Nosocomial transmission of hepatitis C virus within a British dialysis centre

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Abstract Patients on renal replacement therapy are recognized as a group at increased risk of infection with hepatitis C virus (HCV). While the risk has been reduced by the use of erythropoietin for treatment of anaemia and the introduction of HCV screening of blood products and potential renal transplant donors, new cases of HCV are still being documented, with patients on hospital haemodialysis appearing to be particularly at risk. The exact mode of transmission of HCV within dialysis units is unclear, although there is evidence to support nosocomial transmission between patients. Third generation HCV antibody testing was performed on all dialysis patients when a new case of HCV was identified within our unit. Stored monthly serum samples were then examined retrospectively to determine when patients became HCV RNA and HCV antibody positive. Viral typing was carried out to identify the HCV strains responsible for transmission. Four new cases of HCV infection are described within a single dialysis shift. Viral typing identified two distinct strains of HCV as being responsible for these infections, both of which had previously been identified in dialysis patients within the unit known to have HCV infection. This information, taken in conjunction with knowledge of the location of each patient for dialysis, suggests two separate episodes of nosocomial transmission of HCV between haemodialysis patients. While evidence of nosocomial transmission of HCV is accumulating, with modern dialytic procedures evidence of transmission through the dialysis machine or equipment used for dialysis is lacking. This stresses the importance of strict applications of universal precautions as the key to prevention of further transmission of HCV infection. This information is obviously applicable not only to dialysis units but all units that may potentially come in contact with HCV patients.

Key words: haemodialysis; hepatitis C; nosocomial transmission; universal precautions

Introduction

For almost two decades haemodialysis patients have been recognised as an at-risk group for developing non-A, non-B (NANB) hepatitis [1]. Despite significant advances in virology, including the identification of hepatitis C as the principal cause of NANB hepatitis [2], our knowledge on the mode(s) of transmission of this virus and its health implications in the haemodialysis population remain incomplete.

In the dialysis population prevalence of HCV varies widely between countries [3–5] and also within the same country [6,7]. These variations seem not simply to reflect local prevalence of HCV but rather suggest that some aspect(s) of the dialytic process may expose patients to an increased risk of developing HCV. While evidence of nosocomial transmission of HCV is accumulating, with modern dialytic procedures evidence of transmission through the dialysis machine or equipment used for dialysis is lacking. This stresses the importance of strict applications of universal precautions as the key to prevention of further transmission of HCV infection. This information is obviously applicable not only to dialysis units but all units that may potentially come in contact with HCV patients.

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strains of HCV being identified suggesting two separate episodes of nosocomial transmission between patients, the first such episodes to be recorded in the UK, lending further credence to the theory that it is environmental contamination that is responsible for HCV spread within dialysis units.

**Subjects and methods**

**Dialysis conditions**

The unit concerned provides for 96 hospital haemodialysis patients on a thrice weekly basis with two dialysis shifts daily. Dialysis machines are not moved between stations and, wherever possible, patients occupy the same dialysis station. During the time of unknown HCV transmission within the unit two specified machines were used to dialyse all known HCV-positive patients and no patients with hepatitis B were dialysed within the unit. Dialysis was carried out using Fresenius 4008 machines and bicarbonate dialysate (flow rate 500 ml/min) in all patients. Between dialysis sessions machine sterilization was carried out according to manufacturer recommendations using Citrosteril at 3% concentration at 60°C for 5 min. Dialysers used were either Cuprophan (AM-50 and AM-65, Asahi) or polysulfone (F8, Fresenius). Disposable equipment was used for dialysis and dialysers were not reused. Single use heparin vials were used to administer bolus and continuous heparin infusions.

**Virological monitoring**

Prior to November 1994 the policy for virological surveillance within our haemodialysis unit involved sending serum specimens for routine virological testing once per month for hepatitis B virus (HBsAg) and once every 6 months for antibody against hepatitis C virus (HCV) on all patients. Prior to starting haemodialysis HBsAg and anti-HCV status was known on all patients. Anti-HCV testing was not available at that time as an emergency test, although a result was available during the next working day. No routine test is freely available to exclude acute infection in the potentially infectious patient.

The possibility of HCV infection was first raised when two patients were noted to have deranged liver function tests (patients 3 and 4). Although HCV serology was negative when the liver function first became deranged, repeat analysis showed seroconversion (anti-HCV +), confirming, retrospectively, acute infection with HCV to be the cause of this. Sequential specimens (on all patients) sent monthly for HBsAg testing were then analysed retrospectively to establish when seroconversion had occurred and when HCV RNA was detectable. Two further anti-HCV+ patients were thus identified (patients 1 and 2). It is well recognized that renal dialysis patients have reduced immune function and antibody seroconversion dates may be misleading in trying to pinpoint the exact time of transmission. The first detection of HCV RNA was considered to be the most reliable indicator of recent infection.

All sera were screened for antibody against HCV by Ortho third generation enzyme-linked immunosorbent assay (ELISA). Supplemental testing of ELISA-reactive sera was by recombinant immunoblot assay (RIBA-3, Chiron, Ortho). Selected sera from HCV antibody-positive patients taken before and after seroconversion were tested for HCV RNA by the Roche Amplicor PCR (polymerase chain reaction) test (Roche Laboratories). All assays were carried out according to the manufacturers’ guidelines.

**Phylogenetic analysis**

Typing of all positive HCV strains in the unit was carried out by restriction fragment length polymorphism (RFLP) analysis of viral sequences amplified in the 5' non-coding region (NCR) by the method of McOmish et al. [13]. Phylogenetic analysis of sequences derived from patient samples was carried out by comparison of a 222 base-pair fragment derived from the NS5 region of the HCV genome.

Virus was pelleted from samples by spinning 0.5 ml plasma at 100 000g for 1.5 h at 4°C. RNA was extracted from the pellet by a method using lysis buffer containing proteinase K followed by phenol/chloroform extraction. The RNA obtained was reverse-transcribed and amplified using nested primers from conserved regions of the 5’ NCR and the more variable NS5 region of the HCV genome. Product DNA was with the restriction enzymes HaeIII/RsaI and MvaI/HinfI in two separate reactions as described previously [14]. Further analysis of the different genotypes identified was carried out by direct sequencing of positive samples in the NS5 region of HCV genome. Primers used were 1204 and 1203 for the primary reaction followed by amplification of 1 μl of this product with either 123 and biotinylated 518 or 518 and biotinylated 123 as described in Mellor et al. [15]. Sequence comparisons were then carried out using a control group of epidemiologically unrelated blood donors and patients infected with HCV type 1a (n = 79). These are presented as rooted trees.

**Results**

The four patients who seroconverted had been on haemodialysis for between 12 and 60 months. Two of the patients had previously been transfused (patient 3 had received two units of blood electively in 1989 and patient 4 received two units at presentation in September 1991). No blood products had been given to any of the patients in the preceding year. All four patients had been treated exclusively by haemodialysis and none had previously been transplanted. Patients 1 and 3 had both dialysed outside the unit on holiday but neither had done so in the 8 months preceding seroconversion. Patient 3 was sexually active with a regular heterosexual partner while the other three patients denied being sexually active. The daughter of patient 2 is an intravenous drug misuser, otherwise the patients did not admit to lifestyle factors known to be associated with HCV transmission.

In January 1994 there were nine patients on renal replacement therapy (all on haemodialysis) within the unit who were known to be HCV antibody positive.
Table 1. Viral subtype and modality of renal replacement therapy in patients known to have anti-HCV antibodies in January 1994

<table>
<thead>
<tr>
<th>Genotype (+ subtype)</th>
<th>No. of patients</th>
<th>Hospital haemodialysis</th>
<th>Home haemodialysis</th>
<th>CAPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1b</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

All antibody-positive patients were also known to have HCV RNA detectable by PCR. The HCV genotypes and modality of renal replacement are shown in Table 1.

The four patients who seroconverted occupied the same dialysis shift and were found to have the same HCV subtype (1a). Patients 1 and 3 had identical viral cDNA sequences which was also found in a patient known to be anti-HCV+ who dialysed on another shift (patient Y). Patients 2 and 4 were also shown to have the same viral cDNA sequences which was found in a patient on the same dialysis shift known to be anti-HCV+ (patient X). The phylogenetic tree (Figure 1) shows the virus homology in the various patients. With the exception of patient X there were no other patients on the same shift as patients 1–4 who were known to be HCV positive or who have subsequently been diagnosed as such.

Viral cDNA sequencing suggests two independent viruses involved in transmission of HCV but does not identify the mechanism. To attempt to answer this question we studied the geographical location within the dialysis unit and timing of these patients becoming RNA and anti-HCV positive. Figure 2 shows the location of the dialysis stations for the four patients. In early 1994 patients 1 and 2 made a permanent swap of haemodialysis stations (for social reasons). It is notable that patients sharing the same viral cDNA sequence occupied contiguous stations. Figure 3 shows the changes in transaminases and the dates when patients became RNA positive and HCV antibody positive. Observing the sequence of seroconversion between patients X, 2 and 4, in relation to their
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Key
ALT Alanine aminotransferase
AST Aspartate aminotransferase
PCR HCV RNA detectable by polymerase chain reaction
Anti-HCV Antibodies to HCV detectable by third generation assays

Fig. 3. Transaminase levels, HCV RNA and anti-HCV antibody status in patients 1–4. ALT, alanine aminotransferase; AST, aspartate aminotransferase; PCR, HCV RNA detectable by polymerase chain reaction; Anti-HCV, antibodies to HCV detectable by third generation assays.

geographical location, it would appear likely that sequential transmission occurred from X \rightarrow 2 \rightarrow 4, which is strongly suggestive of nosocomial transmission.

The route of transmission in the remaining patients is less easily explained. As is common in most dialysis units, there is frequently a temporal overlap between morning and afternoon shifts which would, therefore, place patients 1 and Y at contiguous stations for a short period of time. During this time patient Y would have been ending dialysis while patient 1 was beginning. It is notable that patient Y received a cadaveric renal transplant in March 1994 and, therefore, would not have been in the dialysis unit when patient 4 became infected. We would postulate that patient 1 became infected while dialysing in the open ward adjacent to patient Y and then moved to the side room where further nosocomial spread occurred between patients 1 and 3.

Discussion

There are several proposed mechanisms to explain the increased risk of HCV in patients treated by haemodialysis. Undoubtedly, the use of non-screened blood
products and organs from HCV-positive donors was a major contributory factor prior to the introduction of screening for HCV in September 1991 [6,8,16,17]. This mode of transmission is now largely historical, as has been confirmed by the fall in HCV prevalence within units since this date [18]. Another possible vector is transmission via the dialysis apparatus. Transmission has been observed when non-disposable dialysis circuits are used [19], although this is easily prevented by using entirely disposable circuits, as is the practice in the UK. While some authors have documented evidence of HCV crossing the dialysis membrane [20], a detailed study by Hubmann et al. [21] found no evidence of this, suggesting it to be an extremely rare occurrence. The clinical implications of this are likely to be further reduced by the fact that the sterilization procedures employed in our unit are known to be virucidal to hepatitis B and, by implication, hepatitis C [22].

Two independent episodes of HCV transmission occurred in patients in close proximity within the unit. The fact that these patients had never previously shared the same dialysis machine suggests that transmission was nosocomial and may have been facilitated either by close proximity allowing direct person-to-person spread through blood spillage (environmental contamination) and/or failure to strictly implement universal precautions. The evidence presented is inconsistent with the suggestion that inadequately sterilized dialysis machines are the vector for HCV transmission. This view is supported by evidence of nosocomial transmission in other dialysis units [23,24] and in other hospital units [25,26].

In view of this and previous evidence to support nosocomial transmission of HCV, emphasis should be placed on application of universal precautions to prevent members of staff facilitating transmission of HCV between patients and also limit the risk of contracting HCV themselves. Studies documenting a reduction in the incidence of HCV following these measures highlight their effectiveness [27,28] and, indeed, since recognizing this problem and reinforcing universal precautions, no further HCV seroconversions have occurred within the unit. The relative contribution of having devoted machines for HCV-positive patients is difficult to establish, however a study by Gilli et al. [29] observed no cases of seroconversion within their unit when allowing HCV-positive and -negative patients to share the same machines while employing strict universal precautions. As for dialysing HCV patients in separate rooms, from our own experience, while isolating patients X and Y (known HCV) may have reduced the chances of nosocomial spread to patients 1 and 2, the source of HCV in patients 3 and 4 would appear to be from patients who, at that time, had no serological evidence (and in the case of patient 2, no biochemical evidence) of HCV infection. Isolation of all known HCV-positive patients (while neglecting universal precautions) may not have prevented spread to patients 3 and 4. Furthermore, as HCV antibodies do not confer immunity against further infection by the same or different HCV genotype, patients who are HCV positive require the same protection as those who are HCV negative, further emphasizing the importance of universal precautions.

Finally, while the importance of HCV screening of haemodialysis patients is recognized, this study illustrates another important aspect of monitoring of HCV infection by highlighting the limitations of indirect indicators of infection, such as the delay of 4 months before seroconversion in patients 2 and 4 and the lack of a significant elevation in transaminases in patient 2. PCR surveillance is not routinely available and is overly expensive to be considered as the recommended screening test for dialysis patients, although this technique could be useful in identifying HCV infection, if suspected, prior to seroconversion in patients with deranged liver function tests. In practical terms all patients (and staff) should be considered as being at risk of contracting HCV and universal precautions against this should be religiously enforced.

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