Hepatitis virus infection in haemodialysis patients from Moldavia

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Abstract

Background. Although the epidemiology of hepatitis B (HBV) and C (HCV) now seems well established for Western European countries, in Central and Eastern Europe <50% of all dialysis centres routinely test for hepatitis C antibodies since testing is not available or is not applied to all patients. This study describes the prevalence, risk factors and clinical significance of HBV and HCV infection for the haemodialysis population of the North Eastern region of Romania, Moldavia.

Methods. The presence of HBV antigens was determined with an ELISA kit (Wellcome, Abbot) and HCV antibodies with the ELISA-3 Ortho-HCV, third generation test. The following individual data were collected: gender, age, duration of dialysis, rural/urban domicile, actual and previous HBV status, actual HCV status, known acute, clinically evident hepatitis episodes in the last 3 years, monthly alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) levels, complete biochemical hepatic assessment at the time of the study, transfusions for the past 3 years and family history.

Results. HBV and HCV prevalences were 17% (stable over the last 3 years) and 75%, respectively; co-infection was seen in 10% of the subjects. Hospitalization (noso-comial infection) for HBV, blood transfusions and duration on dialysis for HCV, emerged as the main risk factors for hepatitis infection. Socio-economic conditions appear to be equally important for HCV infection, since the prevalence was significantly higher among patients from rural, underdeveloped areas than urban areas (80.8 vs 60.3%), and infection was already present in a large proportion of patients (47%) before starting dialysis, without being related to previous disease duration or blood transfusions. HBV and/or HCV was not associated with a worse clinical or biochemical profile at the time of the study. However, infected patients had significantly more previous cytolytic episodes, with higher, transient increases in ALAT and ASAT levels.

Conclusions. HCV infection is endemic among dialysis centres in Moldavia. Apart from previously well-known risk factors for hepatitis infection, our study demonstrates the negative impact of socio-economic underdevelopment. Simple measures such as enforced general asepsia rules, careful disinfection and equipment sterilization, routine testing of patients from economically disadvantaged areas and monthly, serial determination of hepatic enzymes should be the common practice in dialysis centres in Romania.

Key words: epidemiology; haemodialysis; hepatitis B and C prevalence; risk factors

Introduction

The epidemiology of hepatitis B and C now seems well established for countries in Western Europe [1–9]; in Central and Eastern Europe, still <50% of dialysis centres routinely test for hepatitis C antibodies, and testing is not available or applied to all patients [10].

Moldavia is one of the three historical Romanian regions, with six counties, a surface area of 46,070 km² (similar to Belgium, 20% of Romania) and a population of 4 million (17% of Romania). Due to the country’s general dialysis conditions [11] and the region’s economic conditions (mean personal income per month ~100 SUS), only three dialysis centres (‘C. I. Parhon’ University Hospital, Iasi, Suceava County Hospital and Bacau County Hospital) are available for end-stage renal disease (ESRD) patients (10 renal replacement therapy (RRT) patients per million population in 1991, 26 RRT patients per million population in 1995, [11]) although the incidence and prevalence of renal disease and chronic renal failure (CRF) are similar to EDTA figures [11]. Our study describes the hepatitis B and C status for the entire Moldavian haemodialysis population, on January 1, 1997, discussing risk factors for infection and transmission, as well as the clinical and biochemical impact of being hepatitis positive. The analysis benefits from the fact that general and especially the ESRD population
movement in and out the Moldavian region is negligible, and renal transplantation absent.

Subjects and methods

All haemodialysis patients treated for at least 3 months and haemodialysis staff members from the Moldavian region ('C. I. Parhon' University Hospital, Iasi, Suceava County Hospital and Bacau County Hospital) were tested for hepatitis B and C. The 169 patients enrolled in the study were treated with standard 5 h twice weekly or 5 h thrice weekly dialysis sessions, bicarbonate or acetate buffer (40%/60%) and cuprophane or polysulfone filters (61.5%/38.5%) which were not re-used. In the Iasi and Bacau centres, concentrate was prepared in-house (Fresenius automatic mixing systems), but distribution was individual. Standard heparin (multi-dose ampoules) was used for anticoagulation. In none of the three dialysis centres there was a special area dedicated for previously hepatitis-positive patients.

The following individual data were collected: gender, age, CRF aetiology, duration of dialysis, rural/urban domicile, actual and previous hepatitis B status, actual hepatitis C status, known acute, clinically evident hepatitis episodes in the last 3 years, monthly alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) levels, complete biochemical hepatic assessment at the time of the study and transfusions for the past 3 years. Family (husband, spouse) history regarding hepatitis status, surgery and transfusions was also taken.

HBV antigens were detected with an enzyme-linked immunoassay (ELISA) kit (Wellcome, Abbot) and HCV antibodies were also determined with an enzyme immunoassay method (ELISA-3, Ortho HCV), third generation. We chose the ELISA-3 test because this system uses three antigens (c22–3, c200 and NS5) originating from four regions of the viral genome (core, NS3, NS4 and NS5), and because of its superior performance compared with second generation tests.

A CSTAT programme (Oxford Statistics) was used for the statistical analysis. A $\chi^2$ test with Yates correction, unpaired t-test and Wilcoxon test were applied to the data obtained. All differences were considered significant when $P<0.05$.

Results

A total of 169 patients (100 males/69 females, urban areas/rural areas = 92/77) were studied, mean age = 41.3±11.6, range = 20–63 years, with a mean dialysis duration of 25.8 months, range = 3–75 months. HBV antigens were found in 29/169 patients, i.e. a prevalence of hepatitis B of 17%. Hepatitis B prevalence for the previous 3 years is depicted in Figure 1. HCV antibodies were found in 111/148 patients (75%). The relative distribution of hepatitis B and C is presented in Table 1.

A comparison between hepatitis B-positive and negative patients is presented in Table 2. No difference was seen in CRF aetiology. Also, no difference was seen in hepatitis B prevalence among different age groups. From Table 2, only dialysis during the last shift of the day (third) is associated with a greater prevalence of hepatitis B.

A comparison between hepatitis C-positive and negative patients is presented in Table 3. No difference was seen in CRF aetiology. Again, no difference was seen in hepatitis C prevalence among different age groups. From Table 3, a longer duration of dialysis, together with a larger number of blood transfusions, but not the position in the daily programme, are related to a greater prevalence of hepatitis C.

The main possible risk factors for four distinctive categories of patients—positive for markers of hepatitis B and C, positive for C and negative for B, positive for B and negative for C, and negative for both viruses—are compared in Figure 2. All other risk factors analysed in Tables 2 and 3 are essentially identical between the four groups.

No difference was seen in the prevalence of hepatitis B or C among the three centres included in the study; moreover, in our centre (Iasi University Hospital ‘C. I. Parhon’), there was no difference in prevalence among the four main dialysis wards (each isolated one from another). All staff members were hepatitis B or C negative.

Finally, looking for factors not related directly to
Table 1. Prevalence of hepatitis B and C for the Moldavian haemodialysis population (n=169)

<table>
<thead>
<tr>
<th>Hepatitis C status</th>
<th>Hepatitis B positive</th>
<th>Hepatitis B negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>anti-HBC+ 17 (10%)</td>
<td>anti-HBC+ 94 (55.6%)</td>
</tr>
<tr>
<td></td>
<td>anti-HBC− 6 (3.5%)</td>
<td>anti-HBC− 31 (18.4%)</td>
</tr>
<tr>
<td></td>
<td>ND 6 (3.5%)</td>
<td>ND 15 (9%)</td>
</tr>
</tbody>
</table>

ND = not determined.

Table 2. Risk factors for hepatitis B infection: comparison between B-positive and -negative patients

<table>
<thead>
<tr>
<th></th>
<th>Hepatitis B positive</th>
<th>Hepatitis B negative</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42.1 ± 10.3</td>
<td>41.0 ± 12.8</td>
<td>NS</td>
</tr>
<tr>
<td>Male/female</td>
<td>19/10</td>
<td>81/59</td>
<td>NS</td>
</tr>
<tr>
<td>Urban/rural areas</td>
<td>17/12</td>
<td>75/65</td>
<td>NS</td>
</tr>
<tr>
<td>Months on dialysis</td>
<td>30.96 ± 20.9</td>
<td>30.6 ± 23.1</td>
<td>NS</td>
</tr>
<tr>
<td>No. of transfusions</td>
<td>18.7 ± 18.9</td>
<td>17.9 ± 16.2</td>
<td>NS</td>
</tr>
<tr>
<td>Polysulfone/cellulose</td>
<td>19/10</td>
<td>85/55</td>
<td>NS</td>
</tr>
<tr>
<td>Kuf&lt;6/Kuf&gt;9</td>
<td>9/20</td>
<td>50/90</td>
<td>NS</td>
</tr>
<tr>
<td>I/II/III HD shift</td>
<td>6/9/14</td>
<td>64/47/29</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>(20.6%/31.0%/48.3%)</td>
<td>(45.7%/33.6%/20.7%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Risk factors for hepatitis C infection: comparison between C-positive and -negative patients

<table>
<thead>
<tr>
<th></th>
<th>Hepatitis C positive</th>
<th>Hepatitis C negative</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42.1 ± 11.7</td>
<td>39.9 ± 15.2</td>
<td>NS</td>
</tr>
<tr>
<td>Male/female</td>
<td>64/47</td>
<td>25/12</td>
<td>NS</td>
</tr>
<tr>
<td>Urban/rural areas</td>
<td>52/59</td>
<td>23/14</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Months on dialysis</td>
<td>38.21 ± 22.94</td>
<td>16.6 ± 11.02</td>
<td>0.001</td>
</tr>
<tr>
<td>No. of transfusions</td>
<td>20.8 ± 18.4</td>
<td>11.2 ± 5.5</td>
<td>0.001</td>
</tr>
<tr>
<td>HB+/HB−</td>
<td>17/94</td>
<td>6/31</td>
<td>NS</td>
</tr>
<tr>
<td>Polysulfone/cellulose</td>
<td>72/39</td>
<td>20/17</td>
<td>NS</td>
</tr>
<tr>
<td>Kuf&lt;6/Kuf&gt;9</td>
<td>78/33</td>
<td>24/13</td>
<td>NS</td>
</tr>
<tr>
<td>I/II/III HD shift</td>
<td>43/38/30</td>
<td>16/15/6</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(38.7%/34.2%/27.0%)</td>
<td>(43.2%/40.6%/16.2%)</td>
<td>0.056</td>
</tr>
</tbody>
</table>

Fig. 2. Main risk factors for hepatitis infection: comparison between B and C co-infected patients, C infected, B infected and non-infected patients (*P < 0.05).
the dialysis procedure, we analysed hepatitis prevalence at the beginning of the replacement therapy. Thirty four new CRF patients who started dialysis in 1996 were tested for markers of hepatitis B and C. Hepatitis B antigens were absent in all patients, but anti-HCV was detected in 15/17 (47%) before starting HD. No relation of the number of previous blood transfusions to the known duration of CRF was found.

Retrospective analysis identified only 12 episodes which could be classified as acute hepatitis, with asthenia, jaundice and hepatalgias being present in all these cases. Four of the acute episodes were confirmed as having hepatitis B seroconversion, while the others were C positive. Hepatic function, at the time of the study, was similar between the negative, non-infected and the positive, infected subgroups. By analysis of the peak cytolytic level/year and the number of episodes exceeding the upper limits of ALAT and ASAT, a significantly higher proportion of acute hepatic events were recorded in the infected subgroups (data presented in Table 4). All the 12 clinical acute episodes were associated with an acute, important elevation of hepatic enzymes.

Discussion

Our study reports on the prevalence and risk factors of hepatitis B and C in the haemodialysis population of a large Romanian province—Moldavia.

Hepatitis B prevalence varies among studies and geographical areas. It has been reported to be as low as 5–7.2% [3,5] to >80% when antibodies against HBV are examined. Active hepatitis B (HBV-antigen positive) was present in 17% of our patients (unchanged over the last 3 years), similar to the majority of reports in the literature [4,15–19], and almost comparable with that reported for the same area in a high-risk population (dystrophic children from nursing homes, n = 2355): 21.7% [36].

Hepatitis C prevalence in the largest studies in Western Europe determined with second generation ELISA assays ranges between 7% [9] and 23–24% [4,5]. However, in countries close to and similar to Romania, or in developing regions, this figure is significantly higher: 41% in Poland [13], 49% in Turkey [21] and 68% in Saudi Arabia [23]. Thus, the hepatitis C prevalence reported by us is one of the greatest, seen only in a few units from large multicentre studies [4,21,23]. This magnitude was expected based on the socioeconomic conditions in our country. Indeed, a high prevalence was also found in Bucharest’s ‘Carol Davila’ Hospital dialysis centre: 80.6% [26]. Also, hepatitis C prevalence among volunteer blood donors from Moldavia (n = 616 [26]) is 9.4% (when tested with ELISA II) and 4.6% (when tested with RIBA III), i.e. at least seven times higher than in France (0.6%). Finally, third generation assays (used in our study) can detect more positive patients than first and second generation tests [12] used in the majority of the previous studies.

The striking difference in prevalence seen by us between the two viruses (with similar molecular size and transmission routes in the general and the haemodialysis populations [16]) has also been described by others [5,13,14,20,25]. Co-infection with the B and C viruses (10%) was rare in our population compared with much higher rates reported for developed and non-developed countries 41% [22], 54.8% [17], 76% [24], 86.6% [16] and 92.3% [13].

Risk factors for hepatitis infection

From our study, the only risk factor for hepatitis B was dialysis during the last (third) shift of the day. Haemodialysis machine sterilization procedures are similar between the three shifts, but complete disinfection of all surfaces is usually undertaken only at the end of the day, suggesting a role for nosocomial, horizontal transmission. The increasing HBV antigen prevalence found for the first–third shifts might suggest transmission through the dialysis monitor, which has been demonstrated to be possible [27–29], especially for machines with an ultrafiltration control device [30]. In fact, there was no difference between the different dialysis areas (completely separate rooms and even different floors) or centres, supporting the vertical transmission hypothesis. As shown by our study, the type of membrane (polysulfone vs cellulose and low-flux vs larger pores) did not significantly influence virus transmission. Transmission may have occurred in our centres by sharing of multi-dose heparin ampoules (as shown for HCV by Gilli et al. [30]), usually opened in the morning and used throughout the whole day. We changed this procedure after analysing the results of the present study.

Our study confirms previous data [5,7,13,16,21–23,31–35] demonstrating that blood products and duration on haemodialysis emerge as the main risk factors for hepatitis C. These results are not surprising

### Table 4. Distribution of cytolytic episodes and mean highest/year ALAT value as a function of hepatitis (B + vs B −, C + vs C −) status

<table>
<thead>
<tr>
<th></th>
<th>Hepatitis B +</th>
<th>Hepatitis B −</th>
<th>P</th>
<th>Hepatitis C +</th>
<th>Hepatitis C −</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak ALAT 1994 (UI)</td>
<td>72.7 ± 37.03</td>
<td>31.5 ± 32.6</td>
<td>0.001</td>
<td>25.3 ± 18.5</td>
<td>21.4 ± 17.9</td>
<td>NS</td>
</tr>
<tr>
<td>Peak ALAT 1995 (UI)</td>
<td>42.9 ± 40.6</td>
<td>23.2 ± 25.5</td>
<td>0.05</td>
<td>87.7 ± 43.6</td>
<td>36.1 ± 30.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Peak ALAT 1996 (UI)</td>
<td>30.7 ± 22.4</td>
<td>25.8 ± 21.6</td>
<td>NS</td>
<td>44.5 ± 50.2</td>
<td>23.6 ± 14.7</td>
<td>0.05</td>
</tr>
<tr>
<td>No. of cytolytic episodes</td>
<td>26</td>
<td>98</td>
<td></td>
<td>124</td>
<td>9</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The peak cytolytic level/year was calculated as a mean of all single highest/year patient values.
since blood and blood products were not tested routinely for HCV in Romania until 1996, and erythropoetin treatment was available in <10% of our patients.

Other potential risk factors reported by some authors, such as gender [5,23], age [14,32] and hepatitis B or HIV infection [5,24], were not found relevant in our population. Location of dialysis [18,37,38] does not appear to be important in our study since the prevalence was similar between completely separate dialysis areas with completely separate movement of patients. Staff members were not carriers due to a strict vaccination policy and routine testing excluding infected personnel from working in the dialysis centre.

Two other points are relevant for the high prevalence found by us. First, similarly to Gilli et al. [30], a large proportion of our patients may have already been infected, since 47% of the 1996 cohort was HCV positive pre-haemodialysis. Second, the Moldavian region is economically disadvantaged, with half of our patients coming from rural areas with poor sanitation conditions. Socio-economic factors have been shown to be important by Huraib et al. [23] and by the north–south gradient seen in Europe (7% prevalence in the UK [9], 8% in Denmark [7] vs 23% in Italy [4], 24% in the south of France [5]). Moreover, as discussed previously, the hepatitis C prevalence in the normal population of the region is seven times higher than that reported in Western Europe [26], suggesting the importance of transmission in the community [39].

Clinical and biochemical significance

Although infected subjects had more cytolitic episodes in the past (Table 4), probably related to seroconversion, as previously demonstrated in a prospective study by Jadoul et al. [6], their actual clinical and biochemical hepatic profile was similar to the non-infected population, as found by other authors [24,40–43]. Thus, histology is mandatory to define hepatic disease, but fluctuations in serial ASAT and ALAT levels are an indication for detailed viral studies.

Conclusions

Hepatitis C infection but not hepatitis B is very frequent among chronic haemodialysis patients from Moldavia. Blood transfusions and duration on dialysis appear as the main risk factors for hepatitis C, but nosocomial infection represents an equally important source for both type of viruses. Thus, strict adherence to universal hygiene precautions is mandatory in each dialysis unit. Serial, monthly determinations of ALAT and ASAT is a useful follow-up test for seroconversion screening in dialysis patients, but serological confirmation is necessary. Histopathological studies of subgroups at higher risk is warranted, as clinical and biochemical markers for active disease are not sensitive and reliable.

References