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Para Nicolás, el Director, por la confianza inmodificable. Con mi gratitud, [illegible]

# Risk factors for dialysis-associated hepatitis C in Venezuela

GRETE Y. MULLER, MERCEDES E. ZABALETA, ANABELLA ARMINIO, CARMEN J. COLMENARES, FREYA I. CAPRILES, NICOLÁS E. BIANCO, and IRMA V. MACHADO

*Instituto de Inmunologia, Universidad Central de Venezuela and WHO/PAHO Collaborative Center in Clinical Immunology; Unidad de Hemodialisis Cronica de Caracas; and Hospital Universitario, Caracas, Venezuela*

**Risk factors for dialysis-associated hepatitis C in Venezuela.** Utilizing the first and second generation of enzyme immunoassays which detect antibodies to the C virus we investigated the frequency of anti-HCV antibodies in 315 patients undergoing hemodialysis. Other subpopulations at risk were used as reference groups. One hundred and twenty-three samples (39%) from the hemodialysis group repeatedly showed anti-HCV positive antibodies while only 19% and 1% were positive in the reference groups. The rate of anti-HCV reactive patients correlated with time on hemodialysis (< 1 year, 17%; 1 to 5 years 43%; > 5 years, 64%; $r = 0.94$, $P < 0.001$) and with the number of blood transfusions (1 to 10, 40%; > 10, 76%; $r = 0.97$; $P < 0.001$). Length of time on hemodialysis was shown to be the major risk factor in thirty-three anti-HCV positive patients who had no previous record of blood transfusions. Co-infection with HBV was demonstrated in 41% out of 123 anti-HCV reactive patients, and increased alanine aminotransferase (ALT) activity was documented in this co-infected group. Our results further extend the observations on the predisposing factors to HCV spread in hemodialysis units, and suggest that in these renal patients co-infection with C and B viruses is a major cause of rising ALT activity.

Infection with hepatitis C virus (HCV) is currently recognized by the discovery of serum anti-HCV antibodies [1, 2] in patient's blood. The available data suggest that HCV is the major etiologic agent of blood-borne nonA-nonB hepatitis [3, 4]. Patients on long-term hemodialysis are considered to be a population at risk for HCV infection, but the influence of factors such as time on hemodialysis and multiple blood transfusions seems to vary among developed countries [5–7]. To identify characteristics of the predisposing factors of HCV infection in other geographical areas, we assessed the epidemiological pattern of the HCV prevalence in eight Venezuelan urban hemodialysis units, simultaneously exploring reference groups having a high risk to HCV exposure.

## Methods

Study subjects included 315 patients undergoing chronic hemodialysis in eight units located at different hospitals in Caracas, Venezuela. There were 184 men, (mean age 46 years, range 17 to 87) and 131 women, (mean age 44 years, range 16 to 82). At the time of the study all patients were treated without re-use of hemodialysis filters or blood lines. Several types of artificial kidneys were used in the different units (Gambro AK10, Sweden; Monitral, Hospal Laboratories, Italy; SPS 450, Cobe Travenol Lab, USA). Disinfection techniques were also similar including the chemical sterilization procedure (8% sodium hypochlorite) for 35 minutes between each hemodialysis period, or the use of a 2% aqueous glutaraldehyde solution and heating (80C) during the same period of time, depending upon the machine type. None of the patients had a history of intravenous drug abuse and their socio-economic level was essentially similar. A retrospective analysis of the clinical record assessment of each patient was done to determine length of time on chronic hemodialysis, the number of transfusion units received, hepatitis B virus markers profile, and past and recent alanine aminotransferase (ALT) values.

Anti-HCV antibodies were screened twice using the first and second generation enzyme immunoassay developed by Abbott Laboratories (North Chicago, Illinois, USA). The first generation HCV-EIA detects circulating anti-HCV antibodies to the recombinant protein C-100-3 from the C virus, while the second generation HCV-EIA detects antibodies from two additional inmunogenic regions from the HCV genome named 33c and the core [2]. The specificity and sensitivity of the currently available tests for anti-HCV antibodies are not well defined [8]. Therefore, only the repeatedly reactive samples were considered as positive for anti-HCV antibodies. Previous experience employing one of these assays to screen 500 volunteer blood donors [9] was extended by simultaneously testing 100 homosexual men infected with human immunodeficiency virus (HIV). As a reference group, we also included 27 serum samples from patients with the diagnosis of nonA-nonB hepatitis obtained after ruling out HAV, HBV and Epstein-Barr virus infections.

### *Statistical analysis*

Statistical analysis was performed by the correlation coefficient test and by the $\chi^2$ test using $2 \times 2$ and $3 \times 3$ distribution tables.

## Results

### *Prevalence of anti-HCV antibodies in hemodialysis patients*

When the 315 serum samples were assayed by the first generation HCV-EIA, 124 samples were reactive. This group of HCV positive samples was screened twice by the second generation HCV-EIA and only one sample showed negative

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**Table 1.** Correlation between different variables (time on hemodialysis, blood transfusion record) and prevalence of anti-HCV positive patients

| | Time on hemodialysis | | | | | |
|---|---|---|---|---|---|---|
| | <1 year | | 1–5 years | | >5 years | |
| Number of transfusions | *N* | | *N* | (2 ± 0.9) | *N* | (6.8 ± 1) |
| 0 | 29 | 2 (7%)[a] | 71 | 25 (35%)[b] | 12 | 6 (50%)[c] |
| 1–10 | 36 | 9 (25%)[d] | 132 | 56 (42%)[e] | 9 | 6 (67%)[f] |
| >10 | 0 | 0 | 21 | 15 (71%) | 4 | 4 (100%) |

Abbreviations are *N*, number of serum samples; %, percentage of anti-HCV positive samples.
a vs. b vs. c, $r = 0.88$; $P < 0.001$
d vs. e vs. f, $r = 0.98$; $P < 0.001$

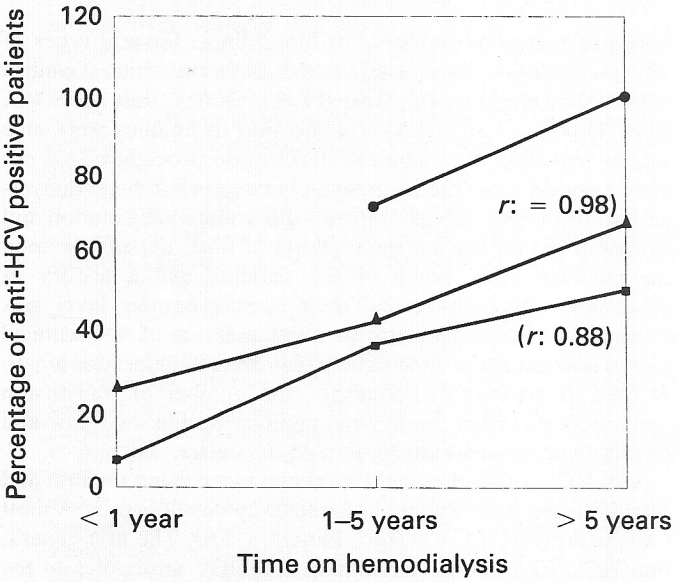


**Fig. 1.** *Correlation between time on hemodialysis, number of blood transfusions and prevalence of anti-HCV positive hemodialysis patients.*

**Table 2.** HBV positive markers in anti-HCV positive and anti-HCV negative hemodialysis patients

| HBV markers | Anti-HCV + ve samples (*N* = 123) | Anti-HCV − ve samples (*N* = 191) |
|---|---|---|
| HBsAg only | 10 (8%) | 15 (8%) |
| Anti-HBs only | 1 (0.8%) | 0 |
| Anti-HBc only | 25 (20%) | 19 (10%) |
| HBsAg +Anti-HBc | 11 (9%) | 7 (4%) |
| Anti-HBs + Anti-HBc | 3 (2%) | 11 (6%) |
| Total | 50 (41%) | 52 (27%) |

**Table 3.** HCV and HBV markers in hemodialysis patients without previous record of blood transfusions

| | Time on hemodialysis | | |
|---|---|---|---|
| Viral markers | <1 year (29) | 1–5 years (71) | >5 years (12) |
| Anti-HCV | 2 | 25 | 6 |
| HBsAg only | 0 | 1 | 0 |
| Anti-HBs only | 0 | 0 | 0 |
| Anti-HBc only | 0 | 3 | 0 |
| HBsAg + Anti-HBc | 0 | 0 | 3 |
| Anti-HBs + Anti-HBc | 0 | 1 | 0 |

results. Therefore, from the analysis of 315 patients' serum, 123 (39%) were repeatedly reactive for anti-HCV antibodies. The proportion of patients who were anti-HCV positive significantly correlated with their length of time on hemodialysis (Table 1). Similar results were observed upon comparison between the transfusion record and the frequency of anti-HCV reactivity (Table 1). Correlation of both variables is depicted in Figure 1. Moreover, analysis of results shown in Table 1 demonstrated that 33 anti-HCV positive samples belonged to patients who were on hemodialysis but who had no previous history of blood transfusions. Twenty-five individuals from this subgroup of patients were on hemodialysis between one and five years, six had been dialyzed for more than five years and only two were on hemodialysis for less than one year. We searched for the possible specific source of contamination. None of the artificial kidneys correlated with a significant number of anti-HCV positive patients. Since this study represents the first HCV screening practiced among Venezuelan hemodialysis patients, typification and isolation of the HCV infected group were made subsequently.

*HBV markers associated with anti-HCV positive antibodies*

Table 2 shows the results of the hepatitis B serology in the subgroup of HCV positive patients. Fifty subjects (41%) showed HBV markers plus anti-HCV antibodies. Fifty-two (27%) patients from the group of 191 anti-HCV negative patients also demonstrated the presence of one or more HBV markers. Table 2 divides both groups according to the identified HBV profile.

Table 3 details the HBV marker's distribution within the anti-HCV positive group who had no previous record of blood transfusions. Only eight subjects demonstrated HBV markers. As occurred with anti-HCV reactivity, all of those HBV positive patients fell in the subgroup of patients who were on hemodialysis more than one year.

*ALT activity, HBV markers and anti-HCV antibodies*

Increased ALT values (twofold increase over the normal upper limit) were documented in 65 (53%) out of 123 anti-HCV positive patients. ALT values ranged between 80 and 888 IU/L. As depicted in Table 4, abnormal ALT activity was documented

**Table 4.** Increased ALT activity and HBV markers in anti-HCV positive and anti-HCV negative hemodialysis patients

| HVB markers | Anti-HCV + ve samples | | Anti-HCV − ve samples | |
|---|---|---|---|---|
| | Number of samples | ALT[a] | Number of samples | ALT[b] |
| HBsAg only | 10 | 3 | 15 | 7 |
| Anti-HBs only | 1 | 0 | 0 | 0 |
| Anti-HBc only | 25 | 12 | 19 | 1 |
| HBsAg + Anti-HBc | 11 | 5 | 7 | 2 |
| Anti-HBSs + Anti-HBc | 3 | 1 | 11 | 0 |
| No HBV markers | 73 | 44 | 139 | 29 |
| Total | 123 | 65 | 191 | 39 |

ALT ≥two-fold increase over the normal upper limit.
[a] ALT ranges 80–888 IU/liter
[b] ALT ranges 76–523 IU/liter

more often in patients who had a coexistence of HCV plus HBV infections than in patients with detectable HBV markers only. So far, chronic persistent hepatitis, chronic active hepatitis and active cirrhosis have been documented in a small number of the HCV-only positive patients (data not shown). Liver biopsy is currently planned for selected patients from both groups.

*Reference groups*

Samples from 5 of 27 patients classified as having nonA-nonB hepatitis repeatedly showed anti-HCV positivity. Three patients were characterized as having sporadic HCV infections and two as having post-transfusional C hepatitis. As in our previous report on Delta virus serological markers [10], anti-HCV antibodies were only demonstrated in 1% of the HIV-infected homosexual population.

## Discussion

NonA-nonB hepatitis is increasingly recognized as a hazard to patients undergoing hemodialysis [11, 12]. The availability of tests which detect antibodies from a nonA-nonB virus designated as HCV has allowed the assessment of the impact of this infection in different at-risk populations [3, 5–7]. In our investigation, we found that the prevalence of anti-HCV reactivity in hemodialysis patients was two to three times higher than the reported rates in some developed countries [5–7, 13]. There was an association between the number of blood transfusions and the frequency of anti-HCV reactive patients. Furthermore, the presence of anti-HCV antibodies was demonstrated even in patients who received fewer than 10 blood units. This finding differs from data reported in some developed countries where the influence of the use and number of blood transfusions or blood products on the rate of HCV positive hemodialysis patients has not been clearly established [6, 7, 14, 15]. At present, we are not able to fully estimate the real incidence of post-transfusional nonA-nonB hepatitis in different Venezuelan populations including renal patients. However, screening of Venezuelan volunteer blood donors has demonstrated a 1.2% prevalence of anti-HCV positive carriers [9], which is similar to that reported in Japan where typical post-transfusional nonA-nonB hepatitis induced by reception of anti-HCV positive blood units has been characterized [16]. This prevalence (1.2%) is also similar to the reported rate of HBV surface antigen in Venezuela, which is classified as a country with an intermediate rate of HBV chronic carriers [10, 17, 18]. Moreover, thus far, Venezuela has not implemented the ALT and anticore antibodies screening at their blood banks, strategies which have demonstrated to be cost effective in preventing up to 30% of post-transfusion nonA-nonB hepatitis in developed countries [19–22]. All these factors taken together support the association demonstrated between the number of blood transfusions and the rate of HCV infection found in Venezuelan hemodialysis units.

There was an important proportion of patients who were positive for anti-HCV antibodies and who were without a history of blood transfusions. This group only showed the length of time on hemodialysis treatment as the main risk factor, which also significantly correlated with the frequency of anti-HCV positivity in the whole group. Except for hemodialysis, we were not able to identify a specific route of transmission for those patients. The observation that long-term hemodialysis plays a major role for HCV infections is similar to reports from Japan, Germany and Italy [13–15, 23]. As occurs with HBV, factors associated with hemodialysis procedures might be implicated in the high rate of HCV infection [6, 11, 24]. In fact, in contrast with reports from other countries [5, 6, 23], we found that an important proportion of the HCV-infected hemodialysis group was also infected with HBV. Furthermore, abnormal ALT activity was mainly demonstrated in this coinfected group and in patients infected only with HCV. In addition to being a marker of inflammatory liver disease, increased levels of alanine aminotransferase indicate HCV infection [25]. Studies of hepatitis C viral sequences have suggested that patients with positive anti-C-100-3 antibodies and chronic nonA-nonB hepatitis probably are in a viremic state [26]. Therefore, those hemodialysis patients with associated anti-HCV antibodies and increased ALT activity are likely to be potentially infectious, and therefore contribute to maintaining the reservoir of HCV infection in renal care units.

At present, specific measures to control the spread of HCV have been taken in Venezuelan blood banks and hemodialysis units. For instance, very recently HCV screening has been introduced in blood banks; in the renal care setting patients are being tested for anti-HCV antibodies prior to entering hemodialysis programs, and patients who test positive have been separated from non-infected individuals. The long-term follow-up should better determine the influence of the hemodialysis procedure on the rate of HCV infection observed in Venezuela.

Our results add further geographical evidence indicating that blood transfusion and chronic hemodialysis are the primary predisposing factors to the spread of HCV in renal patients. HCV and HBV co-infection is a major cause of raised ALT activity in Venezuelan hemodialysis patients.

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*Reprint requests to Irma V. Machado, M.D., Instituto de Inmunologia, Apartado 50109, Caracas 1050A, Venezuela.*

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