems, Vilnius, Lithuania) in the automatic sequencer ABI 3500 (Applied Biosystems). All reactions were developed according to the manufacturers' guidelines. The sequences obtained were edited in the Geneious v8.1.3 program and with the sequences of different HCV genotypes and subgenotypes, available in GenBank. Phylogenetic trees were constructed using the Neighbor-Joining method and Kimura 2-parameter model in the MEGA v7 program.

The chi-square test (χ^2) of homogeneity was used to compare the proportions of the interest variables (clinical manifestations, sex, age, origin) in two or more populations (infected by HCV or not infected by HCV). The residual analysis showed how the various proportions of the contingency table contribute to the final value of the calculated χ^2 ($p = 0.05$). The BioEstat v5.0¹³ program was used.

In compliance with Resolution No. 466 of December 12, 2012, of the National Health Council/MS¹⁴, this project was approved by the Committee for Ethics in Research on Human Beings of the IEC/SVS/MS, under approval opinion no. 1,947,422, on March 3, 2017.

RESULTS

The patients were aged from 1 month to 85 years, with a mean age of 29.9 (± 17.9) and median of 26 years of age, with the majority between 21 and 30 years of age (28.0%). The proportion of men and women was similar, although the frequency for males (55.3%) was bigger. All samples were from patients of the urban area, from the municipalities of Belém and Ananindeua, Metropolitan Region of the state of Pará. The largest number of HCV patients lived in Marco neighborhood (11.1%). Among the records of patients who had information on occupation, the highest frequency was of students (22.2%). However, there were lack of information in the results for the high

frequency of empty fields, e.g. "not informed". The main information on the demographic profile of patients are shown in Table 1.

The clinical manifestations reported by the patients, who sought the SAHEP/IEC in the 1980s and who received the diagnosis by HCV, were collected from 322 of the 396 completed and available forms, highlighting: choluria (180/322; 55.9%), jaundice (161/322; 50.0%), fever (148/322; 46.0%), vomiting (106/322; 32.9%), myalgia (93/322; 28.9%), malaise (90/322; 27.9%), anorexia (76/322; 23.6%), nausea (76/322; 23.6%) diarrhea (41/322; 12.7%).

The individuals who tested positive for anti-HCV living in Belém and were mostly from Marco neighborhood (6/38; 15.8%). Figure 1 highlights neighborhoods with two or more cases of anti-HCV reagents (23/38; 60.5%).

In the municipality of Ananindeua, most positive samples (3/5; 60.0%) were from patients who lived in Cidade Nova neighborhood (Figure 2).

Anti-HCV antibodies were detected in 10.9% (43/396) of the samples, with a similar prevalence between genders. The highest frequencies of anti-HCV were observed in adults over 20 and under 60 years of age, especially the age group from 51 to 60 years. There was no statistical significance between sex and age groups when correlated with the presence of HCV antibody. It was possible to detect the presence of viral RNA in 55.8% (24/43) of the samples seroreactive to HCV. For the positive results, according to sex and age groups, there was no statistical significance when correlated with HCV infection (Table 2).

Through genotyping, two genotypes (1 and 3), subtypes 1b and 3a, respectively, were found in eight (33.3%) of 24 RNA detected (Chart 1). The cladogram developed for HCV samples was based on the sequence alignment of the NS5B region (382 bp). Three samples grouped with sequences of genotype 1, subtype 1b, and one grouped with genotype 3, subtype 3a (Figure 3).

Phylogenetic analysis of the 5'UTR region did not allow the determination of HCV subtypes, because the amplicons of this region presented sequences with a limited number of nucleotide bases (< 230 bp), which made it impossible to infer phylogenetic relationships.

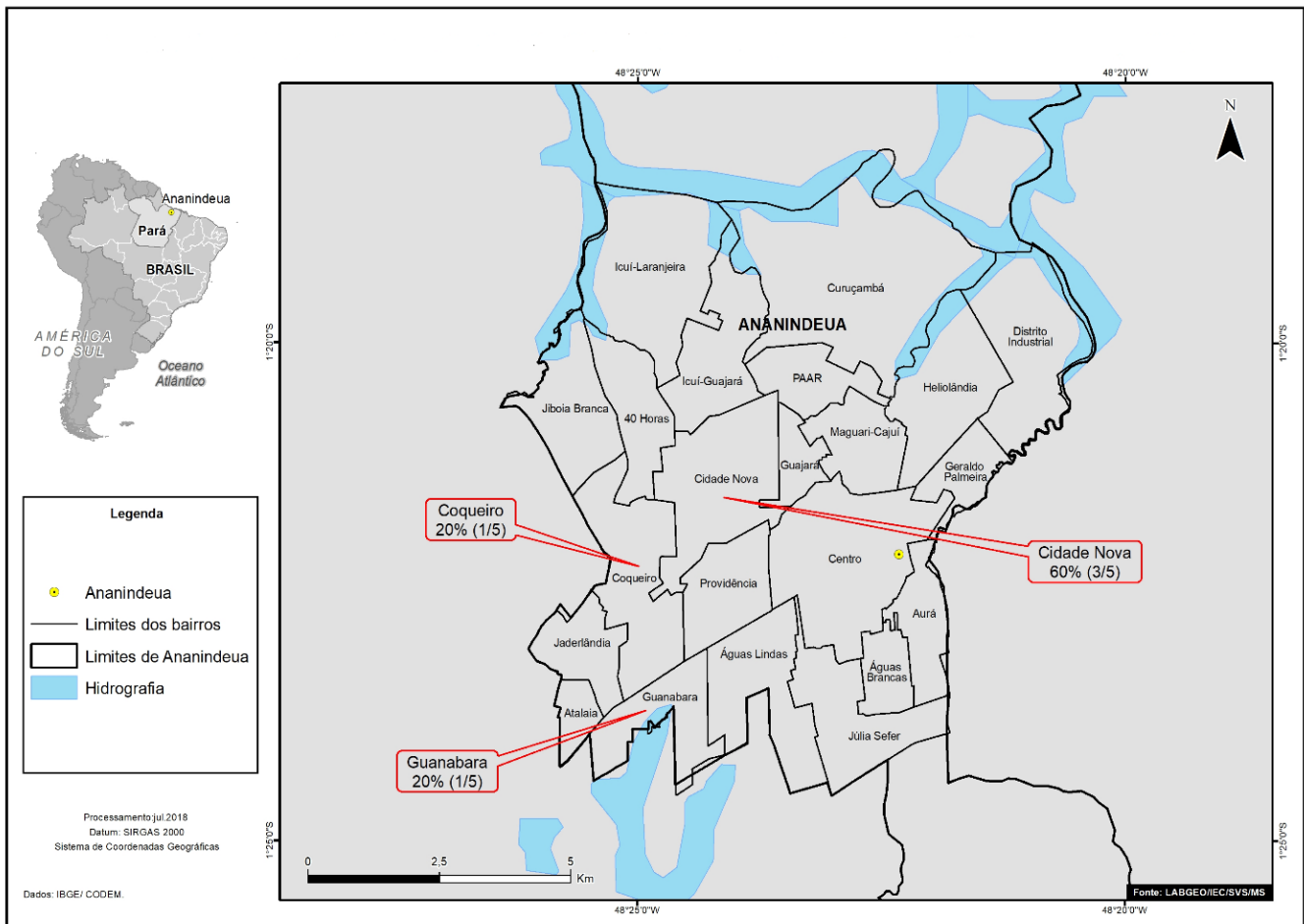
DISCUSSION

Retrospective studies on the prevalence and characterization of HCV are important in order to understand this virus behavior in the population and its dispersion at a time when the specific diagnosis was not available⁹. Despite the sensitive and advanced molecular methods currently available for the diagnosis of viral hepatitis, the long storage period of the samples, although cryopreserved (-20 °C), certainly induced changes at the molecular level capable of impairing laboratory analyses, because it is an RNA virus extremely susceptible to degradation, increasing these circumstantial losses¹⁵. Nevertheless, this study revealed unprecedented results on the occurrence of hepatitis C in the Brazilian Amazon during the analyzed period (1982 to 1988).

Table 1 – Distribution of patients diagnosed with HNANB, according to sex, age group, origin and occupation, from 1982 to 1988, in Belém and Ananindeua, Pará State, Brazil

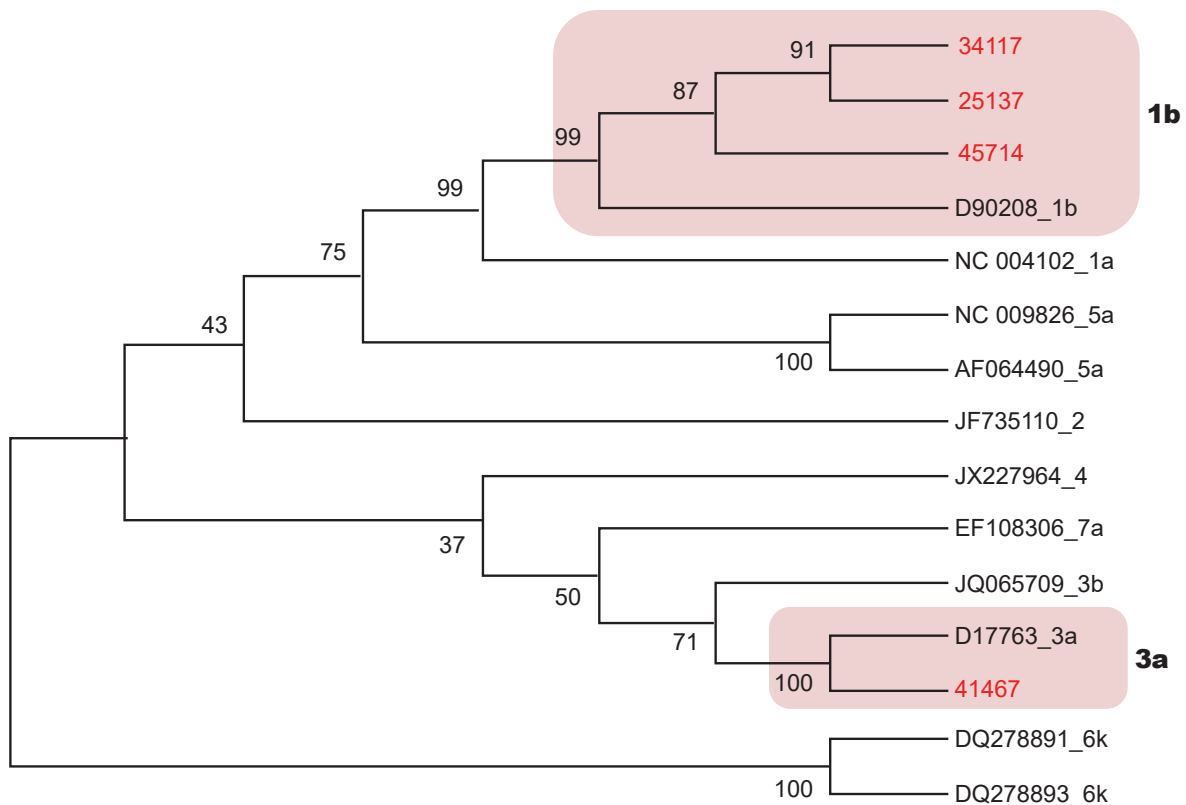
Variables	N = 396	%
Sex		
Male	219	55,3
Female	177	44,7
Age group (years)		
≤ 10	63	15,9
11–20	75	19,0
21–30	111	28,0
31–40	57	14,4
41–50	40	10,1
51–60	23	5,8
≥ 61	27	6,8
Origin		
Belém	349	88,1
Ananindeua	47	11,9
Neighborhoods		
Belém		
Marco	44	11,1
Marambaia	23	5,8
Nazaré	21	5,3
Umarizal	20	5,0
Pedreira	19	4,8
Telégrafo	18	4,5
Jurunas	16	4,0
Canudos	14	3,6
Guamá	14	3,6
Sacramenta	14	3,6
São Brás	14	3,6
Icoaraci	12	3,0
Terra Firme	12	3,0
Batista Campos	11	2,7
Coqueiro	5	1,3
Cremação	9	2,3
Val-de-Cans	5	1,3
Campina	4	1,0
Souza	4	1,0
Other neighborhoods	19	4,8
Not informed	51	12,8
Ananindeua		
Cidade Nova	16	4,0
Coqueiro	6	1,5
Guanabara	5	1,3
Other neighborhoods	14	3,6
Not informed	6	1,5
Occupation		
Student	88	22,2
Housekeeper	38	9,6
Minor without occupation	33	8,3
Homemaker	24	6,1
Merchant	14	3,5
Teacher	9	2,3
Public employee	7	1,8
Driver	7	1,8
Construction worker	7	1,8
Retired	6	1,5
Office assistant	4	1,0
Other occupations	82	20,7
Not informed	77	19,4

N: Sample number; %: Frequency.



Source: Geoprocessing Laboratory (LabGeo/IEC/SVS/MS).

Figure 2 – Distribution by Ananindeua neighborhoods of positive samples for anti-HCV antibodies of patients diagnosed with HNANB treated at SAHEP/IEC, from 1982 to 1988, in Ananindeua, Pará State, Brazil



Sequences obtained from GenBank are identified with the access number.

Figure 3 – Cladogram of the NS5B region of HCV inferred with Neighbor-Joining method (Kimura 2-parameters) in the MEGA v7 program

Samples (record)	Age	Sex	Origin Neighborhood (Belém)	Log (UI/mL)	RT-PCR		Genotypes	Subtypes
					5'UTR	NS5B		
34117	18	F	NI	4,23	+	+	1	1b
41467	24	M	NI	2,80	+	+	3	3a
25137	27	M	Marco	3,35	+	+	1	1b
43444	36	M	Nazaré	2,48	+	-	1	
28387	44	F	Marco	3,99	+	-	3	
45714	52	F	Nazaré	3,68	+	+	1	1b
41064	54	F	Icoaraci	2,82	+	-	1	
17910	82	M	Telégrafo	2,69	+	-	1	

NI: Not informed; +: Positive; -: Negative.

Chart 1 – Demographic characteristics, viral load and HCV genotypes of the eight patients diagnosed with HNANB with detected genotypes, treated at SAHEP/IEC, from 1982 to 1988, in Belém, Pará State, Brazil

Prior to the discovery of hepatitis C and its etiological agent, the incidence of HNANB in the United States of America in the 1980s, was considered stable, presenting an average of 7.1 cases per 100,000 inhabitants, according to the Centers for Disease Control and Prevention, which for a period of seven years (1982–1988) developed a study in four sentinel municipalities, using samples of patients with HNANB, collected between 1985 and 1988, who were tested for anti-HCV, demonstrating 45% positivity¹⁶.

A prospective study conducted in Spain between 1978 and 1984, with 54 patients diagnosed with post-transfusion HNANB, detected 85% of HCV antibodies¹⁷. In Paris, between 1980 and 1989, 39.6% of patients submitted to hemodialysis were infected with HCV and this hepatovirus was responsible for 91% of HNANB cases¹⁸.

In Brazil, Focaccia et al.¹⁹ reported a prevalence of HCV infection of 1.4% in the population of the city of São Paulo. A retrospective study of laboratory reanalysis of HCV infection in samples from 1990 to 1993 in Belém described a prevalence of 62.6% for HCV antibody²⁰. Valois et al.²¹ observed a 1.1% prevalence for anti-HCV among candidates for blood donation in the state capital.

The seroprevalence for anti-HCV in the sample studied was 10.9%, higher than that observed by Fonseca et al.²², who found positivity of 0.2% of anti-HCV, in a retrospective study conducted in the city of Manaus, State of Amazonas, in the period from 1989 to 1998, in patients diagnosed with acute hepatitis. However, similar to that reported by Teixeira²³, who observed positivity of 14.3% of anti-HCV in a research of clinical-laboratory reanalysis of cases reported as HNANB, in the period from 1994 to 1996, also in Belém and Ananindeua.

In the current study, the positivity for anti-HCV was similar between genders, being higher in adults aged between 51 and 60 years. Martins et al.²⁴ suggested that the highest prevalence of hepatitis C, observed after 50 years of age, can be explained by the late diagnosis of HCV exposure.

Regarding sex and age groups, a study conducted by Santos et al.²⁵, with patients diagnosed with HNANB, showed that all individuals reactive to anti-HCV were male, with ages ranging from 27 to 36 years. Simon et al.²⁶, in a research conducted in the city of Paris, France, among hemodialysis patients with HNANB, observed that the mean age of patients with reactive anti-HCV was 50 years, most of them male. Valois et al.²¹, when analyzing a population of candidates for blood donation, in Belém, they found the male sex as predominant among cases of reactive anti-HCV, with a higher prevalence in the age group between 30 and 39 years.

The professional occupation is a relevant variable in epidemiological studies. In this study, professional occupation was heterogeneous, with the group of students being singled out. Moraes and Oliveira²⁷, in a study conducted in southwestern Bahia between 2003 and 2014, reported a prevalence of 0.96% of anti-HCV among students.

In the present study, all samples were from patients of the urban area, from the municipalities of Belém (most of them) and Ananindeua. Marco neighborhood had the highest prevalence of anti-HCV reagent among the neighborhoods of Belém; and Cidade Nova, among the neighborhoods in Ananindeua, which could be explained for being very populous neighborhoods.

Despite the limitations related to the time of storage and conditioning of serum samples, it was possible to detect viral RNA in 55.8% of the 43 seroreagent patients in the present study. Lower results were reported by Fonseca et al.²⁸, who observed a prevalence of 5% of viral RNA detected in the state of Amazonas, with cryopreserved samples collected during the years of 1981 to 1986, from patients with acute HNANB.

In the study by Simon et al.²⁶ conducted in Paris, hemodialysis patients with HNANB, 82.6% (19/23) were positive for HCV RNA.

In Brazil, a retrospective study of laboratory reanalysis of HCV infection in dialysed samples from 1990 to 1993, in the municipality of Belém, described 4.3% of viral RNA detected²⁰.

Oliveira et al.²⁹ observed 62.5% of viral RNA in a study among riverside dwellers in the municipality of Cametá, Pará. Freitas et al.³⁰ reported 5.3% detection for HCV-RNA in patients with anti-HCV in Belém. In the study on the "Epidemiological aspects of HCV infection in users of non-injectable drugs in the state of Pará, eastern Amazonia", there was a prevalence of viral RNA of 28%³¹.

The HCV-RNA detection data from the present study are similar to that found by Oliveira et al.²⁹, with important emphasis on the fact that the serum samples tested in the mentioned research, unlike those tested in the present series, were newly collected. Variations found in different studies can be explained by the differences between the studied groups and/or cryopreserved samples, and by the time elapsed between the collection and performance of the tests.

In addition to the importance for phylogeny, HCV genotyping is an important factor for the management of infected individuals and for epidemiological purposes that indicates the route of acquisition and affects the clinical outcome and response to treatment^{32,33}. Genotype 1, subtype 1b, is more resistant to certain therapies, and genotype 3, subtype 3a, is significantly associated with the more aggressive forms of the disease³².

HCV genotyping of this study was carried out by sequencing the 5'UTR and NS5B regions. Genotyping was possible in eight of the 24 RNAs detected, most of them were genotyped in the 5'UTR region. Two genotypes (1 and 3) were identified in both regions, in the same serum samples, and two subtypes (1b and 3a) were characterized in the NS5B region.

The 5'UTR is highly conserved; consequently, the annealing of primers is much more conserved in relation to NS5B³⁴. Due to the high level of conservation and sensitivity of 5'UTR, this region has been more used by clinical laboratories for HCV genotyping³⁵. Thus, these characteristics may explain the detection of more genotypes in the 5'UTR region than in the NS5B studied.

It was not possible to discriminate subtypes within the genotypes detected in the 5'UTR region. The 5'UTR region, unlike the NS5B, due to its high level of conservation, does not have sufficient variation to discriminate HCV classifications at the level of viral subtype^{34,35,36}. Thus, the phylogenetic trees of 5'UTR are less-abled to group subtypes of the same genotype into clades than NS5B sequence trees, regardless of the method used for phylogenetic inference³⁴.

HCV genotypes 1 and 3 are the most common cause of infections around the world^{1,37}. In Brazil, genotype 1 is considered the most prevalent cause, followed by genotype 3 and genotype 2^{1,12,37}. Among the regions of Brazil, the Northern Region has the highest frequency of genotype 1 (51.7% to 74.1%); the Midwest region, genotype 2 (11.4%); the Southern Region, genotype 3 (43.2%); and in the Southeast Region, in the state of São Paulo, genotypes 4 and 5 were described but rarely found¹².

Perone et al.³⁸ conducted a study with patients with chronic hepatitis C, treated at the National Reference Centers for Viral Hepatitis in Belo Horizonte, State of Minas Gerais, between 2002 and 2006, the study observed a high prevalence of genotype 1 (78.4%) and genotype 3 (17.9%). Oliveira et al.³⁹ reported the detection of 77.1% for genotype 1 and 19.82% for genotype 3 in a study conducted in a reference hospital for infectious diseases in the state of Goiás.

It was possible to genotyping one third of the samples positive for HCV-RNA, with predominance of genotype 1, similar to the study by Sawada et al.³⁶, which concluded that, in the distribution of HCV in different exposure categories in Pará, there is a predominance of genotype 1.

The distribution of genotypes identified in the present study was similar to that found by Baia²⁰ who described the detection of 73% for genotype 1 and 24.3% for genotype 3 in a retrospective study among patients on dialysis, from 1990 to 1993, in Belém.

Data from the current study were also similar to findings of other authors in the Northern Region, demonstrating the importance of genotypes 1 and 3 in the epidemiology of this virus: Campiotto et al.¹² described the detection of 74.1% for genotype 1 and 24.7% for genotype 3 in the Northern Region; Araújo et al.⁴⁰ found a prevalence of 76.1% for genotype 1 and 19.6% for genotype 3 in patients with chronic hepatitis in the state of Amazonas; Oliveira et al.³⁹ observed 76.9% of genotype 1 and 23.1% of genotype 3 in non-injectable drug users in Pará; Guimarães et al.⁴¹ detected 72.4% of genotype 1 and 23.3% of genotype 3 in the general population, in the state of Pará, and described a prevalence of 76.7% for genotype 1 and 20.7% for genotype 3 in the Metropolitan Region of Belém.

A retrospective study conducted in the Amazon also analyzed cryopreserved serum samples at -70 °C, collected from 1981 to 1986, for subsequent analysis of HCV²⁸. Another study also retrospective was conducted by Baia²⁰ in samples placed in biobank (-20 °C) for more than 20 years. These studies corroborate the present research, highlighting the importance of the use of institutional biobanks in health research.

CONCLUSION

Despite the limitations of this research, due to the long storage time of the samples (three decades), it was possible to detect 10.9% of anti-HCV antibodies and characterize HCV in sera from biobank, revealing the circulation of at least two genotypes and two subtypes of the virus: a) genotype 1, detected in most genotyped samples and, currently, exhibiting greater resistance to drugs than other known genotypes; and b) genotype 3, with less frequency in the sample from this study, but currently associated with greater virulence.

It was concluded that the analysis of biobank samples allowed the identification of HCV strains, and may contribute for future studies with the objective of

highlighting evolutionary aspects associated with the resistance and virulence of this pathogen.

It is suggested that the institutions evaluate the best way to packaging serum samples, such as freezing at lower temperatures, lyophilization, etc.; as well as the benefit-cost to modernizing procedures in institutional biobanks aimed at health research, due to its relevance for present and future studies.

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CONFLICTS OF INTEREST

There is no conflict of interest.

AUTHORS' CONTRIBUTION

Kemere Marques Vieira Barbosa, Alex Junior Souza de Souza, Heloisa Marceliano Nunes, Lourdes Maria Garcez and Manoel do Carmo Pereira Soares contributed to the idealization of the study, the analysis and interpretation of the data and the writing of the manuscript. Luiz Vinicius Leão Moreira contributed with the examinations and database, with the bibliographic survey and the critical review of this manuscript. Candida Maria Abrahão de Oliveira helped with the bibliographic survey and participated in the critical review of this article. The authors approved the manuscript and declared that they were responsible for all aspects of this research, ensuring its accuracy and integrity.



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