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BRIEF COMMUNICATION

HEPATITIS C VIRUS GENOTYPES IN HEMODIALYSIS PATIENTS IN THE FEDERAL DISTRICT, BRAZIL

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SUMMARY

Hepatitis C virus (HCV) genotypes and subtypes were determined in hemodialysis patients in the Federal District, Brazil, by sequencing of the 5' noncoding (NC) and nonstructural 5B (NS5B) regions. From 761 patients, 66 anti-HCV-positive samples were tested for HCV RNA. All 51 HCV RNA-positive samples by PCR of the 5' NC region were genotyped as genotypes 1 (90.2%) and 3 (9.8%). Subtype 1a (82.3%) was the most prevalent, followed by subtypes 3a (9.8%), 1b (5.9%) and 1a/1b (2.0%). Forty-two samples could be amplified and genotyped in the NS5B region: 38 (90.5%) as genotype 1, subtypes 1a, and 8 (9.5%) as genotype 3, subtype 3a. For the 42 samples sequenced in both regions, the genotypes and subtypes determined were concordant in 100% and 95.2% of cases, respectively. Two samples presented discrepant results, with the 5' NC region not distinguishing correctly the subtypes 1a and 1b. These findings indicate that the HCV genotype 1, subtype 1a, is the most prevalent among hemodialysis patients in the Federal District, Brazil.

KEYWORDS: Hepatitis C virus; Hemodialysis; Genotypes.

INTRODUCTION

Hemodialysis patients are at high risk of acquiring the hepatitis C virus (HCV) infection. These patients have an increased tendency to become HCV chronic carriers and also to be a potential reservoir for its transmission, possibly contributing to the nosocomial spread of HCV in dialysis centers[5,13]. In addition, hepatitis C seems to increase the mortality rate in this group of patients[4,20].

Based on the nucleotide (nt) sequence divergence, HCV is classified into six major genetic groups, designated genotypes (1 to 6), each one comprising multiple subtypes (designated a, b, c, etc). These genotypes and subtypes have distinct geographical distributions. Genotype determination is a relevant predictive parameter of the response to the antiviral treatment. Information about subtype distribution is required to perform effective HCV molecular and epidemiological surveillance, to study modes of transmission, and improve further vaccine development efforts[22].

In order to determine HCV genotypes and subtypes, the choice of the genome region to be analyzed is crucial. This region must present genotype-specific and subtype-specific motifs. Additionally it must be highly conserved to be detected by most of the available assays, based on nucleic acid amplification. Several assays were developed to identify HCV genotypes and subtypes from the 5' non-coding (NC) region because this region is readily amplified by PCR. However, this region does not contain sufficient information to resolve subtypes. Therefore, sequence analysis of the protein-coding regions such as Core, Envelope (E1) or nonstructural 5B (NS5B) of HCV genome are necessary to discriminating between subtypes[8,14,22].

In Brazil, a country of continental dimensions, the distribution of HCV genotypes and subtypes among hemodialysis patients has not been well documented. Studies carried out in São Paulo[15,16,19], Rio de Janeiro[3], Belo Horizonte[7], Recife[1], Tocantins[23], Goiás[8] and Campo Grande[10] have shown that genotype 1, subtype 1a, was prevalent in those patients. However, the genotyping methods employed in the majority of those studies (except in Goiás State), which were based on 5' NC region analyses, did not permit the correct identification of the HCV subtypes. In the Federal District, Brazil, there are no data about the genetic diversity of HCV isolated in these patients. In the present study, the HCV genotypes and subtypes were determined in hemodialysis patients in the Federal District, Brazil, by nucleotide sequencing analysis of the 5' NC and NS5B regions.

MATERIALS AND METHODS

The study was carried out in the seven dialysis units of the Federal

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District, Central Brazil. Between January and May 2002, blood samples were collected from 761 chronic hemodialysis patients (nearly 86% of this population) and serum samples were screened for anti-HCV by a third-generation enzyme-linked immunoassay (Abbott PrismR, Chicago, IL, USA). The study protocol was approved by the Ethics Committee of the Health Department of the Federal District, and written informed consent was obtained from each patient.

Sixty-six anti-HCV positive samples were subjected to RNA extraction by the commercially standardized reagents (QIAamp Viral RNA Mini Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions, followed by reverse transcription, as described by GINABREDA *et al*.[11]. cDNA was amplified using the primers complementary to the conserved area of the 5' NC and NS5B regions and PCR conditions described by GINABREDA *et al*.[11] and SANDRES-SAUNÉ *et al*.[21], respectively.

HCV genotyping was determined in HCV-RNA-positive samples by analyzing sequences of the 5' NC and NS5B regions. The nested RT-PCR products were purified using the QIAquick gel extraction kit (Qiagen - GmbH, Hilden, Germany) and submitted to a direct nucleotide sequencing reaction using a Big Dye Terminator kit (version 3.1, Applied Biosystems, Foster City, CA, USA) and analyzed with the ABI 3730 automated DNA sequencer. For 5' NC region, the sequence from nucleotide 63 to nucleotide 316 was used for analysis and genotyping. For NS5B region, the sequence from nucleotide 8259 to nucleotide 8625 was used for analysis, and genotyping was performed by phylogenetic analysis using reference sequences retrieved from GenBank. The homology among HCV sequences from the Federal District and those already deposited at HCV Databases was analyzed using the HCV-Blast program (available at http://hcv.lanl.gov). The phylogenetic tree was constructed with PHYLIP version 3.6 software[9] using the neighbor-joining method and the Kimura-two parameter, and its reliability was assessed by bootstrap resampling (1000 pseudo-replicas). To avoid cross-contamination between samples, standard precautions were used in all the manipulations. Separate areas were used for reagents, samples and manipulation of amplified products.

RESULTS AND DISCUSSION

The HCV RNA was detected in 51 samples by RT-nested PCR of the 5'NC region. Direct nucleotide sequencing of 207 nt from the 5' NC region was performed in all HCV RNA-positive samples. Among them, 90.2% were of genotype 1, subtypes 1a (82.3%), 1b (5.9%) and 1a/1b (2.0%). The remaining samples (9.8%) belonged to genotype 3, subtype 3a (Table 1).

Of the 51 HCV RNA-positive samples, 42 (82.3%) samples could be amplified and sequenced in the NS5B region. Using phylogenetic tree analysis of this region 328 nt, 38 sequences (90.5%) were classified as genotype 1, subtype 1a. All genotype 3 sequences (n = 4; 9.5%) were grouped inside the clad of subtype 3a of the phylogenetic tree (Fig. 1 and Table 1).

For the 42 samples which were genotyped by sequencing of the two regions, the genotypes and subtypes determined were concordant in 100% and 95.2% of cases, respectively. Two samples showed discrepant results. These samples (subtype 1b by the analysis of the 5' NC region) were identified as 1a by sequence analysis of the NS5B region. In addition, the 9 samples which were amplified and sequenced in the 5'NC region, but not in the NS5B region, were of subtype 1a (n = 6), 1b (n = 1), 1a/1b (n = 1) and 3a (n = 1).

Table 1
Distribution of hepatitis C virus subtypes isolated from hemodialysis patients in the Federal District using the 5' noncoding (NC) and nonstructural 5B (NS5B) regions sequencing

Region/Subtype	N	Frequency (%)
5' NC (N = 51)		
1a	42	82.3
1b	3	5.9
1a/1b	1	2.0
3a	5	9.8
NS5B (N = 42)		
1a	38	90.5
1b	0	0
3a	4	9.5

The distribution of HCV genotypes in the study population, with a predominance of subtype 1a (90.5%) followed by 3a (9.5%), differs from that of local blood donors, in which subtypes 3a (39.1%), 1a (34.1%) and 1b (26.8%) were detected[2]. However, the predominance of genotype 1, subtype 1a was also found in other Brazilian studies carried out among hemodialysis patients[1,8,10,15,16,19,23]. These data suggest that the high frequency of subtype 1a is probably a consequence of HCV nosocomial transmission in hemodialysis units.

In the present study, all 51 HCV RNA-positive samples were genotyped by sequence analysis of the 5' NC region. Of these, 9 (17.7%) could not be genotyped by NS5B sequence analysis due to failure of the amplification procedure despite successive attempts to amplify cDNA with the NS5B primers. Although these primers are thought to bind highly conserved sequences[21], primer-target mismatching within the NS5B region is still likely because the relative variability of its sequence that may not always be recognized by the used primers. Despite this inability to amplify all HCV RNA positive samples, NS5B sequence analysis was used here as a reference method for accurate genotyping[8,21].

As observed by other authors[6,8,12,18], no difference at the genotype level was found for the 42 samples that could be successfully amplified and sequenced in the the 5' NC and NS5B regions. Also, in accordance with previous studies[6,8,12,14,17,18,21], discrepant results at the subtype level, mainly subtypes 1a and 1b, were found here (two samples identified as 1a by NS5B sequencing were subtyped as 1b by 5' NC sequencing). Although both regions provide similar results at the genotype level, which is adequate for clinical practice, especially in the management of antiviral therapy, the NS5B sequencing appears to be more useful for epidemiological investigations.

In conclusion, by means of 5'NC and NS5B sequence analysis, this study demonstrates that HCV subtype 1a is the most prevalent among hemodialysis patients in the Federal District, Brazil. In addition, the results presented here highlight that 5' NC and NS5B regions provide

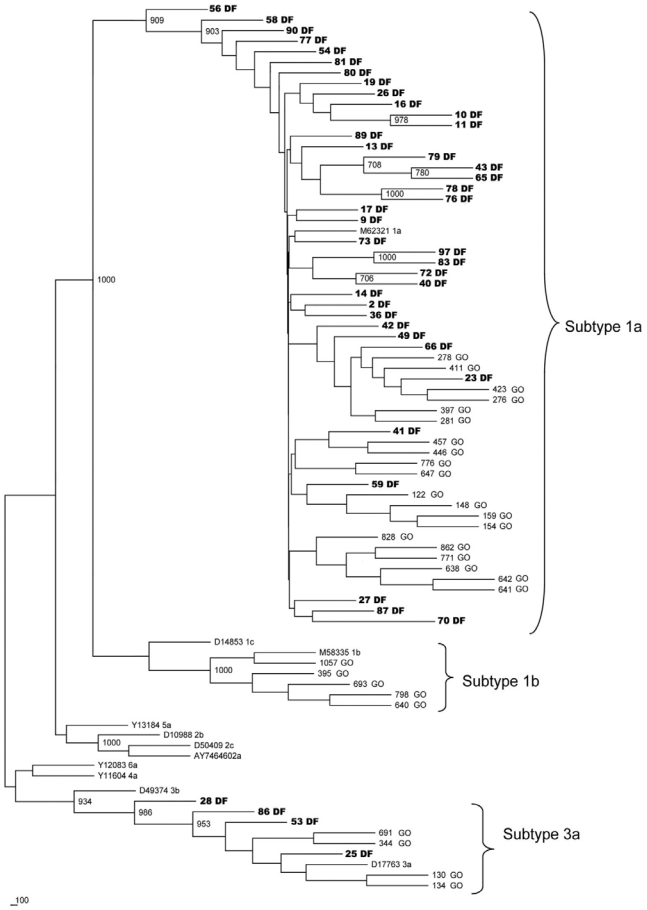

Fig. 1 - Phylogenetic tree analysis of the NS5B region of HCV genome of 42 isolates from hemodialysis patients in the Federal District, 11 references sequences of main HCV subtypes and 28 sequences from hemodialysis patients in Goiás State (GO). Strains belonging to this study are shown in bold and designated by their number and DF, and the references sequences used are: M62321-1a, M58335-1b, D14853-1c, AY746460-2a, D10988-2b, D50409-2c, D17763-3a, D49374-3b, Y11604-4a, Y13184-5a, Y12083-6a. Nodes with bootstrap (1000 pseudo-replicas) support higher than 700 are indicated.

similar HCV genotyping results at the genotype level, which is adequate for clinical purposes, but direct sequencing of the NS5B region is more reliable for HCV subtype identification required in epidemiological studies.

RESUMO

Genótipos do vírus da hepatite C em pacientes em hemodiálise no Distrito Federal, Brasil

Os genótipos e subtipos do vírus da hepatite C (HCV) foram determinados em pacientes em hemodiálise no Distrito Federal, Brasil, pelo sequenciamento das regiões 5' não codificante (NC) e não estrutural 5B (NS5B). De 761 pacientes, 66 amostras anti-HCV positivas foram testadas para RNA-HCV. Todas as 51 amostras RNA-HCV positivas por PCR para a região 5' NC foram genotipadas como dos genótipos 1 (90,2%) e 3 (9,8%). O subtipo 1a (82,3%) foi o mais prevalente, seguido pelos subtipos 3a (9,8%), 1b (5,9%) e 1a/1b (2,0%). Quarenta e duas amostras puderam ser amplificadas e genotipadas na região NS5B: 38 (90,5%) como genótipo 1, subtipo 1a, e 8 (9,5%) como genótipo 3, subtipo 3a. Para as 42 amostras sequenciadas nas duas regiões, os genótipos e subtipos determinados foram concordantes em 100% e 95,2% dos casos, respectivamente. Duas amostras apresentaram resultados discrepantes, sendo que a região 5' NC não diferenciou corretamente os subtipos 1a e 1b. Estes achados indicam que o genótipo 1, subtipo 1a, do HCV é o mais prevalente em pacientes em hemodiálise no Distrito Federal, Brasil.

REFERENCES

1. Albuquerque ACC, Coêlho MRCD, Lopes EPA, Lemos MF, Moreira RC. Prevalence and risk factors of hepatitis C virus infection in hemodialysis patients from one center in Recife, Brazil. Mem Inst Oswaldo Cruz. 2005;100:467-70.

2. Amorim RM, Oliveira CP, Wyant PS, Cerqueira DM, Câmara GN, Flores LS, *et al*. Hepatitis C virus genotypes in blood donors from the Federal District, Central Brazil. Mem Inst Oswaldo Cruz. 2004,99:895-7.

3. Azevedo HA, Villela-Nogueira CA, Perez RM, Segadas-Soares JA, Takahashi C, Gaburo N, *et al*. Similar HCV viral load levels and genotype distribution among end-stage renal disease patients on hemodialysis and HCV-infected patients with normal renal function. J Nephrol. 2007;20:609-16.

4. Baid-Agrawal S, Pascual M, Moradpour D, Frei U, Tolkoff-Rubin N. Hepatitis C virus infection in haemodialysis and kidney transplant patients. Rev med Virol. 2008;18:97-115.

5. Carneiro MA, Teles SA, Lampe E, Espírito Santo MP, Gouveia-Oliveira R, Reis NR, *et al*. Molecular and epidemiological study on nosocomial transmission of HCV in hemodialysis patients in Brazil. J Med Virol. 2007;79:1325-33.

6. Chen Z, Weck KE. Hepatitis C virus genotyping: interrogation of the 5' untranslated region cannot accurately distinguish genotypes 1a and 1b. J Clin Microbiol. 2002;40:3127-34.

7. De Paula Farah K, Carmo RA, De Figueiredo Antunes CM, Serufo JC, Nobre Jr VA, Fonseca de Castro LP, *et al*. Hepatitis C, HCV genotypes and hepatic siderosis in patients with chronic renal failure on haemodialysis in Brazil. Nephrol Dial Transplant. 2007;22:2027-31.

8. Espírito-Santo MP, Carneiro MAS, Reis NRS, Kozeowski AG, Teles SA, Lampe E, *et al.* Genotyping hepatitis C virus from hemodialysis patients in Central Brazil by line probe assay and sequence analysis. Braz J Med Biol Res. 2007;40:545-50.

9. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Molec Evol. 1981;17:368-76.

10. Freitas SZ, Da Cunha RV, Martins RM, Teles SA, Ibanhez ML, Motta-Castro AR. Prevalence, genotypes and risk factors associated with hepatitis C virus infection in hemodialysis patients in Campo Grande, MS, Brazil. Mem Inst Oswaldo Cruz. 2008;103:405-8.

11. Ginabreda MG, Yoshida CF, Niel C. Genomic characterization of Brazilian hepatitis C virus genotypes 1a and 1b. Braz J Med Biol Res. 1997;30:339-45.

12. Halfon P, Trimoulet P, Bourliere M, Khiri H, de Lédinghen V, Couzigou P, *et al*. Hepatitis C virus genotyping based on 5' noncoding sequence analysis (Trugene). J Clin Microbiol. 2001;39:1771-3.

13. Lampe E, Yoshida CF, De Oliveira RV, Lauer GM, Lewis-Ximenez LL. Molecular analysis and patterns of ALT and hepatitis C virus seroconversion in haemodialysis patients with acute hepatitis. Nephrology 2008;13:186-92

14. Laperche S, Lunel F, Izopet J, Alain S, Dény P, Duverlie G, *et al*. Comparison of hepatitis C virus NS5b and 5' noncoding gene sequencing methods in a multicenter study. J Clin Microbiol. 2005;43:733-9.

15. Lemos LB, Perez RM, Matos CA, Silva IS, Silva AE, Ferraz ML. Clinical and laboratory characteristics of acute hepatitis C in patients with end-stage renal disease on hemodialysis. J Clin Gastroent. 2008;42:208-11.

16. Moreira R, Pinho JRR, Fares J, Oba IT, Cardoso MR, Saraceni CP, *et al*. Prospective study of hepatitis C virus infection in hemodialysis patients by monthly analysis of HCV RNA and antibodies. Can J Microbiol. 2003;49:503-7.

17. Nolte FS, Green AM, Fiebelkorn KR, Caliendo AM, Sturchio C, Grunwald A, *et al*. Clinical evaluation of two methods for genotyping hepatitis C virus based on analysis of the 5' noncoding region. J Clin Microbiol. 2003;41:1558-64.

18. Othman SB, Trabelsi A, Monnet A, Bouzgarrou N, Grattard F, Beyou A, *et al*. Evaluation of a prototype HCV NS5b assay for typing strains of hepatitis C virus isolated from Tunisian haemodialysis patients. J Virol Meth. 2004;119:177-81.

19. Perez RM, Ferraz ML, Figueiredo MS, Contado D, Koide S, Ferreira AP, *et al*. Unexpected distribution of hepatitis C virus genotypes in patients on hemodialysis and kidney transplant recipients. J Med Virol. 2003;69:489-94.

20. Perico N, Cattaneo D, Bikbov B, Remuzzi G. Hepatitis C infection and chronic renal diseases. Clin J Am Soc Nephrol. 2009;4:207-20.

21. Sandres-Sauné K, Deny P, Pasquier C, Thibaut V, Duverlie G, Izopet J. Determining hepatitis C genotype by analyzing the sequence of the NS5b region. J Virol Meth. 2003;109:187-93.

22. Simmonds P, Bukh J, Combet C, Deléage C, Enomoto N, Feinstone S, *et al*. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology. 2005;42:962-73.

23. Souza KP, Luz JA, Teles SA, Carneiro MA, Oliveira LA, Gomes AS, *et al*. Hepatitis B and C in the hemodialysis unit of Tocantins, Brazil: serological and molecular profiles. Mem Inst Oswaldo Cruz. 2003;98:599-603.

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