Hepatitis C Is Less Aggressive in Hemodialysis Patients than in Nonuremic Patients

Jose Eduardo Trevizoli,* Raissa de Paula Menezes,† Lara Franciele Ribeiro Velasco,‡ Regina Amorim,§ Mauro Birche de Carvalho,† Liliana Sampaio Mendes,† Columbano Junqueira Neto,† José Roberto de Deus Macedo,¶ and Francisco de Assis Rocha Neves†

*Hospital de Base do Distrito Federal, Secretaria de Estado de Saúde (SES), Brasília, DF, Brazil; and Universidade Católica de Brasília, Brasília, DF, Brazil; †Hospital de Base do Distrito Federal, SES, Brasília, DF, Brazil; ²Sabin Institute and Laboratory of Clinical Analysis, Brasília, DF, Brazil; ³Faculdade de Ciências da Saúde, Universidade de Brasília, Brasília, DF, Brazil; ⃞Hospital Santa Luzia, Brasília, DF, Brazil; and ¶Laboratório de Farmacologia Molecular, Faculdade de Ciências da Saúde, Universidade de Brasília, Brasília, DF, Brazil

Background and objectives: The severity of liver disease among hepatitis C patients on hemodialysis is controversial. The aim of this study was to compare the clinical, biochemical, and liver histologic characteristics of hepatitis C virus (HCV) in hemodialysis patients and in those with normal renal function.

Design, setting, participants, & measurements: A case-control study was carried out with 36 HCV patients on hemodialysis and 37 HCV patients with normal renal function matched for gender, age at infection, and estimated time of infection.

Results: HCV patients on hemodialysis had lower levels of alanina aminotransferase and lower viral load. Hepatic fibrosis was significantly higher in the patients with normal renal function (73%) than in hemodialysis patients (47.2%, P < 0.025); the same was observed for inflammatory activity (control group 59.5% versus hemodialysis patients 27.7%, P = 0.003). In addition, the risk of tissue inflammation was four times lower in hemodialysis patients (odds ratio = 0.23, P < 0.004), and severe inflammatory activity on biopsy was the only independent risk factor for fibrosis (P < 0.001).

Conclusions: The lower biochemical and inflammatory activities observed in hemodialysis patients suggest that hemodialysis and uremia may have a protective role against progression of the disease caused by HCV.


Hepatitis C is the main cause of chronic liver disease in patients with end-stage renal disease (ESRD). The prevalence of hepatitis C virus antibody (anti-HCV) is high, between 3.4% and 32.1%, among hemodialysis (HD) patients and potential kidney transplant candidates (1). Despite the decrease in the incidence of newly acquired HCV infections observed in recent years, which may be attributable to the efficient serologic HCV tests used in blood transfusions and to the use of erythropoietin to treat anemia, contamination by HCV still occurs in HD units (2).

Patients infected with HCV who undergo renal transplantation show a higher risk for progression to severe liver disease, death, and graft failure after transplantation compared with anti-HCV negative recipients (3). Liver disease should consequently be staged at the time of kidney transplantation because patients with cirrhosis or advanced fibrosis will have a poorer prognosis (4).

The natural history of chronic liver disease caused by HCV in HD patients remains unknown (4–6). HCV infection increases mortality rates in uremic patients, and cirrhosis and other liver-related deaths are more frequent in HCV-infected dialysis patients than in those without the virus (5–7).

Results from liver biopsies of HCV-infected dialysis patients are limited (1,2), and the rate of bridging hepatic fibrosis and liver cirrhosis ranges from 5% to 32% (8–15). Whether the liver disease caused by HCV shows a different clinical course in HD patients and in patients with normal renal function is still controversial. Barril (8) reported that progression time to cirrhosis can be much shorter in HCV-infected HD patients than in patients with normal renal function. However, other studies suggest that HCV-infected HD patients present a lower degree of inflammatory activity and a lower stage of liver fibrosis compared with HCV-infected patients with normal renal function (9,12,15). Indeed, the effects of chronic uremia and HD on HCV-related liver disease and on the progression of liver fibrosis in HCV-infected patients with ESRD remain unknown.

To investigate the impact of chronic renal failure and HD on the progression of hepatitis C in patients awaiting kidney transplantation, we compared clinical, biochemical, virologic, and

Copyright © 2008 by the American Society of Nephrology

ISSN: 1555-9041/305–1385
histologic findings in HCV-infected patients with ESRD receiving HD treatment and in HCV-infected patients with normal renal function. Our results showed that HCV-infected patients on HD presented lower levels of alanine aminotransferase (ALT), lower viral load, and less inflammatory activity and fibrosis in their liver biopsies. Moreover, severe inflammatory activity was the only independent risk factor for liver fibrosis. Taken together, our results strongly suggest that chronic hepatitis C is less aggressive in HD patients than in nonuremic patients.

Materials and Methods

This case-control study was conducted from January 2000 to December 2006 and included 36 HD patients enrolled in the kidney transplant waiting list of Hospital de Base do Distrito Federal, Brasília, Distrito Federal, Brazil. Subjects were selected among 761 patients with ESRD who had been treated for at least 6 mo in seven HD units located in the metropolitan area of Brasília. From this population, 101 patients were positive for anti-HCV antibody (enzyme-linked immunosorbent assay 3) and 76 patients were HCV RNA positive. Of these 76 patients, 36 had been submitted to liver biopsy because they were awaiting renal transplantation (case group). The control group comprised 37 HCV-infected patients with normal renal function, defined by creatinine $\leq$ 1 mg/dl.

Date of onset of HCV infection was estimated considering the date when HD was initiated or the year when the patient received a blood transfusion (if before the beginning of HD) in the study group, and the date of the first blood transfusion in the control group. Exclusion criteria for both groups were: coinfection with hepatitis B virus or HIV, chronic use of steroids, interferon, or ribavirin; other liver diseases; intravenous drug use; alcohol consumption of more than 40 g/d for chronic use of steroids, interferon, or ribavirin; other liver diseases; intravenous drug use; alcohol consumption of more than 40 g/d for men and 20 g/d for women. The control group was paired with the study group for age, sex, and estimated time of HCV infection. All patients underwent clinical, biochemical, serologic, virologic, sonographic, and histologic studies, and the results were compared between the two groups.

Clinical Data

Data about age, sex, risk factors, estimated date of onset of HCV infection, possible HCV transmission mode, body mass index and history of diabetes mellitus were obtained from clinical history and review of charts. The diagnosis of diabetes mellitus was made according to reports of use of oral hypoglycemic agents or insulin and fasting glycemia level ($>110$ mg/dl).

Laboratory Studies

Blood samples were collected for the following laboratory tests: glucose, ALT, total proteins, albumin, platelets, transferrin saturation, and prothrombin activity (International Normalized Ratio). ALT levels were calculated as the mean of the three tests performed in the 6 mo before the first biopsy, whereas the other tests were carried out around the date of the liver biopsy.

Serologic and Molecular Tests

Patients were tested for anti-HCV antibody using a third-generation enzyme-linked immunosorbent assay (Abbott Laboratories, Abbott Park, IL). Blood samples were collected from ESRD patients before HD sessions. The samples were centrifuged and sera were frozen at $-70^\circ$C in up to 2 h. HCV RNA was assessed by polymerase chain reaction (PCR) using a commercial kit (Amplicor, Roche Diagnostics, Indianapolis, IN). To measure HCV RNA level, a semiautomated branched DNA (bDNA) assay was used (Versant HCV RNA 3.0 Quantitative, Bayer Corporation, Tarrytown, NY). The genotype was determined with an automated sequencing technique using the ET DYE Terminator kit and the P17 primer for the 5' UTR and S2 for the NS5B region (MegaBACE 1000 GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom). The sequences were analyzed using the basic local algorithm search tool (BLAST software) and aligned with the HCV sequences available in data banks (www.ncbi.nlm.nih.gov, http://hcv.lanl.gov).

Histologic Tests

Liver biopsy specimens were classified as adequate if they were at least 1.5 cm long and had 10 or more portal spaces. Histologic tests were conducted by a pathologist blinded to clinical and laboratory findings. Fibrosis and inflammatory activity were staged according to the Metavir scale (16). The presence of lymphoid follicles, bile duct lesion, liver steatosis, and iron deposits in liver tissue (Perls stain) was also recorded.

Statistical Analysis

Descriptive data were presented as percentages or means with SD and range. Categorical variables were analyzed with the $\chi^2$ test and Fisher exact test. The $t$ test and Mann-Whitney test were used for comparison of numerical variables. Logistic regression models were used to calculate odds ratios (95% confidence interval) and the corresponding $P$ values. The level of significance was set at 0.05 (alpha = 5%). Statistical analyses were performed using the SPSS 13.0 software for Windows.

Approval by Research Ethics Committee

The study protocol followed the Helsinki Declaration and was approved by the Research Ethics Committee at Hospital de Base do Distrito Federal. All patients included signed an informed consent form after receiving information about the study.

Results

Among the 76 HD patients infected by HCV, 36 who were in the kidney transplant list were included in the study, as well as 37 HCV-infected patients with normal renal function. No difference was observed between the 36 HD patients awaiting kidney transplantation and the remaining 40 HD patients who were not enrolled in the kidney transplant list in terms of ALT, total proteins, albumin, platelets, transferrin saturation, prothrombin activity (International Normalized Ratio), and abdominal ultrasound.

Patients with uncontrolled liver disease with ascites, encephalopathy, esophageal varices, or jaundice were not included in the study because isolated kidney transplantation is not indicated for them. Table 1 shows data about sex, age, estimated time since onset of HCV infection, and presence of diabetes; all these parameters were similar in the two groups. Body mass index, ALT, albumin, and total protein levels were significantly lower in uremic patients on HD. The distribution of HCV genotypes was similar in both groups. Notwithstanding, the viral load was significantly lower in HD patients ($P = 0.016$).

The hepatic histologic findings for both groups are shown in Table 2. Liver steatosis and lymphoid follicles, two histologic findings associated with liver disease caused by HCV, were significantly more frequent in the patients with normal renal function than in patients on HD (56.8% versus 16.3% and 43.2%
versus 5.6%, respectively; \( P < 0.001 \)). Iron deposits in liver tissue were more frequent and more intense in patients on HD than in patients with normal renal function \( (P = 0.008) \).

The prevalence of liver fibrosis in patients with ESRD receiving HD treatment was 47.2%, significantly lower than the rate found in patients with normal renal function (73%; \( P = 0.025 \)). Furthermore, significant fibrosis (stages 2 and 3, Metavir) was found in 32.5% of the patients with normal renal function and in only 17.1% of those on HD, although this difference did not reach significance \( (P = 0.118) \). Liver cirrhosis (stage 4 fibrosis) was not found in any of the study groups (Table 2).

Inflammatory activity of the liver was significantly lower in HD patients. As shown in Table 2, absence of or light inflammation (stages 0 and 1) was observed in 39% and 33.3% of the HD patients, respectively, a much higher result than the one found in nonuremic patients: 16.2% and 24.3%, respectively. On the other hand, moderate inflammation (stage 2) was increased in nonuremic patients (40.5%) compared with HD patients (27.7%). It is noteworthy that, whereas severe inflammatory activity (stage 3) was found in 19% of the patients with normal renal function, none of the uremic patients displayed severe inflammatory disease. Consequently, moderate and severe inflammatory activity (stages 2 and 3, Metavir) was twice as frequent in patients with normal renal function as in patients with ESRD on HD (59.5% versus 27.7%; \( P = 0.003 \)). Taken together, these results clearly demonstrate the greater intensity of inflammation and fibrosis in patients with normal renal function.

Univariate analysis revealed four factors correlated with the presence of liver fibrosis: no renal failure \( (P = 0.027) \), severe inflammatory activity \( (P < 0.001) \), lymphoid follicles \( (P = 0.029) \), and no iron in liver tissue \( (P = 0.002) \). Nevertheless, multivariate analysis (Table 3) showed that the only independent risk factor for fibrosis was severe inflammatory activity in liver biopsy \( (P < 0.001) \). Inflammatory activity was associated with an increase of 15.2 in the relative risk of fibrosis.

Interestingly, the analysis of risk factors associated with severe inflammatory activity showed that patients with elevated ALT \( (P = 0.017) \) and with lymphoid follicles \( (P = 0.020) \) had biopsies with stage 2 or 3 inflammation more frequently. However, HD patients showed signs of significantly less severe inflammatory activity than patients with normal renal function \( (9 \text{ of } 36 \text{ versus } 22 \text{ of } 37; P = 0.004) \). Multivariate analysis revealed that chronic kidney disease and HD reduced the occurrence of severe inflammatory activity. Patients with ESRD on HD had a four times lower risk for tissue inflammation than patients with normal renal function (odds ratio = 0.23; \( P < 0.004 \), Table 3).

**Discussion**

The prevalence of HCV infection among HD patients is substantially greater compared with patients with normal renal function. The prevalence of HCV infection among HD patients is substantially greater compared with patients with normal renal function. The prevalence of HCV infection among HD patients is substantially greater compared with patients with normal renal function.
renal function (1). HCV infection is currently the most common cause of chronic liver disease in HD patients (4). The natural history of hepatitis C in patients with ESRD is hard to evaluate because of the high mortality rate among them, the difficulty to make diagnoses in cases of acute hepatitis, and the slow progression of liver disease caused by HCV. Prospective studies have reported greater mortality rates in HCV-infected HD patients than in noninfected patients (6,17,18). The reason for this result has not been defined, but it has been suggested that it may be associated with liver disease and liver cancer (6,17).

Studies assessing liver histology revealed that the inflammatory activity tends to be mild or moderate, whereas the presence of bridging fibrosis ranges from 3% to 11%, and of cirrhosis, from 0% to 24% in uremic patients (2). The rate of progression of liver fibrosis and the identification of which factors affect the natural history of HCV-infected patients have not been established, and some reports suggest that HD may act as a protective factor and reduce the rate of progression of liver disease (19).

In the present study, we compared the natural course of HCV infection in HD patients with patients with normal renal function. ALT level, viral load, prevalence of fibrosis, and inflammatory activity were significantly lower in HD patients than in nonuremic patients. Multivariate analysis revealed that severe inflammatory activity in liver biopsy was the only independent risk factor for liver fibrosis in these patients (relative risk = 15).

### Table 2. Histopathologic changes among hemodialysis and normal renal function patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group with ESRD (n = 36) [mean ± SD (range) or n (%)]</th>
<th>Group with NRF (n = 37) [mean ± SD (range) or n (%)]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis staging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>19 (52.8)</td>
<td>10 (27)</td>
<td>0.025&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>11 (30.6)</td>
<td>15 (40.5)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4 (11.1)</td>
<td>7 (19.5)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 (5.5)</td>
<td>5 (13)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Inflammatory activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14 (39)</td>
<td>6 (16.2)</td>
<td>0.003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>12 (33.3)</td>
<td>9 (24.3)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10 (27.7)</td>
<td>15 (40.5)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0 (0)</td>
<td>7 (19)</td>
<td></td>
</tr>
<tr>
<td>Steatosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>6 (16.3)</td>
<td>21 (56.8)</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>no</td>
<td>30 (83.3)</td>
<td>16 (43.2)</td>
<td></td>
</tr>
<tr>
<td>Lymphoid aggregate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>2 (5.6)</td>
<td>16 (43.2)</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>no</td>
<td>34 (94.4)</td>
<td>21 (56.8)</td>
<td></td>
</tr>
<tr>
<td>Bile duct injury</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>1 (2.8)</td>
<td>4 (10.8)</td>
<td>0.358&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>no</td>
<td>35 (97.2)</td>
<td>33 (89.2)</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14 (38.9)</td>
<td>27 (73)</td>
<td>0.008&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>10 (27.8)</td>
<td>7 (18.9)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7 (19.4)</td>
<td>3 (8.1)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 (11.1)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1 (2.8)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>χ² test.
<sup>b</sup>Fisher’s exact test.

### Table 3. Multivariate analysis of the variables associated with fibrosis (stage I-IV) and inflammatory activity (II-IV) in all patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESRD</td>
<td>—</td>
<td>0.401</td>
</tr>
<tr>
<td>inflammatory activity</td>
<td>15.167 (3.957-58.137)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>lymphoid aggregate</td>
<td>—</td>
<td>0.169</td>
</tr>
<tr>
<td>iron</td>
<td>—</td>
<td>0.129</td>
</tr>
<tr>
<td>Inflammatory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESRD</td>
<td>0.227 (0.084-0.618)</td>
<td>0.004</td>
</tr>
<tr>
<td>lymphoid aggregate</td>
<td>—</td>
<td>0.213</td>
</tr>
<tr>
<td>ALT</td>
<td>—</td>
<td>0.679</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; ESRD, end-stage renal disease; ALT, alanine aminotransferase.
and that uremia and/or HD therapy was a protective factor for severe liver inflammation (relative risk = 0.227).

The lower degree of inflammatory activity and liver fibrosis in our HD patients is similar to findings described by other groups (9,12,13,15). We did not observe any association between inflammation and duration of HD treatment, although Sezer et al. (14) have reported that the longer the time on HD, the lower the degree of liver inflammation. Sterling et al. (15) have also reported that inflammatory activity and fibrosis were less intense in HD patients, although these differences were restricted to patients with elevated ALT as observed in our patients. In addition, HD patients have been described to have less advanced fibrosis and a lower degree of liver inflammation when compared with chronic renal failure patients not requiring dialysis (13,20) or to patients who underwent renal transplantation (19,21,22).

The mechanism by which uremia and HD may exert a protective effect on HCV liver inflammation remains unknown. The dysfunction of B and T cells (23,24), elevated levels of hepatocyte growth factor (25), and changes in the antioxidant system in the serum of HD patients (26) are factors that may be associated with lower liver inflammation. Regardless of the reasons, lower liver inflammation in HD patients may contribute to a delay in the progression of liver disease because the rate of inflammatory activity is associated with fibrosis progression (27).

In this study, no association was found between fibrosis and age, sex, body mass index, age at onset of infection, platelets, albumin, transferrin saturation, steatosis, bile duct lesion, or lymphoid follicles in liver biopsy. These findings were similar to those reported by other authors that studied patients with ESRD (9,13–15).

Iron deposits in the liver, a factor that may stimulate fibrogenesis caused by HCV, were more frequent and more intense in our HD patients ($P = 0.008$). This finding has been associated with blood transfusions and frequent supplementation of iron in patients with ESRD. However, our data did not show a correlation between iron deposits and liver fibrosis in HD patients or in patients with normal renal function.

The distribution of HCV genotypes was similar in the two groups. Contrarily, the viral load was significantly higher in patients with normal renal function ($P = 0.016$). Although dialysis patients have immune compromise resulting from uremia, the HCV viral load of HD patients is usually low, and this may be the result of the clearance of HCV RNA during HD, the adherence of viral particles to the dialysis membrane, or the release of cytokines (28,29). Badalamenti et al. (30) found a reduction in viral load during HD associated with the simultaneous elevation of interferon-alpha levels, and concluded that this might contribute to the fact that the disease is less aggressive in dialysis patients.

Genotype and viral load were not associated with histologic findings of liver biopsy in our study; this result is in accordance with reports by other authors involving both HD patients (13) and those with normal renal function (31).

Similarly to other studies, we found that HD patients had significantly lower ALT levels than patients with normal renal function (9,14). Univariate analysis of data from patients with elevated ALT levels showed more frequent severe inflammatory activity ($P = 0.017$), but significance was not found in the multivariate analysis ($P = 0.679$). Furthermore, we did not find a correlation between elevated ALT and fibrosis ($P = 0.167$), which indicates that enzyme levels do not predict the severity and stage of histologic changes in HD patients. Therefore, liver biopsy remains a valuable tool in the evaluation of liver disease in HCV-infected patients.

The limitations of this study should be carefully considered. HD patients were restricted to candidates for kidney transplantation because, in our service, these patients are submitted to liver biopsy to assess the stage of fibrosis. On the other hand, HCV-positive patients on HD who were not awaiting kidney transplantation are not submitted to liver biopsy because there is no consensus about whether these patients should be treated for HCV. Additionally, although we excluded patients with history of alcohol consumption, hepatitis B virus, or HIV coinfection and uncontrolled severe liver disease, there is a risk that HD patients with more severe illnesses (i.e., with a worse liver function) were not referred to kidney transplantation, in contrast with the group of patients with normal kidney function, who were referred for the accurate management of their liver disease. To examine this possibility, we compared the levels of ALT, total proteins, albumin, platelets, transferrin saturation, prothrombin activity (International Normalized Ratio), and abdominal ultrasound between the 36 HD patients infected by HCV referred to kidney transplantation and the 40 RNA HCV-positive patients on regular HD who were not on the list. Our results did not reveal any difference between the two groups.

Conclusion
This study showed that HCV-infected patients receiving HD treatment had lower ALT levels and viral loads, and their liver biopsies showed a lower degree of inflammatory activity and less fibrosis compared with patients with normal renal function. Less inflammatory activity in serum and liver tissue indicates that HD and uremia may have a protective role against progression of HCV-associated liver disease, and further studies should be conducted to identify the factors in uremic patients that affect the progression of liver disease.

Acknowledgments
This work was supported by Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF).

Disclosures
There are no conflicts of interests associated with the publication of this paper.

References