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High Incidence of Hepatitis C Virus Infection in Hemodialysis Patients in Units with High Prevalence

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The prevalence of hepatitis C virus (HCV) infection was evaluated in 227 hemodialysis patients from four units in Caracas, Venezuela, by using different second- and third-generation enzyme immunoassays (EIAs) and immunoblot assays. HCV antibodies were detected in 162 patients (71%) by the recombinant-based second-generation assays (Abbott and Ortho) and in 161 patients by the synthetic peptide-based EIA (UBI). Of the 162 HCV antibody-positive serum samples, 161 were confirmed to be positive by RIBA 3. HCV RNA was detected in 49 of 68 (72%) of the seropositive patients and in 5 of 21 (24%) of the seronegative ones. HCV RNA was not always correlated with an increase in alanine aminotransferase (ALT) levels. Among 20 patients positive for HCV RNA and for HCV antibodies (without any hepatitis B virus [HBV] marker), only 10 had elevated ALT levels. The possible interference of HBV for HCV replication was evaluated. No significant difference was found between the presence of HCV RNA and the presence of any HBV serological markers. The possible routes of transmission of HCV in hemodialysis patients are multiple, and some of them are still controversial. Of the HCV-positive patients, 30% received a blood transfusion, significantly more than the 15% found for the HCV-negative group. However, blood transfusions alone could not account for the high incidence observed in this group of patients (38% from 1994 to 1995). In conclusion, about one-quarter of the apparently non-HCV-infected patients were probably seroconverting, ALT may not be a useful indicator of HCV infection in hemodialysis patients, and nosocomial transmission of HCV may play a role in the spread of HCV in this group.

Dialysis patients have an increased risk of exposure to parenterally transmitted hepatitis viruses (7, 8, 14). The development of control measures, especially vaccination, has significantly reduced the spread of hepatitis B virus (HBV) among this population. The risk of hepatitis C virus (HCV) infection has now been assessed with the use of screening assays for the detection of antibodies against this new member of the family *Flaviviridae.*

The prevalence of HCV among hemodialysis patients is highly variable between different countries and between different centers in the same locality (8); in Venezuela, a 39% prevalence of HCV infection was found in 1990 in eight hemodialysis units at different hospitals in Caracas by using first-generation tests (17). More recently, a lower prevalence of HCV infection (29%) has been reported in a unit from a private clinic that uses highly stringent precautions for preventing hepatitis virus transmission (22).

The aim of the study described here was to evaluate the prevalence of viral hepatitis markers in some hemodialysis units in Caracas where a high incidence of HCV infection appeared to occur. The objectives of the present work were to evaluate the proficiencies of different tests or serological markers for the detection of HCV infection in hemodialysis patients and to assess some possible routes of transmission of HCV in these patients in a developing country.

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MATERIALS AND METHODS

Sera were collected in April 1994 from patients undergoing hemodialysis at four different units in Caracas, Venezuela. Serum samples were obtained from a total of 227 patients (129 men and 98 women), which represented 89% of the total population attending these units at the time. Sera from some of the anti-HCV-negative patients (40 of 65) were obtained 1 year later. Alanine aminotransferase (ALT) levels were determined in a period of less than 3 months from the time of collection of the sera. ALT values were considered elevated when they were found to be over the normal upper limit (40 IU/liter). HBV surface antigen (HBsAg) was detected by HBsAg Uni-Form II (Organon Teknika, Beerse, Belgium) and AUSZYME (Abbott Diagnostics, North Chicago, Ill.). A sample was considered HBsAg positive when it was reactive by both immunoassays. Antibody to the HBV core antigen (anti-HBc) and HBV e antigen (HBeAg) were detected with the Corzyme (Abbott) and Hepanostika HBeAg/anti-HBe (Organon Teknika) systems, respectively. Antibodies to delta hepatitis virus (HDV) in HBV-positive patients were tested with the anti-HDV EIA (Abbott) and Hepanostika HDV (Organon) systems.

HCV antibodies were tested with the Abbott HCV EIA 2 (Abbott) and the Ortho EIA 2.0 (Ortho Diagnostic Systems, Raritan, N.J.) systems, both of which are based on the use of recombinant proteins, and by the UBI HCV EIA (Organon), which is based on synthetic peptides. Confirmatory assays for anti-HCV were performed with HCV-positive and -negative specimens by RIBA 3 (Ortho). The performance of these tests in terms of sensitivity was found to be equal in a previous study with blood donor sera (23). A specimen was considered seropositive for HCV if it was reactive by all enzyme immunoassays (EIAs) or was confirmed to be positive by RIBA 3. A subset of sera from patients distributed almost equally between the four hemodialysis units and chosen consecutively from the patient lists was evaluated for the presence of HCV RNA by reverse transcription and single-tube nested PCR with primers in the 5′ noncoding region (23). To avoid false-positive results, each test was run in duplicate and two negative controls were added in each step (RNA extraction, cDNA synthesis, and PCR).

Human immunodeficiency virus (HIV) antibodies were detected by HIV-1/HIV-2 recombinant EIA 3 (Abbott). Human T lymphotropic virus type 1 (HTLV-1) antibodies were detected with the Vironostika HTLV-I Microelisa system (Organon) and by an HTLV-1 EIA (Abbott); reactive samples were

TABLE 1. Viral hepatitis serological markers among hemodialyzed patients in Caracas

Unit	No. (%) of patients					No. of CMV (IgM)-positive patients/total no. tested (%)
	Total	HBsAg	Anti-HBc	Anti-HCV	Anti-HDV	
A	54	7 (13)	20 (37)	40 (74)	0	3/25 (12)
B	51	1 (2)	16 (31)	35 (69)	0	4/24 (17)
C	58	0 (0)	22 (38)	42 (72)	0	5/24 (21)
D	64	49 (74)	57 (89)	45 (70)	1 (2)	15/26 (58)
Total	227	57 (25)	115 (51)	162 (71)	1 (0.4)	27/99 (27)

tested by confirmatory assays (Organon). Immunoglobulin M antibodies to cytomegalovirus (CMV) were tested by the Abbott CMV-M EIA.

Statistical differences were evaluated by the chi-square test with Yates' correction, according to a computerized Epi Info program, version 5.01b (Centers for Disease Control and Prevention, Atlanta, Ga., and by the Student *t* test.

RESULTS

A total of 227 patients from four different hemodialysis units in Caracas were analyzed for viral hepatitis serologic markers. A high prevalence of HCV infection was observed among the patients in the four units (162 seropositive patients [71%]), while a high rate of HBV infection was restricted to only one unit, where one patient positive for HDV antibodies was also found (Table 1). No patient was found to be positive for HTLV-1 or HIV antibodies. A high prevalence of immunoglobulin M antibodies to CMV was found in the four units, with a significantly higher prevalence in unit D (Table 1).

A good correlation was found between the three second-generation tests used to detect HCV antibodies. Seropositivity in all 162 seropositive patients was detected by all of the recombinant-based immunoassays and in 161 patients by the synthetic peptide-based assay. The serum specimen that was not recognized by the latter test was reactive by RIBA 3, with an evident reaction with the NS3 antigen and a moderate one with the core antigen. Of these 162 serum specimens, 161 were confirmed to be positive by RIBA 3, while the remaining serum specimen gave an indeterminate pattern.

HCV RNA was detected in 49 of 68 (72%) of the seropositive samples and in 5 of 21 (24%) of the seronegative ones (Table 2). Interestingly, the ALT levels in the sera of these seronegative patients were all normal (data not shown). Likewise, the presence of HCV RNA was not always correlated with an increase in ALT levels. Among 20 patients positive for HCV RNA and HCV antibodies (without any HBV marker), only 10 presented with elevated ALT levels (data not shown), suggesting that ALT levels may not be a useful indicator of HCV infection in these patients. On the other hand, ALT levels were similar in anti-HCV-positive patients and in HBsAg-positive patients, while no significant increase in ALT levels was observed in patients coinfected with HCV and HBV (data not shown). The average ALT level among anti-HCV-positive patients was higher than that among the HBV- and HCV-negative ones. However, only 29 of 162 anti-HCV-positive patients presented with ALT levels more than twofold over the upper limit of normal and 6 of 162 presented with ALT levels more than fivefold over the upper limit of normal. Moreover, 19 of these 29 patients presented with at least one HBV marker.

The possible interference of HBV on HCV replication was evaluated. HCV RNA was more frequently found in the HCV-positive, HBV-negative patients (Table 2) than in patients with any HBV marker, although this difference was not significant ($P = 0.1$). Among the HBsAg-positive patients (Table 1), 53 were tested for HBeAg. This replication marker was found in similar proportions of anti-HCV-positive (22 of 41) and anti-HCV-negative (6 of 12) patients. Preliminary results suggest that HBV DNA was also present among HCV-positive and HCV-negative patients (data not shown).

Of the HCV-seropositive patients, 30% received blood transfusions (23% were polytransfused), significantly more than the 15% of the transfused and 10% of the polytransfused patients found in the HCV-seronegative group (Table 3); however, 70% of the HCV-positive patients did not receive a transfusion. The average time on hemodialysis for the HCV-positive patients was also significantly longer than that for the HCV-negative ones (Table 3). A total of 40 initially HCV-negative patients were retested 1 year later; 15 of 40 were found to be positive for HCV antibodies, implying a high incidence of infection (38%).

DISCUSSION

Viral hepatitis serologic markers were evaluated in the sera of patients from different hemodialysis units in Caracas. A high prevalence of HCV was observed among the four units, while a high rate of HBsAg prevalence was restricted to only one unit, probably because of HBV infection prior to admission to this unit. Because of vaccination against HBV and the segregation of infected patients in one unit, the spread of HBV seems to be controlled in these hemodialysis units.

A good correlation was found between the three second-generation tests used to detect HCV antibodies. Interestingly, all but one specimen could be confirmed to be positive by RIBA 3, which seems to indicate that some unreliability observed previously with RIBA 2 in hemodialysis patients (3, 18, 26) has been corrected with RIBA 3. However, approximately 24% of the seronegative patients were already infected with

TABLE 2. Presence of HCV RNA and correlation with other serologic markers

Category and no. of patients	Presence of marker			No. (%) of patients HCV RNA positive
	Anti-HBc	HBsAg	HBeAg	
HCV EIA positive				
23	−	−	ND[a]	20 (87)
18	+	−	ND	12 (67)
15	+[b]	+	−	10 (67)
12	+	+	+	7 (58)
Total (68)				49 (72)
HCV EIA negative, 21	NS[c]	NS	NS	5 (24)

[a] ND, not determined.
[b] Some HBsAg-positive specimens were anti-HBcAg negative.
[c] NS, not shown.

TABLE 3. Transfusion and HCV infection

HCV serology (total no. of patients)	No. (%) of infected patients with the indicated no. of transfusions[a]			Time on hemodialysis (mo.)
	<5	>5	Total	
Positive (162)	12 (7)	37 (23)	49 (30)	48
Negative (62)	3 (5)	6 (10)	9 (15)	19
Significance[b]	>0.05	<0.05	<0.05	<0.001

[a] Transfused patients were divided into two categories, depending on whether they received fewer than five transfusions or more than five transfusions in their lifetimes; the total is the sum of the two categories.

[b] Statistical differences were evaluated by the Student *t* test.

HCV, as indicated by the presence of RNA in their sera. Because of the increased risk of HCV acquisition, the presence of seroconverting specimens is frequently found in groups of patients undergoing hemodialysis. The immunosuppression found in these patients (9) may also account for a delayed or disturbed anti-HCV response (3, 11). Seronegative and probably seroconverting patients have already been described among this group of patients; Bukh et al. (3) found HCV RNA in 5% of anti-HCV-negative patients, and Huang et al. (12) found HCV RNA in 4% of anti-HCV-negative patients among groups of individuals with anti-HCV prevalences of 8 and 59%, respectively. In our study, HCV RNA was found in the seronegative patients at a frequency higher than that described previously (12), even among groups with a high prevalence of HCV seropositivity. In fact, a high incidence of infection was found in our group of patients, which is compatible with the high frequency of seronegative or seroconverting specimens (Table 2). Of the seropositive samples tested by PCR, only 72% were found to be positive for HCV RNA. This frequency is low if compared with the frequency among hemodialysis patients obtained by others (3), although it is similar to the one obtained among Venezuelan anti-HCV-positive blood donors (23) and is in agreement with the chronicity rate reported for HCV infection. On the other hand, we cannot exclude an intermittent viremic status for some of these patients.

The present study also corroborates previous ones that show that the ALT level is not a reliable marker of HCV infection in hemodialysis patients (10, 21, 24, 26). Only half of the HCV RNA-positive, HBV-negative patients had elevated ALT levels. Likewise, none of the HCV RNA-positive, HCV antibody-negative patients had hypertransaminemia. Thus, even in seroconverting patients, the ALT level does not seem to be a good indicator of HCV infection. It has been reported that ALT levels correlate better with HBV replication than with HCV viremia (19, 21). As stated above, elevations in ALT levels in the sera of these patients are usually not high, probably because of the intrinsic immunosuppression found in these patients (11, 26). It has been proposed that the isolation of patients with elevated transaminase levels could be beneficial for preventing the dissemination of HCV among hemodialysis patients (22). Simon et al. (26) have found a better correlation between long-term HCV infection and elevations in γ-glutamyltranspeptidase (GGT) levels. More studies are needed to evaluate the superior reliability of using GGT levels rather than ALT levels.

Viral interference has been described in patients with dual HBV and HCV infections (15). In the present study, a higher frequency of HCV PCR positivity was found among HBV-negative patients than HBV-positive ones, although this difference was not significant. More precise studies, such as quantitation of both viral genomes, are needed to evaluate the interference of replication between these two viruses (1, 19). The mechanism(s) of viral interference is not known, but the host immune response could be involved in mediating the suppressive effect of one virus on the other (15). If this is the case, viral interference between HBV and HCV might not occur in immunocompromised hosts like hemodialyzed patients.

The possible routes of transmission of HCV in hemodialysis patients are multiple and some of them are still controversial. The frequent blood transfusions in this group of patients have been an important route of infection before blood testing became available. There is, however, increasing evidence of the nosocomial transmission of HCV, as described previously for HBV. The sharing of equipment as a mode of HCV transmission is still controversial. Recent studies argue against HCV transmission through the hemodialysis ultrafiltrate (4). Others have shown the spread of virus between patients not sharing equipment (2). On the other hand, the beneficial effect of isolating equipment for HCV-positive patients has been described (5, 8, 22). In fact, lower prevalences of HCV positivity have been found in other Venezuelan hemodialysis units where more strict aseptic norms, such as isolation of equipment, are enforced (22).

The significantly higher frequency of blood recipients among HCV-positive patients compared with that among the uninfected group (Table 3) suggests that blood transfusion remains an important mode of exposure to HCV (6). However, blood transfusions alone cannot account for the high prevalence and incidence of HCV infection that was observed, and nosocomial transmission of HCV may play a role in the spread of HCV in this group. As described by others (12, 13, 17), we found that the duration of hemodialysis correlates with HCV positivity (Table 3). Nosocomial transmission among hemodialysis patients has recently been documented by molecular analysis (25, 27).

The hemodialysis machine used in the units studied might also play a role in HCV dissemination because of accidental contamination of the membrane on the device for pressure testing and inadequate subsequent disinfection. On the other hand, even if no disposable equipment or syringes were shared in these units, the multiple parenteral exposures and the sharing of drugs (heparin) among different patients could be involved in HCV transmission.

In conclusion, a high prevalence of HCV infection was observed by each second-generation assay. Despite a good correlation between the different tests, a high frequency of HCV-infected patients determined by the available tests can still be misleading, especially among immunocompromised patients undergoing hemodialysis. Although third-generation tests were not available in Venezuela for use in the present study, RIBA 3 did not, however, detect any seroconverting patient, and only inconsistent indeterminate results were observed (data not shown). ALT levels also are not useful for identifying these seronegative patients. Other serum markers, such as GGT, might be needed to monitor patients for HCV infection more efficiently. On the other hand, even if transfusion has been the main mode of HCV transmission in the past (testing for HCV in blood banks was implemented in Venezuela in 1992), nosocomial transmission now seems to play a role in the dissemination of HCV among these patients. Recent studies have shown that strict aseptic measures can virtually eliminate HCV contamination, even in units with a high prevalence of HCV infection (20), preventing the consequences of infection not only by HCV but by other non-A, non-B, and non-C viruses that could be circulating in these renally compromised patients (14, 16).

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REFERENCES

1. **Alberti, A., P. Pontisso, L. Chemello, G. Fattovich, L. Benvegnu, F. Belussi, and M. S. DeMitri.** 1995. The interaction between hepatitis B virus and hepatitis C virus in acute and chronic liver disease. J. Hepatol. **22**(Suppl.1)**:** 38–41.
2. **Allander, T., C. Medin, S. H. Jacobson, L. Grillner, and M. A. Persson.** 1994. Hepatitis C transmission in a hemodialysis unit: molecular evidence for spread of virus among patients not sharing equipment. J. Med. Virol. **43:** 415–419.
3. **Bukh, J., P. Wantzin, K. Krogsgaard, F. Khudsen, R. H. Purcell, R. H. Miller, and the Copenhagen Dialysis HCV Study Group.** 1993. High prevalence of hepatitis C virus (HCV) RNA in dialysis patients: failure of commercially available antibody tests to identify a significant number of patients with HCV infection. J. Infect. Dis. **168:**1343–1348.
4. **Caramelo, C., S. Navas, M. L. Alberola, T. Bermejillo, A. Reyero, and V. Carreño.** 1994. Evidence against transmission of hepatitis C virus through hemodialysis ultrafiltrate and peritoneal fluid. Nephron **66:**470–473.
5. **Chiaramonte, S., A. Tagger, M. L. Ribero, A. Grossi, M. Milan, and G. La Greca.** 1992. Prevention of viral hepatitis in dialysis units: isolation and technical management of dialysis. Nephron **61:**287–289.
6. **Dentico, P., A. Volpe, R. Buongiorno, A. Carlone, M. Carbone, and M. Manno.** 1992. Hepatitis C virus in hemodialysis patients. Nephron **61:**307–308.
7. **Dienstag, J. L., and H. J. Alter.** 1986. Non-A, non-B hepatitis: evolving epidemiologic and clinical perspective. Semin. Liver Dis. **6:**67–81.
8. **Druwe, P. M., P. P. Michielsen, A. M. Ramon, and M. E. DeBroe.** 1994. Hepatitis C and nephrology. Nephrol. Dial. Transplant. **9:**230–237.
9. **Goldblum, S. E., and W. P. Reed.** 1980. Host defenses and immunological alterations associated with chronic hemodialysis. Ann. Intern. Med. **93:**597–613.
10. **Gubertini, G., D. Scorza, M. Beccari, G. Buccianti, A. Constantino, D. Spotti, and G. Graziani.** 1992. Prevalence of hepatitis C virus antibodies in hemodialysis patients in the area of Milan. Nephron **61:**271–272.
11. **Guerrero, E., A. Guerrero, L. Gil, R. Montes, J. Mateos, M. Cunningham, D. Vallari, J. Casey, S. Watanabe, B. Zeck, S. Desei, and S. Devare.** 1994. Serological response to hepatitis C virus (HCV) in serial bleeds from hemodialysis patients, p. 485–488. *In* K. Nishioka, H. Suzuki, S. Mishiro, and T. Odo (ed.), Viral hepatitis and liver disease. Springer-Verlag, Tokyo.
12. **Huang, C.-S., M.-S. Ho, C.-S. Yang, C.-L. Lee, and C. A. Tan.** 1993. Hepatitis C markers in hemodialysis patients. J. Clin. Microbiol. **31:**1764–1769.
13. **Irie, Y., H. Hayashi, K. Yokozeki, T. Kashima, and K. Okuda.** 1994. Hepatitis C infection unrelated to blood transfusion in hemodialysis patients. J. Hepatol. **20:**557–559.
14. **Johnson, R. J., R. Wilson, H. Yamabe, W. Couser, C. E. Alpers, M. H. Wener, C. Davis, and D. R. Gretch.** 1994. Renal manifestations of hepatitis C virus infection. Kidney Int. **46:**1255–1263.
15. **Liaw, Y.-F.** 1995. Role of hepatitis C virus in dual and triple hepatitis virus infection. Hepatology **22:**1101–1108.
16. **Mioli, V. A., E. Balestra, L. Bibiano, P. Carletti, S. Della Bella, E. Fanciulli, G. Gaffi, R. Marinelli, R. Perilli, A. M. Ricciatti, D. Taruscia, and E. Pisani.** 1992. Epidemiology of viral hepatitis in dialysis centers: a national survey. Nephron **61:**278–283.
17. **Muller, G. Y., M. E. Zabaleta, A. Arminio, C. J. Colmenares, F. I. Capriles, N. E. Bianco, and I. V. Machado.** 1992. Risk factors for dialysis-associated hepatitis C in Venezuela. Kidney Int. **41:**1055–1058.
18. **Nordenfelt, E., B. Lofgren, B. A. Widell, B.-G. Hansson, Y. Y. Zhang, K.-E. Hagstam, and J. Kurkus.** 1993. Hepatitis C infection in hemodialysis patients in southern Sweden: epidemiological, clinical, and diagnostic aspects. J. Med. Virol. **40:**266–270.
19. **Ohkawa, K., N. Hayashi, N. Yuki, M. Masuzawa, M. Kato, K. Yamamoto, H. Hosotsubo, M. Deguchi, K. Katayama, A. Kasahara, H. Fusamoto, and T. Kamada.** 1995. Long-term follow-up of hepatitis B virus and hepatitis C virus replicative levels in chronic hepatitis patients coinfected with both viruses. J. Med. Virol. **46:**258–264.
20. **Okuda, K., H. Hayashi, S. Kobayashi, and Y. Irie.** 1995. Mode of hepatitis C infection not associated with blood transfusion among chronic hemodialysis patients. J. Hepatol. **21:**28–31.
21. **Pol, S., R. Romeo, B. Zins, F. Driss, B. Lebkiri, F. Carnot, P. Berthelot, and C. Brechot.** 1993. Hepatitis C virus RNA in anti-HCV positive hemodialyzed patients: significance and therapeutic implications. Kidney Int. **44:**1097–1100.
22. **Pru, C. E., C. Cuervo, M. Ardila, and M. Teran.** 1994. Hepatitis C transmission through dialysis machines. ASAIO (Am. Soc. Artif. Itern. Organs) J. **40:**M889–M891.
23. **Pujol, F. H., L. Blitz, G. León, G. F. Monsalve, J. M. Echevarría, and F. Liprandi.** 1995. Efficacy of different second- and third-generation assays for detection of hepatitis C virus antibodies in plasma and sera also tested by polymerase chain reaction. Serod. Immunother. Infect. Dis. **7:**51–54.
24. **Sakamoto, N., N. Enomoto, F. Marumo, and C. Sato.** 1993. Prevalence of hepatitis C virus infection among long-term hemodialysis patients: detection of hepatitis C virus RNA in plasma. J. Med. Virol. **39:**11–15.
25. **Sampietro, M., S. Badalamenti, S. Salvadori, N. Corbetta, G. Graziani, G. Como, G. Fiorelli, and C. Ponticelli.** 1995. High prevalence of a rare hepatitis C virus in patients treated in the same hemodialysis unit: evidence for nosocomial transmission of HCV. Kidney Int. **47:**911–917.
26. **Simon, N., A.-M. Courouce, N. Lemarrec, C. Trepo, and S. Ducamp.** 1994. A twelve year natural history of hepatitis C virus infection in hemodialyzed patients. Kidney Int. **46:**504–551.
27. **Stuyver, L., H. Claeys, A. Wyseur, W. van Arnhem, H. De Beenhouwer, S. Uyttendale, J. Beckers, G. Leroux-Roels, G. Maertens, and M. De Paepe.** 1996. Hepatitis C virus in a hemodialysis unit: molecular evidence for nosocomial transmission. Kidney Int. **49:**889–895.