

DISTRIBUTION OF HEPATITIS C VIRUS (HCV) GENOTYPES IN SEROPOSITIVE PATIENTS IN THE STATE OF ALAGOAS, BRAZIL

Rosa Maria S. Gonzaga¹; Itatiana F. Rodart²; Mitermayer Galvão Reis²; Cícero Eduardo Ramalho Neto¹; Denise Wanderlei Silva^{3*}

¹Laboratório de Genética Molecular, Genômica e Proteômica, Universidade Federal de Alagoas, Maceió, AL, Brasil; ²Laboratório de Patologia e Biologia Molecular, Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, BA, Brasil;

³Laboratório de Microbiologia Molecular, Setor de Microbiologia, Parasitologia e Patologia, Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas, Maceió, AL, Brasil

Submitted: October 08, 2007; Returned to authors for corrections: February 25, 2008; Approved: November 02, 2008.

SHORT COMMUNICATION

ABSTRACT

We determined the frequency of hepatitis C virus (HCV) genotypes in anti-HCV seropositive patients in the state of Alagoas, Brazil, by means of nested-reverse transcription-polymerase chain reaction (RT-nested-PCR) followed by restriction fragment length polymorphism (RFLP) of amplified fragments of the 5' NCR. The nested-PCR with genotype-specific primers from the core region was carried out when detection was not possible by the first approach. Detectable HCV-RNA was present in 115 (74.7%) of 154 serum samples. Genotype 1 was the most frequent (77.4%), against 20.9% of genotype 3 and 0.8% of genotype 2. Subtype 1b was predominant (65.2%), followed by subtypes 1a (8.7%), and 3a (6.1%). Coinfection (1a/3a) was detected in 0.8% of the samples. Indeed, there was no significant differences in the prevalence of genotype 1 compared to what has been obtained from anti-HCV seropositive patients from other locations in Brazil. Here we report for the first time the genotype 2 in the state of Alagoas.

Key words: Hepatitis C virus, 5'NCR, core region, RT-nested-PCR, genotypes

Hepatitis C virus (HCV) is a major cause of parenterally transmitted hepatitis (17) and has become a major emerging infectious disease problem, whereas it has been estimated that 3% of the world population (14) are infected with the virus.

Infection by the virus C could lead to chronicity in 85% of the cases, with possible progression to hepatocellular carcinoma (HCC), or complications requiring liver transplantation, such as liver cirrhosis (13).

There is an extensive genetic heterogeneity among different HCV strains (21), with at least six major genotypes further divided into subtypes (12), whose frequency and incidence in the population seem to be variable both geographically and temporally due to the distribution and evolution of risk factors (17).

Previous data about the frequencies of HCV genotypes in different geographical regions of Brazil (2) found types 1, 2, 3, 4, and 5 in samples from 1,688 patients with chronic hepatitis C. In all regions genotype 1 was the most frequent, whereas types 4 and 5 were rare and detected only in the Southeast. However, genotype 4 of the virus was recently described in Salvador, Bahia, Northeastern area of Brazil (19).

Studies have suggested that genotypes 1, especially subtype 1b, and 4 are associated with a less favorable outcome after interferon (INF) therapy (4,18). It has been also determined that patients infected with these genotypes and with viral loads higher than 800,000 UI/mL should be treated for one year, whereas patients with genotype 2 or 3, may be treated for only 6 months, in spite of the viremia degree (5,22).

*Corresponding Author. Mailing address: Setor de Microbiologia, Parasitologia e Patologia, Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas, Praça Afrânio Jorge, s/n. Prado. 57020-720. Maceió, AL, Brasil. Telefax: +55 82 3221 2105. E-mail: dmws@cobi.ufal.br

To our knowledge, the only information about the prevalence of HCV genotypes in the State of Alagoas, northeastern Brazil, is based on 28 samples (2), where the investigators found 23 of them infected with genotype 1 and five with genotype 3, but none have been reported for subtypes.

Therefore, the objective of the present study was to use a larger number of samples in order to determine the prevalence of genotypes of the virus circulating in anti-HCV seropositive patients in the state of Alagoas. We used a nested-reverse transcription-polymerase chain reaction (RT-nested-PCR) followed by restriction fragment length polymorphism (RFLP) of amplified fragments of the 5' NCR and genotype-specific primers from the core region when genotyping was not possible by the first approach.

We evaluated sera from 154 anti-HCV seropositive patients (age range = 18-72 years; male:female ratio = 121:33) who attended the Day Hospital of University of Alagoas (UFAL) and Central Laboratory of Alagoas (LACEN), in Maceió, Alagoas state, Brazil, between January 2003 to December 2005. The project was approved by the Institution ethical committee of the University of Alagoas and informed consent was obtained from each subject. RNA isolation from 200 µL of serum was performed using a commercial viral RNA isolation kit TRIzol® *LS Reagent* (Life Technologies™) following manufacturer's instructions. Single-stranded cDNA was immediately synthesized by using reverse transcription of the RNA sample with 200U of Moloney murine reverse transcriptase (MMLV), 2 µM of random primer, 0.4 mM of each dideoxynucleotide (dNTP), 8 µM of dithiothreitol (DTT), 10U RNasin, and 1X supplied PCR buffer. cDNA amplification by amplification-polymerase chain reaction (RT-PCR) and HCV genotyping was performed by two systems. In the first one, based on Chan *et al.* (3), a first-round PCR was carried out with primers 939 and 209, whereas in the second-round PCR, primers 940 and 211. HCV genotyping and subtyping were accomplished by double digestion of the nested PCR products with *HaeIII-RsaI* and *HinfI-MvaI*. Samples with pattern consistent with type 1 were further digested with *BstUI*, and of type 2 or 3 with *ScrFI*. The second system was based on Lerat *et al.* (7), which was used when detection was not possible by the first approach. The first PCR was performed using the antisense primer 186 NTER and the amplified product was subjected to a second PCR. Combinations of primers 104/132Nbis, 104/123Nbis, 104IIIIa/134Nbis1-2, 104IIIIa/339Nbis, and 104IVa/465 generated product sizes corresponding to genotypes/subtypes 1a, 1b, 2, 3a, and 4a, respectively. In both systems, PCR products were analysed by electrophoresis on a 4% metaphor agarose gel and stained with ethidium bromide.

Hepatitis C accurate genotyping has been shown to have an important role in epidemiological studies and clinical setting of the liver disease. HCV-RNA was detected in serum samples from 115 out of 154 patients (74.7%). The frequency of HCV-RNA from anti-HCV-positive patients was similar to that reported in other studies (1,2,6,9,11).

The overall distribution of HCV genotypes and subtypes from patients attending the Day Hospital of University of Alagoas (UFAL) and Central Laboratory of Alagoas (LACEN) is summarized in Table 1.

It has been suggested that the pattern of HCV genotypes distribution in Brazil is similar to the one found in many European countries, with prevalence of types 1 and 3, with epidemiological behavior typical of an exponential spread in recent years, probably through blood transfusions (2).

Here, genotypes 1, 2, and 3 of the virus were found among the 115 HCV-RNA positive samples evaluated, where genotype 1 was by far the most frequent (77.4%), followed by genotype 3 (20.9%), genotype 2 (0.8%), and mixed infection 1a/3a (0.8%). Indeed, there was no substantial differences in prevalence of genotype 1 compared to frequencies obtained from anti-HCV positive patients in other Brazilian locations (6,8,11,13).

In a study carried out to investigate HCV prevalence in different regions of Brazil (2), genotype 1 was predominant, with lower frequency of the genotype in the Southern region (Rio Grande do Sul), 37.5%, when compared to the other regions, whose prevalence ranged from 50 to 82.2%. In the Northeastern population the authors found genotype 1 the most prevalent, with higher frequency in Alagoas (82.1%), comparable to the one found in our study results using a larger number of samples.

Here we report for the first time genotype 2 in the state of Alagoas. However, the prevalence of the genotype was lower to those previously reported in other geographic regions of Brazil, 2.4% in Pernambuco (2), 6.7% in Bahia (20), 1.6% in Paraná (16), as well as 25.8% in Mato Grosso and 62.5% in Rio Grande do Sul (2).

Campiotto and collaborators (2) found that the frequency of genotype 3 varied among the different Brazilian regions, with its highest value in Santa Catarina (50%) and Paraná (41.7%), Southern region, as well as Goiás, Center-West region, (37.1%), and Pernambuco (36.9%), Northeast region. In our study, the frequency of genotype was similar to the one obtained the investigators for the state (17.9%).

Genotype 1 is predominant worldwide and is comprised of two major subtypes, 1a and 1b (9,15). Furthermore, it has been demonstrated that the growth rates of these subtypes are greater than those of genotypes 4 and 6 (10).

In our population, the prevalence of subtype 1b (65.2%) was higher than what was observed in Salvador, Bahia state (11), where the subtype only occurred in 38.6% of the anti-HCV-positive samples. However, it is very similar to the results obtained from HCV seropositive samples from the Brazilian states of Acre, Amazonas, and Pará (North region), as well as from Bahia and Pernambuco, with frequencies of 60% and 71% for each region, respectively (8).

Despite not being reported frequently, one sample had a mixed pattern 1a/3a, what could be explained by mutations in the core region or by coinfection (11). In our study, subtypes

Table 1. Distribution of Hepatitis C virus genotypes in seropositive patients in the state of Alagoas, Brazil

No. (%) of isolates of the genotypes							
Type 1			Type 2	Type 3		Mixed	All
1a	1b	Undet. ^a	(2a)	3a	Undet. ^a	(1a/3a)	
10 (8.7)	75 (65.2)	4 (3.5)	1 (0.8)	7 (6.1)	17 (14.8)	1 (0.8)	115 (100.0)

^a Undetermined= subtype no further determined.

related to four genotype 1 (3.5%) and 17 genotype 3 (14.8%) could not be determined by the methods used.

Brazil Health Ministry recommends the accurate identification of infecting HCV in order to distinguish genotype 1 and 4 from the others, thus limiting costs and improving clinical outcomes. Therefore, this study demonstrates that genotype 1 was the most frequent (77.4%) in Alagoas, which will require longer antiviral therapy.

ACKNOWLEDGMENTS

This work was supported by the National Council of Technological and Scientific Development (CNPq), Research Foundation for the State of Alagoas (Fapeal), and Brazil Health Ministry (MS).

RESUMO

Distribuição de genótipos do Vírus da hepatite C (HCV) em pacientes soropositivos no Estado de Alagoas, Brasil

A frequência de genótipos do vírus da hepatite C (HCV) em pacientes soropositivos anti-HCV no estado de Alagoas, Brasil, foi determinada através da RT-PCR aninhada da região 5'NCR seguida pela análise do polimorfismo de comprimento dos fragmentos de restrição (RFLP). A RT-PCR aninhada utilizando primers genótipo-específicos da região core foi efetuada quando não foi possível determinar o genótipo pelo primeiro método. Níveis detectáveis de HCV-RNA estavam presentes em 115 (74,7%) das 154 amostras de soro. O genótipo 1 foi o mais freqüente (77,4%), contra 20,9% do genótipo 3 e 0,8% do genótipo 2. O subtipo 1b foi predominante (65,2%), seguido pelos subtipos 1a (8,7%) e 3a (6,1%). Co-infecção (1a/3a) foi detectada em 0,8% das amostras. Não foram encontradas diferenças significativas quanto à prevalência do genótipo 1 em relação ao que tem sido obtido de pacientes soropositivos anti-HCV de outras localidades do Brasil. Este é o primeiro relato da presença do genótipo 2 no estado.

Palavras-chave: Vírus da hepatite C, 5'NCR, região core, RT-PCR aninhada, genótipos

REFERENCES

- Bezerra, C.S.; Lima, M.C.; Vilar, J.L.; Moreira, J.L.B.; Frota, C.C. (2007). Viral hepatitis C in a leading Brazilian hospital: epidemiological factors and genotyping. *Braz. J. Microbiol.*, 38 (4), 656-666.
- Campiotto, S.; Pinho, J.R.R.; Carrilho, F.J.; Da Silva, L.C.; Souto, F.J.D.; Spinelli, V.; Pereira, L.M.M.B.; Coelho, H.S.M.; Silva, A.O.; Fonseca, J.C.; Rosa, H.; Lacet, C.M.C.; Bernardini, A.P. (2005). Geographic distribution of Hepatitis C virus genotypes in Brazil. *Braz. J. Med. Biol. Res.*, 38 (1), 41-49.
- Chan, S.W.; McOmish, F.; Holmes, E.C.; Dow, B.; Peutherer, J.F.; Follet, E.; Yap, P.L.; Simmonds, P. (1992). Analysis of a new hepatitis C virus type and its phylogenetic relationship to existing variants. *J. Gen. Virol.*, 73 (Pt 5), 1131-1141.
- Davis, G.L.; Lau, J.Y. (1997). Factors predictive of a beneficial response to therapy of hepatitis C. *Hepatology*, 26 (3 Suppl 1), 122S-127S.
- EASL International Consensus Conference on Hepatitis C. (1999). Consensus statement. *J. Hepatology*, 31 (Suppl 1), 3-8.
- Gonçalves, P.L.; Cunha, C.B.; Busek, S.C.U.; Oliveira, G.C.; Ribeiro-Rodrigues, R.; Pereira, F. (2005). Detection of hepatitis C virus RNA in saliva samples from patients with seric anti-HCV antibodies. *Braz. J. Infect. Dis.*, 9 (1), 28-34.
- Lerat, H.; Rumin, S.; Habersetzer, F.; Berby, F.; Traub, M.A.; Trépo, C.; Inchauspé, G. (1998). In vivo tropism of hepatitis C virus genomic sequences in hematopoietic cells: Influence of viral load, viral genotype, and cell phenotype. *Blood*, 91 (10), 3841-3849.
- Lunge, V.R.; Ikuta, N.; Fonseca, A.S.K.; Marques, E.K.; Cheinquer, H. (1999). Prevalence of hepatitis C virus genotypes in north and northeast regions from Brazil. XX World Congress of Pathology and Laboratory Medicine, São Paulo, SP, Brazil, p. 81-86.
- Oliveira, M.L.A.; Bastos, F.I.; Sabino, R.R.; Paetzold, U.; Schreier, E.; Pauli, G.; Yoshida, C.F.T. (1999). Distribution of HCV genotypes among different exposure categories in Brazil. *Braz. J. Med. Biol. Res.*, 32 (3), 279-282.
- Pybus, O.G.; Charleston, M.A.; Gupta, S.; Rambaut, A.; Holmes, E.C.; Harvey, P.H. (2001). The epidemic behavior of the hepatitis C virus. *Science*, 292 (22), 2323-2325.
- Silva, L.K.; Paraná, R.; Souza, S.P.; Berby, F.; Kay, A.; Trépo, C.; Santana, N.; Cotrim, H.; Lyra, L.G.; Reis, M.G. (2000). Hepatitis C virus genotypes in a northeastern area of Brazil. *Am. J. Trop. Med. Hyg.*, 62 (2), 257-260.
- Simmonds, P.; Alberti, A.; Alter, H.J.; Bonino, F.; Bradley, D.W.; Brechot, C.; Brouwer, J.T.; Chan, S.W.; Chayama, K.; Chen, D.S.; Choo, Q.-L.; Colombo, M.; Cuyppers, H.T.M.; Date, T.; Dusheiko, G.M.; Esteban, J.I.; Fay, O.; Hadziyannis, S.J.; Han, J.; Hatzakis, A.; Holmes, E.C.; Hotta, H.; Houghton, M.; Irvine, B.; Kohara, M.; Kolberg, J.A.; Kuo, G.; Lau, J.Y.N.; Lelie, P.N.; Maertens, G.; McOmish, F.; Miyamura, T.; Mizokami, M.; Nomoto, A.; Prince, A.M.; Reesink, H.W.; Rice, C.; Roggendorf, M.; Schalm, S.W.; Shikata, T.; Shimotohno, K.; Stuyver, L.; Trépo, C.; Weiner, A.;

- Yap, P.L.; Urdea, M.S. (1994). A proposed system for the nomenclature of hepatitis C viral genotypes. *Hepatology*, 19 (5), 1321-1324.
13. Souza, M.; Paraná, R.; Trépo, C.; Barbosa-Jr, A.A.; Oliveira, I.R.; Andrade, Z.A. (2001). Effect of interferon-alpha on experimental fibrosis of the liver-study with a new model. *Mem. Inst. Oswaldo Cruz*, 96 (3), 343-348.
 14. Strauss, E. (2001). Hepatite C. *Rev. Soc. Bras. Med. Trop.* 34 (1), 69-82.
 15. Takahashi, M.; Yamada, G.; Miyamoto, R.; Doi, T.; Endo, H.; Tsuji, T. (1993). Natural course of chronic hepatitis C. *Am. J. Gastroenterol.*, 88 (2), 240-243.
 16. Vogler, I.H.; Nishiya, A.; Morimoto, H.K.; Reiche, E.M.V.; Bortoliero, A.L.; Matsuo, T.; Sabino, E.C.; Vaz, A.J. (2004). Serological, epidemiological and molecular aspects of hepatitis C virus infection in a population from Londrina, PR, Brazil, 2001-2002. *Rev. Inst. Med. Trop. São Paulo*, 46 (6), 303-308.
 17. Wasley, A.; Alter, M.T. (2000). Epidemiology of hepatitis C: geographic differences and temporal trends. *Semin. Liver Dis.*, 20 (1), 1-16.
 18. Webster, G.; Barnes, E.; Brow, D.; Dusheiko, G. (2000). HCV genotypes-role in pathogenesis of disease and response to therapy. *Baillieres Best Pract. Res. in Clin. Gastroenterol.*, 14 (2), 229-240.
 19. Zarife, M.A.S.; Oliveira, E.C.P.; Romeu, J.M.S.L.; Reis, M.G. (2006). Detecção do genótipo 4 do vírus da hepatite C em Salvador, Bahia. *R. Soc. Bras. Med. Trop.*, 39 (6), 567-569.
 20. Zarife, M.A.S.; Silva, L.K.; Silva, M.B.; Lopes, G.B.; Barreto, M.L.; Teixeira, M. da G.; Dourado, I.; Reis, M.G. (2006). Prevalence of Hepatitis C virus infection in north-eastern Brazil: a population-based study. *Trans. R. Soc. Trop. Med. Hyg.*, 100 (7), 663-668.
 21. Zein, N.N. (2000). Clinical significance of hepatitis C virus genotypes. *Clin. Microbiol. Rev.*, 13 (2), 223-235.
 22. Zylberberg, H.; Chaix, M.L.; Bréchet, C. (2000). Infection with hepatitis C virus genotype 4 is associated with a poor response to interferon alpha. *Ann. of Intern. Med.*, 132 (10), 845-846.