Hepatitis C virus infection in Jordanian haemodialysis units: serological diagnosis and genotyping

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The seroprevalence and genotypes of hepatitis C virus (HCV) were studied in 283 patients attending six haemodialysis units in Jordan. In all, 98 (34.6%) patients were anti-HCV-positive by EIA, 92 (93.9%) of whom were also reactive in an immunoblot assay. The prevalence of anti-HCV was correlated with a history of blood transfusion before the introduction of blood donor screening for HCV and with duration of haemodialysis. HCV RNA was detected in 30 (30.6%) of 98 anti-HCV-positive sera. HCV viraemia was not associated with a particular antibody for the six HCV antigens studied by the immunoblot assay, although reactivity to the core antigens was greater in the HCV RNA-positive sera than in negative sera. Two HCV genotypes (1 and 4) were identified for the first time in Jordan by restriction fragment length polymorphism analysis of HCV 5'-NCR. The predominant genotype was HCV 1a (12 of 30). Genotypes 1b and 4 were detected in 10 and 8 patients, respectively. The antibody response to HCV antigens was genotype-dependent, with a wider range of antibody specificities detected in the immunoblot assay in the 12 patients with genotype 1a infection than in the 8 patients with genotype 4. However, there was no significant difference in the prevalence of antibodies to HCV antigens among patients infected with either genotype 1a or 1b. In conclusion, the prevalence of anti-HCV, blood transfusion, duration of dialysis and HCV genotypes suggest possible nosocomial HCV transmission among patients which needs confirmation by phylogenetic analysis of subgenomic HCV regions.

Introduction

Haemodialysis patients have a high risk of acquiring hepatitis C virus (HCV) infection. Transfusion of unscreened blood [1], duration of dialysis [2] and nosocomial transmission within haemodialysis units [3, 4] are implicated as the main transmission routes of HCV in haemodialysis patients. In these patients, HCV infection is usually asymptomatic [5] and can be diagnosed by serological methods and by amplification of HCV RNA by reverse transcriptase-PCR (RT-PCR) [6], which distinguishes between viraemic and non-viraemic HCV patients and also is used for HCV genotyping [7]. HCV isolates have been classified into six major genotypes, many of which contain a number of closely related subtypes [8]. New HCV variants from Vietnam, Jakarta and Indonesia have been described as genotypes 7, 8, 9, 10 and 11 [9–10]. The distribution of HCV genotypes varies in different countries as documented in blood donors, and haemodialysis and chronic hepatitis patients [11–13]. There has been no report on HCV genotypes in Jordan. Therefore, this study used both serological and molecular methods to assess (i) the prevalence of anti-HCV and HCV RNA among haemodialysis patients in different dialysis units in Jordan, (ii) the influence of blood transfusion and dialytic age on HCV infection, (iii) the HCV genotypes and (iv) the correlation between HCV genotypes, viraemia and the antibody response to various HCV antigens.

Materials and methods

Patients

In all, 283 patients from haemodialysis units at six government hospitals were studied, with informed, written consent. Plasma samples were separated from
the whole blood within 1–3 h of vein puncture, divided into small volumes and stored at −70°C.

Information on the age of patients, duration of haemodialysis, number of blood transfusions and renal diagnosis was obtained from patient records and interviews. The renal diagnoses were: chronic renal failure and hypertension (n = 81), chronic glomerulonephritis (46), diabetic nephropathy (42), small kidney (33), failed kidney transplant (12), polycystic kidney (6) and others (63).

Biochemical tests
Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured by spectrophotometry with kits from Menagent (Italy) according to the manufacturer’s instructions.

Serology
All plasma samples were tested by the Innotest HCV Ab IV (Innogenetics, Belgium) enzyme immunosassay (EIA) for the presence of antibodies against proteins derived from four regions of the HCV genome: core, NS3, NS4 and NS5. EIA-positive samples were re-tested by the Inno-EIA HCV Ab III immunoblot system (Innogenetics), according to the manufacturer’s instructions. Each strip incorporates HCV antigens derived from the core (C1, C2), E2, NS3, NS4A, NS4B and NS5 regions.

Reverse transcriptase nested PCR (RT-nested PCR)
RNA was extracted from 200–400 µl of all seropositive plasma samples with the commercially available high pure RNA Kit (Boehringer Mannheim, Germany). To detect contamination, an HCV-negative plasma was extracted with each batch of five RNA extractions. Extracted RNA was reverse transcribed and amplified with nested primers matching the conserved sequences in the 5’ non-coding region (5’-NCR) of HCV RNA [14]. A negative PCR blank with distilled water instead of RNA was included with each set of five reactions. The PCR products (5 µl) were subjected to electrophoresis in agarose (Promega, USA) 3% and the band size was assessed by direct comparison with a 100-bp DNA marker (Promega).

Genotyping analysis
HCV genotypes were determined by restriction fragment length polymorphism (RFLP) analysis of the 5’-NCR. The positive RT-nested PCR products were digested separately by two pairs of restriction endonucleases (HaeIII/RsaI and MvaI/Hinfl; Boehringer Mannheim) for differentiation into genotypes 1–6 [15]. Subtypes 1a/c and 1b were further differentiated by digestion with the restriction endonuclease BstUI (New England Biolabs, USA). Band size was assessed by direct comparison with a 20-bp DNA marker (BioRad, USA).

Statistical analysis
The χ² test was used for statistical analysis, with Fisher’s exact test where any one value was <5; p values <0.05 were considered significant. Correlation coefficients were determined with an Excel programme.

Results
In all, 174 male and 109 female patients aged 9–80 years were enrolled into the study (Table 1). Sera from 98 patients (34.6%) were anti-HCV positive. Seropositivity rates did not differ between male (36.8%) and female (31.2%) patients. The duration of haemodialysis treatment was grouped into, 12, 12–48 and >48 months. Rates of anti-HCV seropositivity were corre-

### Table 1. Characteristics of haemodialysis patients tested by EIA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total (n = 283)</th>
<th>Anti-HCV-positive (n = 98)</th>
<th>Anti-HCV-negative (n = 185)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>174</td>
<td>64 (36.8)</td>
<td>110 (63.2)</td>
</tr>
<tr>
<td>Female</td>
<td>109</td>
<td>34 (31.2)</td>
<td>75 (68.8)</td>
</tr>
<tr>
<td>Duration of haemodialysis (months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>45</td>
<td>3 (6.7)</td>
<td>42 (93.3)</td>
</tr>
<tr>
<td>12–48</td>
<td>135</td>
<td>27 (20)</td>
<td>108 (80)</td>
</tr>
<tr>
<td>&gt;48</td>
<td>103</td>
<td>68 (66)</td>
<td>35 (34)*</td>
</tr>
<tr>
<td>Previous blood transfusion before 1993</td>
<td>191</td>
<td>62 (32.5)</td>
<td>129 (67.5)</td>
</tr>
<tr>
<td>after 1993</td>
<td>16</td>
<td>11 (68.7)</td>
<td>5 (31.3)</td>
</tr>
<tr>
<td>elevated</td>
<td>175</td>
<td>51 (29.2)</td>
<td>124 (70.8)*</td>
</tr>
<tr>
<td>ALT</td>
<td>7</td>
<td>6 (85.7)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>AST</td>
<td>7</td>
<td>5 (71.4)</td>
<td>2 (28.8)</td>
</tr>
</tbody>
</table>

*p <0.001 for <12 months dialysis versus >48 months, and for receipt of blood transfusion before and after 1993.
lated with the duration of haemodialysis ($r^2 = 0.988$).
The prevalence of anti-HCV in those who had under-
gone haemodialysis for $>$48 months was significantly higher than in those who had undergone dialysis for
$<$48 months ($p < 0.001$). In Jordan, anti-HCV screen-
ing was introduced to blood banks in 1993. The prevalence of anti-HCV was significantly higher in
patients who received blood transfusion before 1993 (68.7%) than those who received blood transfusion
after 1993 (29.2%, $p < 0.001$). Only six (6.1%) and five
(5.1%) of anti-HCV positive patients had elevated ALT
and AST levels, respectively.

Variation in the prevalence of anti-HCV was observed
between the haemodialysis units (range 13.6–47.1%) (Table 2).

HCV RNA was detected in 30 (30.6%) of the 98 anti-
HCV-positive patients, with the prevalence of HCV RNA varying from 0% to 41.6% in the six dialysis
units.

In 92 of 98 anti-HCV EIA-positive sera, the result was
confirmed by immunoblot; 29 of these sera were HCV
RNA-positive. Four sera gave indeterminate results on
immunoblotting (one was HCV RNA-positive) and two
were negative (one was HCV RNA-positive).

There were no significant differences in the prevalence
of antibodies to different HCV antigens between HCV
RNA-positive and HCV RNA-negative sera. However, HCV RNA-positive sera were more likely to have high
intensity antibody bands (3+ or 4+) to core antigens
(C1 20 30 versus 10 68, C2 17 30 versus 9 68; $p < 0.001$) than HCV RNA-negative sera.

The predominant HCV genotype was 1a, detected in
40% of HCV RNA-positive patients, whilst genotypes
1b and 4 were found in 33.3% and 26.6% of patients,
respectively. All isolates from patients in Unit 3 were
genotype 1 (Table 2).

Patients infected with genotype 1a were more likely
than those infected with type 4 to have detectable
antibodies in the immunoblot assay to core 1 (12/12
versus 5/8, $p = 0.049$), E2 (10/12 versus 3/8,
$p = 0.06$), NS4 (11/12 versus 2/8, $p = 0.004$) and
NS5 (6/12 versus 0/8, $p = 0.04$) antigens. There were
no other significant differences in antibody distribution
between patients with genotypes 1a, 1b or 4.

Discussion

This is the first study to report on the seroprevalence
and genotyping of HCV in haemodialysis patients in Jordan. The seroprevalence of 34.6% compares with
41.9% in Saudi Arabia [16], 45% in Tunisia [17] and
45% in Syria [18]. HCV RNA was detected in only
30.6% of anti-HCV positive patients (Table 2). Possible
explanations for this low percentage of viraemic patients include (i) false positive serology, (ii) inter-
mittent viraemia, (iii) unusually high rates of clearance
of infection in these dialysis patients or (iv) levels of
viraemia in these patients below the lower limit of
detection of the genome detection assay. The low rates
of strong positive (3+ or 4+) antibody bands in the
immunoblot assay in the HCV RNA-negative sera
support the first hypothesis. However, it has also been
reported that 7–68% of haemodialysis patients have
intermittent viraemia with periods of undetectable HCV
RNA for up to 4 weeks [7, 19–21]. The viral load is
relatively low in this group of patients and long-term
maintenance haemodialysis decreases the HCV RNA
level but does not produce clearance of viraemia
[22–24]. It is not possible to discern for certain which
of the four suggested hypotheses pertains in this group
of patients.

HCV viraemia was not associated with a particular antibody specificity, as also reported elsewhere [25,
26]. In contrast, others have reported that antibodies to
capsid antigen (C22) [27] or NS3 region [28] are more
likely to be correlated with viraemia. However, in the
present study, HCV RNA was associated with stronger
reactivity (3+, 4+) to HCV core (C1, C2) antigens in
the immunoblot assay, as compared with the HCV
RNA-negative sera. The low reactivity (1+) to C1 and
C2 in HCV RNA-negative patients suggests resolved
infection with loss of antibodies. Such a profile has
been reported by other investigators [29].

The ALT and AST values in haemodialysis patients
are reported to be lower than in healthy individuals [30–

\[ Table 2. \text{Prevalence of HCV RNA and HCV genotypes in anti-HCV-positive haemodialysis patients in Jordan} \]

<table>
<thead>
<tr>
<th>Haemodialysis units</th>
<th>Total</th>
<th>Anti-HCV-positive</th>
<th>HCV RNA-positive</th>
<th>HCV genotypes (% of RNA-positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Princess Basma Hospital</td>
<td>65</td>
<td>23 (35.4)</td>
<td>7 (30.4)</td>
<td>2 (28.5) 1 (14.3) 4 (57)</td>
</tr>
<tr>
<td>2. Jarash Government Hospital</td>
<td>22</td>
<td>3 (13.6)</td>
<td>0 (0)</td>
<td>0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>3. Al-Salt Government Hospital</td>
<td>38</td>
<td>12 (31.6)</td>
<td>5 (41.6)</td>
<td>3 (60) 2 (40) 0 (0)</td>
</tr>
<tr>
<td>4. Al-Basheer Hospital</td>
<td>70</td>
<td>22 (31.4)</td>
<td>7 (31.8)</td>
<td>4 (57) 2 (28.5) 1 (14.2)</td>
</tr>
<tr>
<td>5. Al-Nadeem Hospital</td>
<td>21</td>
<td>6 (28.6)</td>
<td>0 (0)</td>
<td>0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>6. Zarqa Government Hospital</td>
<td>68</td>
<td>32 (47.1)</td>
<td>11 (34.4)</td>
<td>3 (27.3) 5 (45.4) 3 (27.3)</td>
</tr>
<tr>
<td>Total</td>
<td>283</td>
<td>98 (34.6)</td>
<td>30 (30.6)</td>
<td>12 (40) 10 (33.3) 8 (26.3)</td>
</tr>
</tbody>
</table>
The prevalence of anti-HCV in Jordanian haemodialysis patients was correlated with history of blood transfusion before the introduction of anti-HCV screening for Jordanian blood banks in 1993, suggesting this as an important route of infection. The seropositivity to HCV also increased significantly with increase in the duration of haemodialysis (Table 1), suggesting possible nosocomial transmission between patients. Numerous studies in recent years have provided molecular evidence for the nosocomial transmission of HCV within haemodialysis units [3, 4]. The greatest risk of transmission of HCV within dialysis units is via dialysis next to an HCV-positive patient [33]. In the present study, the route by which the haemodialysis patients acquired HCV was not determined. All Jordanian haemodialysis units use hypex and formalin to disinfect the haemodialysis machines at the end of each session. Filters and tubes are discarded after each use. Nurses do not regularly wear gloves when dealing with patients. The hands of dialysis personnel have been implicated as a potential mode of facilitating transmission of HCV between haemodialysis patients [34]. Furthermore, variations in the level of hygienic standards may explain the differences in prevalence of anti-HCV positivity among the dialysis units (Table 2). To limit the spread of HCV infection in haemodialysis units, precautionary aseptic measures should be improved. Anti-HCV-positive patients should be considered potentially infectious and should be isolated from the anti-HCV-negative patients – dialysing HCV-positive patients in a separate unit or in a defined sector of a dialysis unit significantly reduces nosocomial HCV infection [33]. Furthermore, education programmes for staff on the risk of transmission of blood-borne viruses should be considered.

HCV genotype 1a is the most prevalent genotype in Jordanian patients (Table 2). This genotype is also predominant in Jordanian blood donors (unpublished data) and in haemodialysis patients of some Middle Eastern countries including Lebanon, Turkey, Cyprus [11] and Syria [18]. In contrast, HCV genotype 4 is the most prevalent genotype in other Middle Eastern countries including Saudi Arabia, Egypt, Yemen and Bahrain [11, 15, 36, 35].

The prevalence of antibodies to different HCV antigens in patients infected with genotype 4 was significantly lower than in patients infected with genotype 1. The difference in this serological response may be due to differences in virus replication capacities or in the immune response to viral antigens, or to the presence of different epitopes in the two genotypes. Genotype dependence of antibody to HCV core, NS3 and NS4 antigens has been reported by others [36, 37].

References


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