Prevalence and risk factors of hepatitis C virus infection in hemodialysis patients from one center in Recife, Brazil

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A hemodialysis population from a dialysis unit in the city of Recife, Northeastern Brazil, was screened to assess the prevalence of hepatitis C virus (HCV) infection and to investigate the associated risk factors. Hemodialysis patients (n = 250) were interviewed and serum samples tested for anti-HCV antibodies by enzyme-linked immunosorbent assay (ELISA). All samples were also tested for HCV RNA by reverse transcriptase nested polymerase chain reaction (RT-nested-PCR). Out of 250 patients, 21 (8.4%) were found to be seropositive by ELISA, and 19 (7.6%) patients were HCV RNA positive. HCV viraemia was present in 90.5% of the anti-HCV positive patients. The predominant genotype was HCV 1a (8/19), followed by 3a (7/19), and 1b (4/19). None of the anti-HCV negative patients were shown to be viraemic by the PCR. Univariate analysis of risk factors showed that time spent on hemodialysis, the number of blood transfusions and a blood transfusion before November 1993 were associated with HCV positivity. However, multivariate analysis revealed that blood transfusions before November 1993 were significantly associated with HCV infection in this population. Low prevalence levels were encountered in this center, however prospective studies are necessary to confirm these findings.

Key words: prevalence - risk factors - hemodialysis - genotypes - hepatitis C virus - polymerase chain reaction - Recife - Brazil

Hemodialysis patients are at high risk of hepatitis C infection. Some factors are especially related with these high prevalence rates, such as blood transfusions and length of dialysis time (Cardoso et al. 1994, Salama et al. 2000, Carneiro et al. 2001, Santana et al. 2001, Hinrichsen et al. 2002).

In 2000, the Brazilian Ministry of Health published guidelines for dialysis units, setting rigorous rules to improve this treatment procedure and thus lower mortality and morbidity rates (Ministério da Saúde 2004). These rules determine that hepatitis C serology had to be analyzed semestrally to avoid viral spreading throughout these units.

The guidelines had to be implemented correctly in order to reduce potential virus cross-contamination among patients and staff.

The aims of this study were: (1) to determine the prevalence of hepatitis C virus (HCV) infection in hemodialysis patients from a center in the city of Recife (Brazil); (2) to detect HCV infection by serology and polymerase chain reaction (PCR) among these patients; (3) to investigate associated risk factors, such as the number of blood transfusions and time spent on hemodialysis; and (4) to identify the HCV genotypes in this population by molecular method.

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MATERIALS AND METHODS

Subjects - The study was carried out at a dialysis unit in the city of Recife, Northeastern Brazil, between March and June 2002; 257 chronic hemodialysis patients were interviewed at the unit to establish any risk factors for HCV infection. The studied population ranged in age from 17 to 92 years (average 46 years); 147 of the patients were males (58.8%) and 103 were females (41.2%).

A standardized form was used to collect data regarding age, sex, length of time spent on hemodialysis, the number of blood transfusions, any blood transfusion before November 1993, any tattooing, and intravenous drug use. The study protocol was submitted and approved by the Ethical Committee of the Adolfo Lutz Institute and of the Health Science Center of the Federal University of Pernambuco. Consent was obtained from all patients.

Dialysis center - The HCV positive patients of the center studied realized the hemodialysis treatment in the reserved machines and separate rooms to reprocess dialyzers from anti-HCV positive and anti-HCV negative patients were observed.

Serum samples - Blood samples were collected from 250 patients. These samples were separated into three aliquots, one for serological tests, another for PCR and the third reserved for any necessary result confirmation. The sera were stored at –20ºC and –70ºC.

Anti-HCV serological tests - To study the prevalence of HCV infection in this population, all serum samples were analyzed by a third-generation enzyme immunoassay (Murex® anti-HCV version 4.0) following manufacturer's instructions. Samples with results ranging from 0.667 to 0.815 (signal/cutoff ratio) were consid-

ered indeterminate ELISA results. Samples with indeterminate results were tested by a third-generation recombinant Immunoblot (IB, CHIRON RIBA HCV 3.0 SIA). These tests included recombinants antigens (c33C e NS5) and synthetic peptide (5-1-1, c100, c22). Samples with a negative PCR result and reactive in the ELISA were also submitted to IB testing.

PCR technique - HCV-RNA detection by PCR was carried out on all serum samples. A reverse transcriptase-nested PCR was used to amplify a fragment of the 5' untranslated region (5'UTR) of the HCV genome. Serum RNA was extracted using Trizol® (Life Technologies) with primers PTC1 (5'CGTTAGTATGAGTGTCGTGC3') and NCR2 (5'ATACTCGAGGTGCACGGTCTACGAGACCT3') in the first amplification round. For the second amplification round, primers PTC3 (5'AGTGTCGTGCA GCCTCCAGG3') and NCR4 (5'CACTCTCGAGCACCC TATCAGGCAGT3') were used (Garson et al. 1990a,b). In each reaction set, water samples were used as negative controls to exclude possible cross contaminations due to amplicon carry-over.

HCV genotyping - PCR products were submitted to cycle sequencing reactions, using the second round primers described above and the ABI prism® BigDye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Applied Biosystems, Foster City, CA, US). Nucleotide sequences from both strands were determined using an automated DNA sequencer model ABI 377 (Applied Biosystems). For genotyping, sequences were compared with those previously published using the Lasergene package (DNAstar, Madison, WI, US).

Prevalence - The following criterion was used to consider a patient as HCV infected: any sample with a positive result in at least two of three tests (PCR, ELISA, IB). Prevalence was determined by the serological result obtained.

Statistical analysis - The degree of agreement between the techniques employed was measured by Kappa statistics (Gordis 1996), which takes into account the agreement that might be expected to occur by chance. Prevalence and 95% confidence intervals (95% CI) were calculated. Chi-square tests were performed to evaluate risk factors associated with HCV infection. Statistical significance was assessed at the 0.05 probability level in all analysis. Risk factors detected by univariate analysis were analyzed by multiple logistic regression. Statistical evaluations were performed using the Epi-Info 6.0 program and SPSS PC program version 8.0.

RESULTS

Hundred forty seven male and 103 female patients aged between 17-92 years were enrolled on the study. Of the 250 patients only one patient revealed an indeterminate result by the ELISA and IB and negative result by the RT-PCR in two samples collected during the following seven month period. The result of the 250 patients tested for HCV antibodies by third generation ELISA and for HCV-RNA by RT/PCR with a detection limit of 100-genomes/ml are shown in Table I. The seropositivity of the two patients only anti-HCV-positive by ELISA was confirmed by the IB.

Of the 21 HCV positive patients, 14 of whom were men (9.6%) and 14 had 40 years old and more (8.3%) $p > 0.05$. The positivity rates did not differ between patients who received blood transfusions (64.6%) and patients who did not receive blood transfusions (35.3%). However, univariate analysis showned that the length of time spent on hemodialysis, the number of blood transfusions, and blood transfusion before November 1993 were associated with HCV positivity (Table II). Multivariate analysis revealed that only blood transfusions before screening for anti-HCV were significantly associated with HCV infection in this population (Table III).

HCV genotypes were identified in 19 HCV RNA positive samples. These results are shown in Table IV.

TABLE I

Comparison between ELISA and polymerase chain reaction (PCR) results

	PCR			
ELISA	Positive	Negative	Total	%
Positive	19	2	21	8.4
Negative	-	228	228	91.6
Total	19	230	249	100

ELISA: enzyme immunoassay; Kappa = 0,946 (0,908; 0,984); -: not detected

DISCUSSION

This is the first study to report on the prevalence and genotyping of HCV in hemodialysis patients in the state of Pernambuco, Brazil. The prevalence of 8.4% found in the present study can be considered low when compared with the prevalence of São Paulo, 14.6% (Moreira et al. 2003), of Curitiba, PR, 39.2% (Carvalho et al. 1999), of Goiânia, GO, 46.7% (Carneiro et al. 2001), and of Belo Horizonte, MG, 20% (Busek et al. 2002). However, in comparison with the prevalence index of hepatitis C throughout the general population, the index observed in this study was seven times higher (Ministério da Saúde 2003).

In the present study, PCR was used to screen for the presence of HCV RNA in all 250 serum samples. The IB test was used to confirm the presence of antibodies anti-HCV in two patients with an absence of viraemia. More recently, a transcription-mediated amplification (TMA) assay has been developed with a lower limit of detection on the order of 5 to 10 IU/ml. A follow-up qualitative HCV RNA should be performed in the sample studied to confirm the absence of active HCV replication. The HCV RNA was not detected in seronegative samples. The presence of HCV viraemia in anti-HCV-negative hemodialysis patients has been frequently reported by other authors (Cardoso et al. 1994, Fernández et al. 1996, Salama et al. 2000, Carneiro et al. 2001, Hinrichsen et al. 2002, Busek et al. 2002, Moreira et al. 2003). However, some researchers

TABLE II

Distribution of hemodialysis patients at a center in Recife, Brazil, during March to July 2002, according to the number of blood transfusions, blood transfusions before 1993, time spent on hemodialysis, and hepatitis C virus (HCV) positivity

	HCV[a/b]				RR (CI)	χ^2	P value
	Positive		Negative				
	n	%	N	%			
Transfusion time					2.73 (1.12; 6.64)	3.98	0.026
Before 1993	9	19.1	38	80.9			
After 1993	8	7	106	93			
Total	17	8.4	144	91.6			
Number of lood transfusions					12.88	0.004	
None	4	4.5	84	95.5	1.0		
1 - 5	11	8.5	118	91.5	1.88 (0.62; 5.7)		
6 - 15	3	12	22	88	2.64 (0.63; 11.03)		
> 15	3	42.9	4	67.1	9.43 (2.61; 34.04)		
Total	17	63.4	144	246.6			
Time on hemodialysis					2.73 (1.17; 6.33)	4.28	0.028
5 and more	13	14.0	80	86			
< 5	8	5.1	148	94.9			
Total	21	8.4	228	91.6			

a: HCV = anti-HCV; *b*: HCV = RNA/HCV; RR: relative risk (Confidence Interval); χ^2: Chi-square test

TABLE III

Model finished of the multiple logistic regression between the time on hemodialysis, number of blood transfusions, transfusion period, and the hepatitis C virus (HCV) positivity in the hemodialysis patients at a clinic in Recife, during the period of March to July 2002

Variable	Odds ratio	Confidence interval	P-value
Transfusion period			
After 1993	3.13	(1.12;8.71)	0.0295
Before 1993	1		

TABLE IV

Genotypes identified in the hemodialysis population studied

Genotype	N (%)
1a	8 (42)
1b	4 (21)
3a	7 (37)
Total	19

have not observed anti-HCV-negative hemodialysis patients with HCV RNA in the serum (Dalekos et al. 1998).

Multivariate analysis revealed that blood transfusions before screening for anti-HCV, were significantly associated to HCV infection in this population. In addition, revealed that patients who did receive blood transfusion before November 1993 had a 2,73 fold (95% CI: 1,17-6,33) greater risk of HCV positivity compared to subjects who had received blood transfusion after November 1993.

The extensive use of recombinant erythropoietin to correct renal anaemia in hemodialysis patients resulted in a significant reduction in blood transfusions. The prevalence in the studied center was low, although a prospective study is necessary to confirm these findings.

A very important role must be attributed to the scrupulous and strict application of universal precautions (Gilli et al. 1995) to reduce of environmental diffusions of C hepatitis in dialysis units. A genuine transmission could be caused by the leakage of blood or blood components through the dialysis filter.

Alterations of pore size and micro-fractures of the membrane are possible events in the course of manufacturing, during the dialysis session or with dialyzer re-use. A second type of contamination is caused by occasional or systematic contaminations of machine and instruments (Sampietro et al. 1996).

In this study, an excellent use of universal precautions was observed as well as the fact that reprocessed dialyzers for anti-HCV positive and anti-HCV negative patients were placed in separate rooms. Dialysis units that placed reprocessed dialyzers in separate rooms had the lowest incidence of C hepatitis (Pereira et al. 1999). The anti-HCV positive hemodialysis patients of the studied center received the treatment on reserved machines and the staff members of this study paid careful attention to hygiene and the strict sterilization of dialysis machines.

Molecular studies are useful to detect nosocomial transmission of viral infections. The HCV strains were characterized by genotyping the samples with positive PCR by sequencing the 5'UTR. This is not the best region to be sequenced since it represents the most conserved region among HCV genotypes, but by choosing primers from this region, it is possible to obtain a high sensitivity test in a population in which multiple genotypes are represented (Zein et al. 2000).

In this study, genotypes 1a and 3a were detected in the majority of samples. Genotype 1 is the most prevalent genotype in the general population in Brazil. Differences between viral genotypes may have major implications for the current use of HCV diagnostic tests, especially in geographic areas with a high prevalence of HCV genotypes that are phylogenetically distant from genotype 1a (Zein et al. 2000).

This study does not demonstrate the occurrence of false serological results (negative and positive), however the effectiveness of PCR for early recognition of HCV infection in patients undergoing hemodialysis treatment is very important, and the spread of viral strains with the same genotypes, could confirm eventually the possibility of the HCV dissemination in the hemodialysis setting. The homogeneity of the genotypes reflects a transmission nosocomial. However, it is necessary to carry out retrospective and prospective studies at this center in order to evaluate the time during which the patients acquire this infection.

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REFERENCES

Busek SU, Baba EH, Tavares Filho HA, Pimenta L, Salomão A, Correa-Oliveira R, Oliveira GC 2002. Hepatitis C and hepatitis B virus infection in different hemodialysis units in Belo Horizonte, Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz 97*: 775-778.

Cardoso MS, Koerner M, Epple S, Krämer R, Bundschu D, Kubanek B 1994. Prevalence of HCV-RNA-positive patients in a dialysis unit in Germany. *Nephron 68*: 517-518.

Carneiro MAS, Martins RMB, Teles SA, Silva SA, Lopes CL, Cardoso DDP, Vanderborght BOM, Yoshida CFT 2001. Hepatitis C prevalence and factors in hemodialysis patients in Central Brazil: a survey by polymerase chain reaction and serological methods. *Mem Inst Oswaldo Cruz 96*: 765-769.

Carvalho M, Branco PB, Luvizotto ML, Valderrama DE, Rabone S, Doi E, Ivantes C, Muzzilo DA 1999. High prevalence of hepatitis C virus infection in chronic hemodialysis patients. *Braz J Infect Dis 3*: 144-148.

Dalekos GN, Boumba DS, Katopodis K, Zervou E, Sferopoulos G, Elisaf M, Tsianos EV, Siamopoulos KC 1998. Absence of HCV viraemia in anti-HCV-negative hemodialysis patients. *Nephrol Dial Transplant 13*: 1804-1806.

Fernández JL, Del Pino N, Lef L, Valtuille R, Berridi J, Rendo P, Viola L 1996. Serum hepatitis C virus RNA in anti-HCV negative hemodialysis patients. *Dialysis & Transplantation 25*: 14-18.

Garson JA, Tedder RS, Briggs M, Tuke P, Glazebrook JA, Trute A, Parker D, Barbara JAJ, Contreras M, Aloysius S 1990a. Detection of hepatitis C viral sequences in blood donation by "nested" polymerase chain reaction and prediction of infectivity. *Lancet 335*: 1419-1422.

Garson JA, Tuke PW, Makris M, Briggs M, Machin SJ, Preston FE, Tedder RS 1990b. Demonstration of viraemia patterns in hemophiliacs treated with hepatitis-C-virus-contaminated factor VIII concentrates. *Lancet 336*: 1002-1025.

Gilli P, Soffritti S, Vitali De P, Bedani PL 1995. Prevention of hepatitis C virus in dialysis units. *Nephron 70*: 301-306.

Gordis L 1996. Assessing the validity and reliability of diagnostic and screening tests. In *Epidemiology*, WB Saunders, Philadelphia, p. 58-76.

Hinrichsen H, Leimenstoll G, Stegen G, Schrader H, Fölsch UR, Schmidt WE 2002. Prevalence and risk factors of hepatitis C virus infection in hemodialysis patients: a multicentre study in 2796 patients. *Liver Disease 51*: 429-433.

Ministério da Saúde 2004. Resolução – RDC nº 154, de 15 de junho de 2004, Diário Oficial da União 110 – E, Seção I.

Ministério da Saúde. 2003. Fundação Nacional de Saúde. Hepatites. Disponível em:<http://www.funasa.gov.br/guia_epi/htm/doenças/hepatite/situação_doença.htm> acess 13 Mar 2003.

Moreira R, Pinho JRR, Fares J, Oba IT, Cardoso MR, Saraceni CP, Granato C 2003. Prospective study of hepatitis C virus infection in hemodialysis patients by monthly analysis of HCV RNA and antibodies. *Can J Microbiol 49*: 503-507.

Pereira BJG 1999. Hepatitis C virus infection in dialysis: a continuing problem. *Artif Organs 23*: 51-60.

Salama G, Rostaing I, Sandres K, Izopet J 2000. Hepatitis C virus infection in French hemodialysis units: a multicenter study. *J Med Virol 61*: 44-51.

Sampietro M, Badalamenti S, Graziani G 1996. Nosocomial hepatitis C in dialysis units. *Nephron 74*: 251-260.

Santana GO, Cotrim HP, Mota E, Paraná NP, Lyra l 2001. Antibodies to hepatitis C virus in patients undergoing hemodialysis in Salvador, BA, Brazil. *Arq Gastroenterol 38*: 24-31.

Zein NN 2000. Clinical significance of hepatitis C virus genotypes. *Clin Microbiol Rev 13*: 223-235.