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Prevalence of hepatitis C virus (HCV) infection and HCV genotypes of hemodialysis patients in Salvador, Northeastern Brazil

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Abstract

Hepatitis C virus (HCV) infection has been identified as the major cause of chronic liver disease among patients on chronic hemodialysis (HD), despite the important reduction in risks obtained by testing candidate blood donors for anti-HCV antibodies and the use of recombinant erythropoietin to treat anemia. A cross-sectional study was performed to estimate the prevalence of HCV infection and genotypes among HD patients in Salvador, Northeastern Brazil. Anti-HCV seroprevalence was determined by ELISA in 1243 HD patients from all ten different dialysis centers of the city. HCV infection was confirmed by RT-PCR and genotyping was performed by restriction fragment length polymorphism. Anti-HCV seroprevalence among HD patients was 10.5% (95% CI: 8.8-12.3) (Murex anti-HCV, Abbott Murex, Chicago, IL, USA). Blood samples for qualitative HCV detection and genotyping were collected from 125/130 seropositive HD patients (96.2%). HCV-RNA was detected in 92/125 (73.6%) of the anti-HCV-positive patients. HCV genotype 1 (77.9%) was the most prevalent, followed by genotype 3 (10.5%) and genotype 2 (4.6%). Mixed infections of genotypes 1 and 3 were found in 7.0% of the total number of patients. The present results indicate a significant decrease in anti-HCV prevalence from 23.8% detected in a study carried out in 1994 to 10.5% in the present study. The HCV genotype distribution was closely similar to that observed in other hemodialysis populations in Brazil, in local candidate blood donors and in other groups at risk of transfusion-transmitted infection.

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- Hepatitis C
- Seroprevalence
- Viremia
- Hemodialysis
- Genotype
- Brazil

Introduction

Hepatitis C virus (HCV) is a small enveloped virus first isolated in 1989 (1) which belongs to the family Flaviviridae. Its genome is composed of a positive-sense, single-stranded RNA encoding a polyprotein comprising structural (core and envelope glycoproteins E1 and E2) and non-structural (NS2, NS3a/b, NS4a/b, and NS5a/b) proteins. In 50% or more of cases, acute HCV infections result in chronic hepatitis, which may lead to cirrhosis and hepatocellular carcinoma (2).

Diagnosis of this infection has evolved remarkably, progressing from the simple detection of anti-HCV antibodies by ELISA to molecular methods. With early serological assays, especially when applied to immunodepressed patients such as those under hemodialysis, false-negative results were obtained for a considerable number of patients. The latest development is the qualitative determination of HCV-RNA which allows early diagnosis and detection of viremia and provides products for viral genotyping (3).

Recently, HCV infection has been identified as the major cause of chronic liver disease among patients on chronic hemodialysis (HD), despite the important reductions in risk obtained by testing candidate blood donors for anti-HCV antibodies and the use of recombinant erythropoietin to treat anemia. Among HD patients, the prevalence of the antibody to HCV infection (anti-HCV) may vary greatly according to the country and dialysis center, ranging from less than 1% to more than 50% in Brazil (4-10), Southern Europe (11), USA (12), and Japan (13), and 1-10% in Northern European countries (14). However, in some cases HCV infection is not identified by using anti-HCV antibodies (15). At present, routine screening for HCV infection by the polymerase chain reaction (PCR) is recommended for identifying false-seronegative patients. The measurement of aspartate and alanine aminotransferases has served as an auxiliary but less specific test (16).

The study of genetic variability of HCV strains has led to a consensus classification into six major genotypes, many of which include a number of closely related subtypes (3). Some studies suggest that the clinical features of liver disease depend on HCV genotypes (17), but this has not been accepted by many investigators (18). It is also noteworthy that the success of interferon treatment seems to be related to genotype (19). These observations have raised interest in the identification of infecting HCV genotypes from different geographical regions and groups at risk. Furthermore, genotyping is a useful tool for the purposes of molecular epidemiology.

The primary objective of the present study was to update the information about the anti-HCV seroprevalence and to determine the HCV viremia and genotypes in a group of HD patients belonging to all ten dialysis centers in the city of Salvador, BA, in Northeastern Brazil.

Patients and Methods

Patients

A cross-sectional study was conducted at all ten dialysis centers between April and July 2002, and 130 anti-HCV positive HD patients were enrolled. The Institutional Ethics Committee of the Gonçalo Moniz Research Center, Oswaldo Cruz Foundation, Bahia, approved the study protocol. Written informed consent was obtained from all subjects who participated in the study.

Serum samples for the assays were collected from 125 participants, representing 96.2% of eligible subjects, who were also interviewed. Five patients did not follow this protocol for personal reasons and were excluded. Clinical and laboratory data were collected from the medical records of the patients.

Serological data

Anti-HCV antibody results for the HD patients were available at the hemodialysis centers. Since 1996, the Brazilian Health Ministry has required all patients undergoing HD treatment to be screened for anti-HCV every 3 months. All serum samples used for molecular assays were anti-HCV positive as identified by a third-generation ELISA according to manufacturer instructions (Murex anti-HCV, Abbott Murex, Chicago, IL, USA).

Samples for molecular assays

Blood samples were always collected at the beginning of the week before heparin introduction to avoid false-negative results due to the presence of this PCR-inhibiting component (20). Within 2 h after venipuncture, all samples were aliquoted and stored immediately at -70ºC until use. Aliquots were not thawed more than once prior to analysis to avoid RNA degradation.

Extraction of HCV-RNA and cDNA synthesis

Two hundred microliters of serum was used for HCV-RNA extraction using Trisol LS reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to manufacturer instructions, precipitated with ethanol, and dried (21). HCV-RNA was immediately transcribed into cDNA using random primers (Amersham Biosciences, Piscataway, NJ, USA).

HCV-RNA detection and genotyping

cDNA was targeted by a nested-PCR directed at the 5' untranslated region using specific primers 939, 209, 940, and 211, as previously described (22). The 251-bp (unlabeled) second PCR product was submitted to electrophoresis using a 1.5% routine agarose gel in 1X Tris borate buffer and visualized by ethidium bromide staining under ultraviolet light. Positive samples were genotyped by restriction fragment length polymorphism (RFLP) according to Davidson et al. (23). Briefly, restriction digestions were carried out for 4-16 h after adjustment with 10X enzyme reaction buffer as appropriate. Reactions were carried out at 37ºC in the presence of 10 units each of a) *Rsa*I and *Hae*III, and b) *Hin*fI and *Mva*I. Digestion products were visualized under ultraviolet light after 4% Metaphor agarose gel electrophoresis (BMA, Rockland, ME, USA) in 1X Tris borate buffer containing 0.5 µg/mL ethidium bromide. Figure 1 illustrates the band pattern consistently produced by RFLP in different genotypes. Previously genotyped samples from our laboratory were used as positive controls for genotypes 1 and 3. Genotypes were determined by the method of Simmonds et al. (3). Samples with undetectable HCV-RNA by nested-PCR were extracted at least twice in different experiments. Even when confirmed to be negative, all patients were instructed to repeat blood collection within 6 months after the first exam to avoid false-negative results.

Statistical analysis

Data were analyzed using the statistical database package Epi-Info Version 6.04d (Center for Disease Control, GA, USA) and PEPI Version 4.0 (25). The 95% confidence intervals (95% CI) of prevalence were estimated using the Mid-p algorithm. Fisher's exact test and the χ^2 test with Yates correction were used to compare frequencies between groups when appropriate. ANOVA was used to compare means, and the Kruskal-Wallis test was used when the variances were heterogeneous. P values <0.05 were considered to be statistically significant under the respective degree of freedom and critical value of the tests.

Results

Serological data collected from each di-

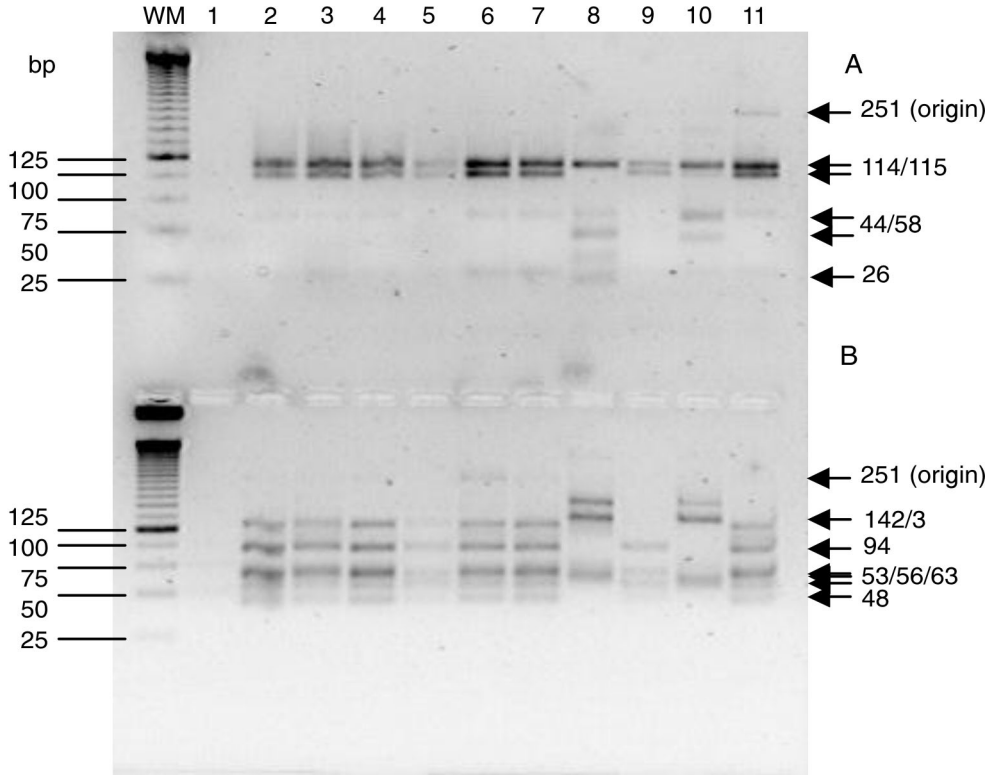

Figure 1. Agarose gel electrophoresis of restriction digests carried out in 251 base pairs (bp) of secondary PCR. Reactions were carried out at 37ºC in the presence of 10 units each of A) *Rsa*I and *Hae*III and B) *Hin*fI and *Mva*I as described by McOmish et al. (24) and Davidson et al. (23). Lane weight marker (WM), 25-bp DNA ladder; *lane 1*, blank control; *lanes 2-7* and *9*, genotype 1 samples; *lane 8*, genotype 3 sample; *lane 10*, genotype 3 control; *lane 11*, genotype 1 control. Genotype was deduced by the combination of the banding patterns produced by the two restriction reactions.

alysis center revealed an overall seroprevalence of anti-HCV of 10.5% (95% CI: 8.8-12.3), ranging from 4.1% (95% CI: 1.3-9.6) to 22.4% (95% CI: 17.2-28.4) according to the center. The risk of anti-HCV positivity was significantly higher ($P < 0.05$) at dialysis center 3 compared to centers 5 to 10, but not to centers 1, 2, and 4 (Table 1). None of the centers reported new seroconversions during the study period.

Table 1. Seroprevalence of the anti-hepatitis C virus (HCV) antibody in the dialysis centers in the city of Salvador, Northeastern Brazil.

Center	No. of patients	anti-HCV-positive patients (%)[a]	95% CI
1	72	11 (15.3)	8.3-25.0
2	149	17 (11.4)	7.0-17.3
3	214	48 (22.4)	17.2-28.4
4	99	16 (16.2)	9.9-24.4
5	135	6 (4.4)	1.8-9.0
6	117	8 (6.8)	3.2-12.6
7	143	7 (4.9)	2.2-9.4
8	98	4 (4.1)	1.3-9.6
9	64	5 (7.8)	2.9-16.5
10	152	8 (5.3)	2.5-9.7
Total	1243	130 (10.5)	8.8-12.3

[a]Pearson chi-square test (degree of freedom = 9; critical value = 58.8; $P < 0.01$). CI = confidence interval.

HCV viremia was found in 73.6% (92/125) of the anti-HCV-positive HD patients. The positivity of HCV-RNA did not vary significantly among different HD centers (Table 2).

No significantly different prevalence of a particular genotype emerges from the cross-sectional study related to the comparison of the genotypes in each dialysis center (Table 3). HCV genotype distribution was similar to the distribution among local candidate blood donors and other groups at transfusion-transmitted risk of infection (data not shown), with the predominance of genotype 1 (77.9%), followed by genotype 3 (10.5%) and genotype 2 (4.6%). Mixed infections were found in 7.0% of the total number of patients, all associating genotypes 1 and 3.

Six samples were not considered to be typable because the amplification was not strong enough (Table 3).

Discussion

The present investigation updates the data of the seroprevalence of anti-HCV reported in a 1994 study by Santana et al. (4) and provides information about HCV viremia and the genotypes circulating among HD patients in the city of Salvador. Since the study by Santana et al. almost a decade ago, the number of HD patients practically quadrupled to a total of about 1200 while the anti-HCV prevalence significantly decreased from 23.8% in 1994 to 10.5% in 2002 ($P < 0.01$). The anti-HCV prevalence observed is still very high when compared to rates found among candidate blood donors from the same region (1.5%; Hemotransfusion and Hemotherapy Foundation of Bahia, HEMOBA, personal communication in 1999). Nevertheless, the anti-HCV prevalence was the lowest compared to other Brazilian hemodialysis populations studied previously from Minas Gerais (20.3%), Porto Alegre (29.8%), Goiânia (46.7%), São Paulo (42.5%), and Rio de Janeiro (65%) (5-10). Taken together, these studies demonstrate that HCV infection is still a significant problem in Brazilian dialysis units.

The causes and source of infection in patients with chronic renal failure on hemodialysis are multiple. Before the introduc-

Table 2. Hepatitis C virus (HCV) RNA positivity among hemodialysis patients in the dialysis centers in the city of Salvador, Northeastern Brazil.

Center	No. of samples tested[a]	HCV-RNA-positive patients (%)[b]
1	10	7 (70.0)
2	17	14 (82.4)
3	48	32 (66.6)
4	16	11 (68.8)
5	6	6 (100.0)
6	8	5 (62.5)
7	7	6 (85.7)
8	4	4 (100.0)
9	5	4 (80.0)
10	4	3 (75.0)
Total	125	92 (73.6)

The total number of patients tested for anti-HCV antibody was 1243 (see Table 1). [a]The total number of samples reported was limited by data availability. [b]Pearson chi-square test (degree of freedom = 9; critical value = 6.8; P = 0.65).

Table 3. Hepatitis C virus genotypes isolated from hemodialysis patients in the dialysis centers in the city of Salvador, Northeastern Brazil.

Centers	No. of samples[a]	Genotype (%)[b]				
		1	2	3	Mix	NT
1	7	7 (100.0)	-	-	-	-
2	14	10 (76.9)	3 (23.1)	-	-	1
3	32	25 (80.6)	1 (3.2)	2 (6.5)	3 (9.7)	1
4	11	5 (62.5)	-	1 (12.5)	2 (25.0)	3
5	6	4 (66.6)	-	1 (16.7)	1 (16.7)	-
6	5	2 (50.0)	-	2 (50.0)	-	1
7	6	5 (83.3)	-	1 (16.7)	-	-
8	4	3 (75.0)	-	1 (25.0)	-	-
9	4	3 (75.0)	-	1 (25.0)	-	-
10	3	3 (100.0)	-	-	-	-
Total	92	67 (77.9)	4 (4.6)	9 (10.5)	6 (7.0)	6

The total number of patients tested for anti-HCV antibody was 1243 (see Table 1). NT = not typable. [a]The total number of samples reported was limited by data availability. [b]Pearson chi-square test (degree of freedom = 27; critical value = 31.2; P = 0.24).

tion of routine screening of candidate blood donors for anti-HCV, blood transfusions were an important risk factor for the acquisition of hepatitis C (5). On the other hand, several studies pointed to risk factors in the hemodialysis environment suggesting nosocomial transmission, such as increased number of patients under treatment per unit, patients attending more than one treatment unit, previous or present contact with HBV infection, type of dialysis equipment used, hygiene and sterilization of the equipment, number of times the dialysis lines and filters are reused, and duration of hemodialysis treatment (6,26). Last, patients on peritoneal dialysis and those on home hemodialysis are at lower risk of HCV infection than are patients dialyzed at a center (27). Nevertheless, only a few studies were successful in identifying molecular evidence of this mode of transmission (14), an issue that was not addressed in the present investigation.

As expected, anti-HCV prevalence varied widely according to HD centers. Since none of the centers reported new seroconversions during the study period, this variability may be related to how the patients differed at each clinic and the risk of nosocomial transmission in the centers in the past. It is known that different methods of control, cleaning and disinfection of the hemodialysis membranes, equipment, instruments, and surfaces may affect prevalences (26). In the relatively short period between the study by Santana et al. (4) and the present study, many small clinics merged and others expanded to accommodate the increased demand for dialysis. While the older centers concentrated on patients with established hemodialysis treatment, newer centers sought new patients, perhaps contributing to discrepancies in the prevalence by center.

The general reduction in HCV seroprevalence points to advances in the management of chronic renal treatment and in the control of HCV transmission (11). Some of these advances were the introduction of recombinant erythropoietin for the treatment of anemia that eliminated the need for regular blood transfusion, the routine anti-HCV screening test implemented at the blood centers preventing the transfusion route of HCV transmission, the modernization of new dialysis equipment, and the requirement of periodic anti-HCV screening of all patients undergoing hemodialysis treatment that permitted the individualization of treatment. Calabrese et al. (28) and others (29) showed that by dialyzing HCV-positive patients using separate equipment in a dedicated area (but not a separate room) led to a striking reduction in the incidence of HCV infection.

In the present study, PCR was used to confirm infection by detecting HCV-RNA in the serum. Although viremia did not differ among dialysis centers, the frequency can be considered low when compared to those observed in other groups of patients and hemodialysis populations (5,6,30). HCV-RNA-negative cases may be considered to be patients with either past infection or intermittent viremia status. In fact, the biological dynamics of HCV viremia is well known and the possibility that some of these HD patients have viral replication below the detection limit of PCR in serum should not be excluded (31). In HD patients, membrane-dependent adsorption of HCV can occur during hemodialysis, causing a transient decrease in HCV load in the circulation of patients (32). To increase the sensitivity of PCR, some investigators have suggested the application of PCR to detect HCV viremia in whole blood instead of serum (33,34). Furthermore, new promising methodology based on transcription-mediated amplification became available for more accurate HCV-RNA detection (35-37). On the other hand, false-negative ELISA has been reported (5,6). The presence of HCV viremia in anti-HCV-negative HD patients may play a role in the spread of HCV in dialysis units. In a study performed by our group on a smaller HD population from the city of Campina Gran-

de, State of Paraíba, the prevalence of HCV-RNA among anti-HCV-negative HD patients was found to be as high as 34.1% (38). Thus, the overall prevalence of HCV infection might have been much higher if we had been able to evaluate this group of patients. Such a potential problem might be discussed in terms of better validation of anti-HCV screening tests in this risk group or the necessity of incorporation of complementary molecular exams in the hemodialysis service (15,39). Previous studies have confirmed the importance of HCV-RNA and alanine aminotransferase levels for HCV screening in HD patients (16,40).

Prevalent genotypes were similar to those of local candidate blood donors (data not shown). This observation confirms the primary findings of Santana et al. (4) that HCV infection in HD patients is primarily associated with post-transfusion infection (4). However, mixed infections were found in 7.0% of the total patient population, all associating genotypes 1 and 3, the most prevalent genotypes. Despite the control of nosocomial transmission in the majority of centers in Salvador, the possibility of transmission among HCV-infected HD patients who use the same dialysis equipment should be better investigated to confirm the failure of the decontamination procedures between turns of use of the same equipment.

The present study confirms the expected decline in anti-HCV seroprevalence in HD patients in the city of Salvador. However, there still are many viremic patients demanding surveillance procedures to avoid outbreaks by nosocomial transmission. HCV genotypes were similar to those described for the candidate blood donor population and other groups at transfusion-transmitted risk of infection, like hemophiliacs and patients with sickle cell disease (data not shown). Further investigations using molecular epidemiology should be carried out in the hemodialysis environment to explain the presence of mixed infection among HD patients.

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References

1. Choo QL, Kuo G, Weiner AJ et al. (1989). Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science*, 244: 359-362.
2. Hoofnagle JH (1997). Hepatitis C: the clinical spectrum of disease. *Hepatology*, 26: 15S-20S.
3. Simmonds P, Holmes EC, Cha TA et al. (1993). Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *Journal of General Virology*, 74: 2391-2399.
4. Santana GO, Cotrim HP, Mota E et al. (2001). Antibodies to hepatitis C virus in patients undergoing hemodialysis in Salvador, BA, Brazil. *Arquivos de Gastroenterologia*, 38: 24-31.
5. Carneiro MA, Martins RM, Teles SA et al. (2001). Hepatitis C prevalence and risk factors in hemodialysis patients in Central Brazil: a survey by polymerase chain reaction and serological methods. *Memórias do Instituto Oswaldo Cruz*, 96: 765-769.
6. Busek SU, Baba EH, Tavares Filho HA et al. (2002). Hepatitis C and hepatitis B virus infection in different hemodialysis units in Belo Horizonte, Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz*, 97: 775-778.
7. Vanderborght BO, Rouzere C, Ginuino CF et al. (1995). High prevalence of hepatitis C infection among Brazilian hemodialysis patients in Rio de Janeiro: a one-year follow-up study. *Revista do Instituto de Medicina Tropical de São Paulo*, 37: 75-79.
8. Góngora DVN (1998). Marcadores sorológicos da infecção pelo vírus da hepatite C em trabalhadores e pacientes da Unidade de Diálise do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. *Revista da Sociedade Brasileira de*

Medicina Tropical, 31: 585-586.
9. Karohl C, Manfro RC, Senger MB et al. (1995). Prevalência de anticorpos antivírus da hepatite C em pacientes em hemodiálise crônica de Porto Alegre. *Jornal Brasileiro de Nefrologia*, 17: 40-46.
10. Oliveira ML, Bastos FI, Sabino RR et al. (1999). Distribution of HCV genotypes among different exposure categories in Brazil. *Brazilian Journal of Medical and Biological Research*, 32: 279-282.
11. Djukanovic L, Radovic M, Bakovic J et al. (2002). Epidemiology of end-stage renal disease and current status of hemodialysis in Yugoslavia. *International Journal of Artificial Organs*, 25: 852-859.
12. Sivapalasingam S, Malak SF, Sullivan JF et al. (2002). High prevalence of hepatitis C infection among patients receiving hemodialysis at an urban dialysis center. *Infection Control and Hospital Epidemiology*, 23: 319-324.
13. Yamaji K, Hayashi J, Kawakami Y et al. (1996). Long term survey of hepatitis C virus infection in hemodialysis units in Fukuoka, Japan. *Journal of Epidemiology*, 6: 166-171.
14. Allander T, Medin C, Jacobson SH et al. (1994). Hepatitis C transmission in a hemodialysis unit: molecular evidence for spread of virus among patients not sharing equipment. *Journal of Medical Virology*, 43: 415-419.
15. Bassit L, Van Heuverswyn H, De Bosschere K et al. (2002). Comparative study of two anti-HCV screening tests in a large genotyped population of Brazilian dialysis patients. *European Journal of Clinical Microbiology and Infectious Diseases*, 21: 404-406.
16. Caramelo C, Bartolome J, Albalate M et al. (1996). Undiagnosed hepatitis C virus infection in hemodialysis patients: value of HCV-RNA and liver enzyme levels. *Kidney International*, 50: 2027-2031.
17. Silini E, Bono F, Cividini A et al. (1995). Differential distribution of hepatitis C virus genotypes in patients with and without liver function abnormalities. *Hepatology*, 21: 285-290.
18. Benvegnu L, Pontisso P, Cavalletto D et al. (1997). Lack of correlation between hepatitis C virus genotypes and clinical course of hepatitis C virus-related cirrhosis. *Hepatology*, 25: 211-215.
19. Kohara M, Tanaka T, Tsukiyama-Kohara K et al. (1995). Hepatitis C virus genotypes 1 and 2 respond to interferon-alpha with different virologic kinetics. *Journal of Infectious Diseases*, 172: 934-938.
20. Masukawa A, Miyachi H, Ohshima T et al. (1997). Monitoring of inhibitors of the polymerase chain reaction for the detection of hepatitis C virus using the positive internal control. *Rinsho Byori*, 45: 673-678.
21. Sambrook J, Fritsch EF & Maniatis T (1989). *Molecular Cloning: A Laboratory Manual*. 2nd edn. Cold Spring Harbor Laboratory Press, New York.
22. Chan SW, McOmish F, Holmes EC et al. (1992). Analysis of a new hepatitis C virus type and its phylogenetic relationship to existing variants. *Journal of General Virology*, 73: 1131-1141.
23. Davidson F, Simmonds P, Ferguson JC et al. (1995). Survey of major genotypes and subtypes of hepatitis C virus using RFLP of sequences amplified from the 5' non-coding region. *Journal of General Virology*, 76: 1197-1204.
24. McOmish F, Yap PL, Dow BC et al. (1994). Geographical distribution of hepatitis C virus genotypes in blood donors: an international collaborative survey. *Journal of Clinical Microbiology*, 32: 884-892.
25. Abramson JH & Gahlinger PM (2001). Computer Programs for Epidemiologic Analyses: PEPI Version 4.0. [http://sagebrushpress.com/pepibook.html]. Accessed December 9, 2004.
26. Carrilho FJ, Moraes CR, Pinho JR et al. (2004). Hepatitis B virus infection in Haemodialysis Centres from Santa Catarina State, Southern Brazil. Predictive risk factors for infection and molecular epidemiology. *BMC Public Health*, 4: 13.
27. Cendoroglo Neto M, Draibe SA, Silva AE et al. (1995). Incidence of and risk factors for hepatitis B virus and hepatitis C virus infection among haemodialysis and CAPD patients: evidence for environmental transmission. *Nephrology, Dialysis, Transplantation*, 10: 240-246.
28. Calabrese G, Vagelli G, Guaschino R et al. (1991). Transmission of anti-HCV within the household of haemodialysis patients. *Lancet*, 338: 1466.
29. Harmankaya O, Cetin B, Obek A et al. (2002). Low prevalence of hepatitis C virus infection in hemodialysis units: effect of isolation? *Renal Failure*, 24: 639-644.
30. Silva LK, Parana R, Souza SP et al. (2000). Hepatitis C virus genotypes in a northeastern area of Brazil. *American Journal of Tropical Medicine and Hygiene*, 62: 257-260.
31. Fabrizi F, Martin P, Dixit V et al. (2000). Biological dynamics of viral load in hemodialysis patients with hepatitis C virus. *American Journal of Kidney Diseases*, 35: 122-129.
32. Mizuno M, Higuchi T, Yanai M et al. (2002). Dialysis-membrane-dependent reduction and adsorption of circulating hepatitis C virus during hemodialysis. *Nephron*, 91: 235-242.
33. Mazur W, Mazurek U, Jurzak M et al. (2001). Positive and negative strands of HCV-RNA in sera and peripheral blood mononuclear cells of chronically hemodialyzed patients. *Medical Science Monitor*, 7: 108-115.
34. Schmidt WN, Wu P, Cederna J et al. (1997). Surreptitious hepatitis C virus (HCV) infection detected in the majority of patients with cryptogenic chronic hepatitis and negative HCV antibody tests. *Journal of Infectious Diseases*, 176: 27-33.
35. Saback FL, Gomes SA, de Paula VS et al. (1999). Age-specific prevalence and transmission of TT virus. *Journal of Medical Virology*, 59: 318-322.
36. Sarrazin C (2002). Highly sensitive hepatitis C virus RNA detection methods: molecular backgrounds and clinical significance. *Journal of Clinical Virology*, 25 (Suppl 3): S23-S29.
37. Sarrazin C, Teuber G, Kokka R et al. (2000). Detection of residual hepatitis C virus RNA by transcription-mediated amplification in patients with complete virologic response according to polymerase chain reaction-based assays. *Hepatology*, 32: 818-823.
38. Florentino GSA (2004). Discordância entre os métodos de biologia molecular e ELISA no diagnóstico da hepatite C em pacientes hemodializados [Discordance between molecular biologic methods and ELISA in the diagnosis of hepatitis C in hemodialysis patients]. Master's thesis, Pós-graduação em Medicina, Universidade Federal da Bahia, Salvador, BA, Brazil.
39. Fabrizi F, Martin P, Dixit V et al. (1999). Detection of *de novo* hepatitis C virus infection by polymerase chain reaction in hemodialysis patients. *American Journal of Nephrology*, 19: 383-388.
40. Moreira R, Pinho JR, Fares J et al. (2003). Prospective study of hepatitis C virus infection in hemodialysis patients by monthly analysis of HCV-RNA and antibodies. *Canadian Journal of Microbiology*, 49: 503-507.