Absence of HCV viraemia in anti-HCV-negative haemodialysis patients

George N. Dalekos1,2, Dimitra S. Boumba1, Costas Katopodis3, Eleftheria Zervou1,2, George Sferopoulos3, Moses Elisaf1,3, Epameinondas V. Tsianos1 and Kostas C. Siamopoulos3

Divisions of 1Internal Medicine and 3Nephrology, Department of Internal Medicine, Medical School, University of Ioannina and 2Blood Bank, University Hospital of Ioannina, 451 10 Ioannina, Greece

Abstract

Background. Immunologic alterations have been reported in chronic haemodialysis (HD) patients. Some HD patients may have, therefore, an inability to produce detectable amounts of serum antibodies to hepatitis C virus (anti-HCV). Previous studies have shown the presence of HCV viraemia in anti-HCV-negative HD patients (ranging from 1 to 15%). However, the universal epidemiologic impact of these cases remains uncertain since there are conflicting results. In this context, we conducted a study in an attempt to investigate the presence of HCV viraemia among anti-HCV-negative HD patients in a well-defined geographic area of the northwestern part of Greece.

Methods. During a 6 month period, 81 anti-HCV-negative HD patients were tested twice for the presence of HCV RNA, using the reverse transcriptase polymerase chain reaction (RT-PCR) combined with a DNA enzyme immunoassay (DEIA). At the same time, periodic testing for anti-HCV by two commercially available third generation assays was done. In addition, 15 anti-HCV-positive HD patients and 20 non-HD patients with well established chronic HCV infection used as internal controls were tested for the presence of HCV RNA and anti-HCV.

Results. None of the anti-HCV-negative HD patients were shown to be viraemic by the combined RT-PCR and DEIA method. During the same time period, all remained anti-HCV negative by the third generation assays. By contrast, all the patients with known HCV infection were positive by the two enzyme immunoassays, whereas 13 anti-HCV-positive HD patients (86.7%) and 18 non-HD patients (90%) were viraemic by RT-PCR.

Conclusions. This study demonstrated that routine HCV RNA testing in anti-HCV-negative HD patients appears not to be necessary particularly when third generation assays are used for the detection of anti-HCV.

Key words: chronic haemodialysis; HCV RNA; hepatitis C virus; viraemia

Introduction

Patients on haemodialysis (HD) are considered to be a high risk group for contracting hepatitis C virus (HCV) infection [1–3]. The prevalence of antibodies to HCV (anti-HCV) in HD patients ranges worldwide from 1% in the UK to 62% in Portugal [1–3]. This variability appears to be dependent on the particular country, the strategy of precautions in various dialysis centres, the presence of other viral markers and the use of first or second generation assays for the detection of anti-HCV. In our region, where epidemiologic studies can be done with good accuracy [4–6], we have already reported a prevalence rate of 12–17% [7,8].

However, since immunological alterations are associated with chronic HD, the possibility of an inability to produce detectable amounts of serum anti-HCV [9] in the same way as is observed in transplant recipients [10] seems rational. In this context, Fernandez et al. [11] in Argentina recently showed that ~13% of anti-HCV-negative HD patients were viraemic by polymerase chain reaction (PCR) testing. The latter study confirmed previous observations, although rates vary considerably, ranging from 1 to 15% [12–14]. However, the universal epidemiologic impact of these cases in HD units remains uncertain since other authors failed to reveal similar findings [15].

This study was conducted in an attempt to address these intriguing findings by investigating the presence of HCV RNA in a cohort of anti-HCV-negative HD patients who reside in a well-characterized geographic area in the Northwestern part of Greece [4–6].

Subjects and methods

Eighty one chronic HD patients (54 male, 27 female, age range 17–76 years, median age 61 years) were investigated for the presence of HCV RNA. Among them, 20 patients started HD before 1991 (the year of obligatory examination
for anti-HCV in blood banks). The clinical and demographic characteristics of the patients are shown in Table 1. All of them were repeatedly negative by two commercially available third generation enzyme immunoassays for at least 1 year before the beginning of the study (Murex Diagnostics Ltd, Central Road Temple Hill, UK, and Abbott Laboratories, Wiesbaden Germany). These immunoassays utilize microplates coated with a combination of HCV antigens from the putative core (structural), protease/helicase ([NS3, non-structural], and [NS4 non-structural]) and replicase (NS5, non-structural) regions of the HCV. For the detection of HCV RNA, a combination of two well-established techniques was also available. The latter included the reverse transcription nested PCR (RT-PCR) and a DNA enzyme immunoassay (DEIA, GEN-ETI-K, Sorin Biomedica, Saluggia, Italy) as described previously [16]. The lower detection limit by this assay is between 10 and 10^3 RNA copies present in the initial sample used for reverse transcription. The patients were evaluated twice for the detection of HCV RNA with a time interval of 6 months. At the same time, examination for anti-HCV antibodies every 2 months was done in serial serum samples of the patients. In addition, 15 anti-HCV-positive HD patients (10 male, five female, age range 20–70 years, median age 55 years, duration of HD (mean ± SD), 81.2 ± 32.5 months) and 20 non-HD patients (10 male, 10 female, age range 20–65 years, median age 58 years) with well-established chronic HCV infection based on clinical, laboratory and histological assessment were used as internal controls in order to evaluate the accuracy of the assays used in this study.

## Results

All HD patients remained anti-HCV negative by the third generation enzyme immunoassays during the 6 months period of the study. None of them was shown to be viraemic by the highly specific combined assay (RT-PCR and DEIA). By contrast, all the patients with known HCV infection tested positive for anti-HCV by the third generation enzyme immunoassays, whereas 13 anti-HCV-positive HD patients (86.7%) and 18 non-HD patients (90%) were also viraemic by the RT-PCR.

### Table 1. The clinical and demographic characteristics of 81 anti-HCV negative chronic haemodialysis patients

<table>
<thead>
<tr>
<th>Starting time</th>
<th>Before 1991</th>
<th>After 1991</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (M/F)</td>
<td>20 (10/10)</td>
<td>61 (44/17)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.2 ± 17.9</td>
<td>56.7 ± 13.2</td>
</tr>
<tr>
<td>Duration (months)</td>
<td>107.2 ± 39.1</td>
<td>7.9 ± 18.8</td>
</tr>
<tr>
<td>History of transfusion (%)</td>
<td>65</td>
<td>42.6</td>
</tr>
<tr>
<td>Number of blood units transfused</td>
<td>10.2 ± 14.3</td>
<td>3.9 ± 1.9</td>
</tr>
<tr>
<td>Erythropoietin administration (%)</td>
<td>65</td>
<td>83.6</td>
</tr>
<tr>
<td>Duration of continuous erythropoietin administration (months)</td>
<td>72 ± 12.9</td>
<td>35.9 ± 9.9</td>
</tr>
<tr>
<td>History of increased AST or ALT in the past year (%)</td>
<td>15</td>
<td>18</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD.

AST = aspartate aminotransferase; ALT = alanine aminotransferase.

## Discussion

Contrary to what has been observed by others [11–14], this study failed to confirm the presence of viraemia in anti-HCV-negative HD patients. It is interesting to notify that the absence of HCV viraemia in this study was observed even among the anti-HCV-negative HD patients who started HD before 1991 (Table 1). The previous studies, however, underscored the fact that the available serodiagnostic tests may underestimate the prevalence of HCV in HD patients. As possible explanations, the prolonged viraemia before seroconversion to anti-HCV in HD patients [17] and their inability to produce antibodies towards different HCV antigens, as has been shown by Lok et al. [9], probably due to their immunosuppression, have already been discussed.

The discrepancy between our findings and the previous studies [11–14] may be due to the serological tests used for the detection of anti-HCV. In all of the previous studies, first or second generation enzyme immunoassays were used for the detection of anti-HCV. By contrast, in this study, two commercially available third generation assays were used. The latter assays incorporate (in addition to the antigens of the previous assays) antigen encoded by the NS5 region of the HCV RNA, and contain a biochemically modified C33c recombinant protein of the HCV genome. It is believed that these enzyme immunoassays, in general, are more sensitive and specific than the previous ones [3,18]. Another possible explanation may be genetic or other factors reflecting the known differences in our population in general [4–6,19,20]. Whatever the causes, this study provides evidence that the periodic determinations of anti-HCV remain mandatory and that routine HCV RNA detection in anti-HCV-negative HD patients appears rather unnecessary. The duration of our study (6 months) could be considered as short to reveal the absence of HCV viraemia. However, taking into account our findings, as well as the increased cost and the technical difficulty of carrying out PCR analysis, we cannot suggest that this scientific tool should be an additional screening test for the possible detection of viraemia in anti-HCV-negative HD patients. In our opinion, the monitoring of aminotransferase, even in low titres, every month as suggested by the guidelines of the Centres for Disease Control and Prevention to control HCV infection in HD centres [21], the use of third generation enzyme immunoassays for serum anti-HCV detection, the transfusional prevention of contamination in general such as increased use of erythropoietin, the presence of strict criteria for the collection of blood donors by blood banks and the careful serological investigation for anti-HCV, as well as universal measures to avoid viral transmission, appear to be the only reliable schedules in order to reduce the high prevalence of HCV in HD patients.

Acknowledgements. The authors wish to thank Miss Aleka Papageorgiou for excellent secretarial assistance.
References


Received for publication: 13.11.97
Accepted in revised form: 25.2.98