Hepatitis C Prevalence and Risk Factors in Hemodialysis Patients in Central Brazil: a Survey by Polymerase Chain Reaction and Serological Methods

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An hemodialysis population in Central Brazil was screened by polymerase chain reaction (PCR) and serological methods to assess the prevalence of hepatitis C virus (HCV) infection and to investigate associated risk factors. All hemodialysis patients (n=428) were interviewed in eight dialysis units in Goiânia city. Blood samples were collected and serum samples screened for anti-HCV antibodies by an enzyme-linked immunosorbent assay (ELISA). Positive samples were retested for confirmation with a line immunoassay (LIA). All samples were also tested for HCV RNA by the PCR. An overall prevalence of 46.7% (CI 95%: 42-51.5) was found, ranging from 20.7% (CI 95%: 8.8-38.1) to 90.4% (CI 95%: 79.9-96.4) depending on the dialysis unit. Of the 428 patients, 185 were found to be seropositive by ELISA, and 167 were confirmed positive by LIA, resulting in an anti-HCV prevalence of 39%. A total of 131 patients were HCV RNA-positive. HCV viremia was present in 63.5% of the anti-HCV-positive patients and in 10.3% of the anti-HCV-negative patients. Univariate analysis of risk factors showed that the number of previous blood transfusions, transfusion of blood before mandatory screening for anti-HCV, length of time on hemodialysis, and treatment in multiple units were associated with HCV positivity. However, multivariate analysis revealed that blood transfusion before screening for anti-HCV and length of time on hemodialysis were significantly associated with HCV infection in this population. These data suggest that nosocomial transmission may play a role in the spread of HCV in the dialysis units studied. In addition to anti-HCV screening, HCV RNA detection is necessary for the diagnosis of HCV infection in hemodialysis patients.

Key words: hepatitis C - hemodialysis - prevalence - risk factors - Central Brazil

Hemodialysis patients are at high risk of infection by hepatitis C virus (HCV). Such factors as blood transfusion, partial immunosuppression, and frequent parenteral interventions have been associated with an increased risk for infection (Olmer et al. 1996). The duration of hemodialysis treatment, and the possibility of nosocomial HCV transmission have also been suggested as additional contributing elements (Masuko et al. 1994, Sampietro et al. 1995, Lamballerie et al. 1996, Sandhu et al. 1999, Scotto et al. 1999, Grethe et al. 2000).

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The prevalence of HCV antibodies (anti-HCV) has been reported to range from 2% in northwestern Europe to 76.3% in Indonesia (Soetjipto et al. 1996, Schneeberger et al. 1998). In Brazil, surveys have shown that hemodialysis patients have high anti-HCV prevalence rates (29.4%-65%) (Karohl et al. 1995, Vanderborght et al. 1995, Naghettini et al. 1997, Góngora 1998). However, there are strong indications that studies of hemodialysis patients which rely solely on serological screening could underestimate the prevalence of HCV infection considerably (Bukh et al. 1993, Caramelo et al. 1996). Partial immunosuppression in these patients, resulting in a poor antibody response, may be a contributing factor (Goldblum & Reed 1980). Such shortcomings could overcome by determining HCV RNA, which may be required to identify all infected patients (Seelig et al. 1994, Pujol et al. 1996, Schroter et al. 1997, Fabrizi et al. 1999).

The present study describes a survey among hemodialysis patients in Central Brazil, by means of both serological (anti-HCV) and molecular (HCV RNA) methods to screen for HCV infection. We sought to: (i) assess the prevalence of HCV infection in a hemodialysis population of Central Brazil, (ii) compare serological (ELISA and LIA) and molecular (PCR) methods for detection of HCV infection, and (iii) study possible risk factors for HCV infection in this population.

MATERIALS AND METHODS

Subjects - Our study was carried out at all dialysis units (n=8) in Goiânia city, Central Brazil (1,000,000 inhabitants). Between January and March 1998, all chronic hemodialysis patients (n=428) were interviewed for risk factors to HCV infection. The studied population ranged in age from 9 to 70 years (average 46.1 years). One hundred sixty-five were males (38.6%) and 263 were females (61.4%).

A standardized form was used to collect data on age, sex, length of time on hemodialysis, treatment or not in multiple dialysis units, number of previous blood transfusions, transfusion before November 1993 (blood not screened for anti-HCV), acupuncture, tattooing, intravenous drug use, multiple sex partners, sexually transmitted diseases, and possible household contact with hepatitis. Permission for carrying out the study was granted by the institutions involved and informed consent was obtained from all participants.

Serological tests - Blood samples were collected from all patients and sera were stored at -20ºC. The samples were screened by ELISA for the presence of anti-HCV antibodies (INNOTEST HCV Ab III, Innogenetics NV, Belgium). Positive samples were retested for confirmation using a line immunoassay (INNO-LIA HCV Ab III, Innogenetics).

Detection of RNA HCV - All samples were submitted to RNA extraction, reverse transcription, and a nested PCR with primers complementary to the conserved area of the 5' non-coding region of HCV, essentially as described by Ginabreda et al. (1997).

Statistical analysis - Prevalence and 95% confidence intervals (95% CI) were calculated. Chi-square test or Chi-square for trend test were performed to evaluate risk factors associated with HCV infection. Statistical significance was assessed at the 0.05 probability level in all analyses. Risk factors detected by univariate analysis were analyzed by multiple logistic regression. Statistical evaluations were performed using the Epiinfo 6.0 program developed by the Centers for Disease Control and Prevention (Atlanta, GA) and "EGRET" ("Epidemiological, Graphics, Estimation and Testing Package", 1991).

RESULTS

As shown in Table I, an overall prevalence of 46.7% (CI 95%: 42-51.5) was found, ranging from 20.7% (CI 95%: 8.8-38.1) to 90.4% (CI 95%: 79.9-96.4) depending on the dialysis unit.

Of the 428 hemodialysis patients, 185 were found to be seropositive by ELISA, and 167 (90.3%) were subsequently confirmed as being positive by LIA, resulting in an anti-HCV prevalence of 39%. In 18 patients, ELISA was positive but LIA was either negative (4/18) or indeterminate (14/18). A total of 131 patients were HCV RNA positive. HCV viremia was present in 63.5% (106/167) of the anti-HCV-positive patients and in 10.3% (25/243) of the anti-HCV-negative patients (Table II).

Analysis of all risk factors studied showed that number of previous blood transfusions, transfusion of blood before mandatory screening for anti-HCV, length of time on hemodialysis, and treatment in multiple units were significantly associated with HCV positivity by univariate analysis. However, multivariate analysis revealed that only blood transfusion before screening for anti-HCV and length of time on hemodialysis were significantly associated with HCV infection in this population (Table III). In addition, patients who received blood transfusion before screening for anti-HCV had a 6.5-fold (95% CI: 3.4-12.6) greater risk of HCV positivity compared to those that were transfused with blood which had been screened for anti-HCV. Hemodialysis patients under treatment for more than three years had a 13.6-fold (95% CI: 5.8-32.3) greater risk of acquiring HCV positivity compared to subjects who had undergone less than one year of treatment.

TABLE I

Prevalence of hepatitis C virus (HCV) infection in eight hemodialysis units in Central Brazil

Units	No. patients	HCV Pos.	(%)	CI 95%
A	52	47	(90.4)	79.9-96.4
B	19	6	(31.6)	13.9-54.5
C	32	14	(43.8)	27.5-61.1
D	29	6	(20.7)	8.8-38.1
E	41	17	(41.5)	27.2-56.9
F	48	19	(39.6)	26.5-53.8
G	70	16	(22.9)	14.2-35.2
H	137	75	(54.7)	46.3-62.9
Total	428	200	(46.7)	42.0-51.5

CI: confidence interval; Pos.: positives

TABLE II

Comparison between serological (ELISA/INNO-LIA) and molecular (PCR) tests in detecting hepatitis C virus infection among 428 hemodialysis patients in Central Brazil

ELISA		LIA						PCR			
		Positive		Negative		Indeterminate		Positive		Negative	
	n	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Pos.	185	167	(90.3)	4	(2.1)	14	(7.6)	106	(57.3)	79	(42.7)
Neg.	243	_	_	_	_	_	_	25	(10.3)	218	(89.7)

n: no. patients

TABLE III

Risk factors associated with hepatitis C virus (HCV) infection in an hemodialysis population of Central Brazil

Risk factor	Crude OR (CI 95%)	Adjusted OR (CI 95%)
Number of previous transfusions		
0	1.0	1.0
< 5	1.5 (0.8-3.0)	1.3 (0.7-2.6) [a]
≥ 5	2.8 (1.3-5.8)	1.5 (0.7-3.2)
Screening of blood for anti-HCV		
After	1.0	1.0
Before	9.5 (5.2-17.6)	6.5 (3.4-12.6) [a]
Length of time on hemodialysis		
< 1 year	1.0	1.0
1 a 3 years	2.3 (1.3-4.0)	2.2 (1.3-3.7) [b]
> 3 years	16.5 (6.8-40.8)	13.6 (5.8-32.3)
Treatment in multiple dialysis units		
No	1.0	1.0
Yes	1.8 (1.2-2.8)	1.2 (0.8-1.9) [c]

OR: odds rate; CI: confidence interval; *a*: adjusted for gender, age and length of time on hemodialysis; *b*: adjusted for gender, age, previous transfusion and treatment in multiple dialysis units; *c:* adjusted for gender, age, previous transfusion and length of time on hemodialysis

DISCUSSION

The present investigation showed high prevalence rates of HCV infection in hemodialysis patients from Goiânia city, when compared to rates found in blood donors (1.4%) and the female population (0.9%) from the same region (Martins et al. 1994, 1995). Nevertheless, with reference to other Brazilian hemodialysis populations, anti-HCV prevalence (39%) was higher than those observed previously in Goiânia and Porto Alegre (Karohl et al. 1995, Naghettini et al. 1997), but similar to that obtained in São Paulo (Góngora 1998), and lower than the prevalence found in Rio de Janeiro (Vanderborght et al. 1995). This demonstrates that HCV infection is a significant problem in Brazilian dialysis units.

In the present study, PCR was used to screen for the presence of HCV RNA in all 428 serum samples. HCV RNA was detected in 10.3% of the seronegative samples. Retesting confirmed this result. The presence of HCV viremia in anti-HCV-negative hemodialysis patients has been frequently reported by others researchers (Bukh et al. 1993, Masuko et al. 1994, Seelig et al. 1994, Pujol et al. 1996, Schneeberger et al. 1998). Just as in others studies (Caramelo et al. 1996, Schroter et al. 1997, Fabrizi et al. 1999, Grethe et al. 2000), we can not exclude recent infections, where the sample was collected during the seronegative window phase, or the existence of impaired immune responses in some of these patients. In addition, 8 of the unconfirmed serological results (1 LIA-negative and 7 LIA-indeterminates) were positive for HCV RNA. All these LIA indeterminate/PCR- positive samples reacted weakly (reactivity rating of 1+ or +/-) with NS3 antigen line on LIA test strip (data not shown). These results point to the additional advantages of molecular diagnostic methods, especially in pa-

tients who have inadequate antibody responses to HCV antigens.

On the other hand, RNA HCV was detected in 63.5% of the seropositive samples. This frequency is low when compared to those observed in other hemodialysis populations (Bukh et al. 1993, Masuko et al. 1994, Schroter et al. 1997, Schneeberger et al. 1998), but generally higher than the frequencies reported elsewhere (Lamballerie et al. 1996, Olmer et al. 1996). Some of these cases may be considered either as patients with past infections or intermittent viremia status (Seelig et al. 1994, Umlauft et al. 1997).

Previous studies have also indicated that the duration of dialysis treatment is clearly correlated with HCV seropositivity (Olmer et al. 1996, Lamballerie et al. 1996, Naghettini et al. 1997, Sandhu et al. 1999, Scotto et al. 1999). In the present study, this association was also confirmed. Multivariate analysis revealed that patients under treatment for more than three years had a 13.6-fold (95% CI: 5.8-32.3) greater risk of HCV positivity compared to subjects who had undergone less than one year of treatment.

Regarding previous blood transfusion, our results revealed that hemodialysis patients who received blood that had not been screened for anti-HCV had a 6.5-fold (95% CI: 3.4-12.6) greater risk for HCV positivity compared to patients that were transfused with blood screened for anti-HCV. The screening of blood units for anti-HCV became mandatory in 1993 in Brazilian blood banks, a requirement which has contributed substantially to lower HCV infection prevalence in these transfused patients. Also, transfusion should be restricted to the minimum by the administration of recombinant human erythropoietin (rHuEPO) in hemodialysis patients. However, a high HCV infection prevalence (34.7%) was observed in non-transfused patients (data not shown). These findings suggest a substantial risk in hemodialysis procedures. Moreover, nosocomial transmission among hemodialysis patients has been reently documented by molecular analysis (Sampietro et al. 1995, Lamballerie et al. 1996, Grethe et al. 2000).

In conclusion, along with the high endemicity of HCV in patients at hemodialysis units in Goiânia, this study confirmed the expected decline in transfusion-acquired HCV infection in this population and showed that the length of time on hemodialysis treatment seems to be the main risk factor, suggesting the nosocomial transmission of HCV. These data emphasize the need for stricter adherence to infection control measures in dialysis units and reinforce the importance of screening by both PCR and serological methods at regular intervals to identify all HCV-infected patients.

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