SUPERINFECTION WITH DIFFERENT HCV GENOTYPES IN A HEMODIALYSIS PATIENT

ABSTRACT

Hepatitis C vírus (HCV) is a serious health problem worldwide. Hemodialysis patients are at a high risk of HCV infection, although seroconversion is not always observed. We report here a case of superinfection by genotype 3 in an anti-HCV negative, HCV-genotype 1 chronic carrier, with no clinical evidence of hepatitis. All biochemical parameters were normal, including alanine aminotransferase (ALT) levels. Seroconversion to anti-HCV was detected after a six month follow-up, concomitant with an increase of serum ALT levels. Viremia with a different genotype (genotype 3) was demonstrated after amplification of a 187 base pair (bp) fragment of the 5′ non-coding region followed by restriction fragment length polymorphism (RFLP) analysis. We believe this patient had a chronic HCV genotype 1 infection, with no or undetectable immunologic response (anti-HCV negative) and no liver damage (normal ALT levels). When the infection with HCV genotype 3 occurred, a consistent immune response was induced, detected by anti-HCV seroconversion, with liver damage, reflected by the elevation of transaminases and y-GT levels.

INTRODUCTION

Hepatitis C Virus (HCV) was cloned by Choo et al. In 1989, and it was the first virus to be identified by means of DNA recombinant technology. It is a non-enveloped icosahedral virus, with an RNA genome of 9400bp (Choo et al. 1989). Many genotypes have since been identified, with different distributions in the various regions of the planet (Simmonds et al. 1993, McOmish et al. 1994, Davidson et al. 1995, Flamm 2003). For many years, HCV has been known as the main cause of post-transfusional non-A-non-B hepatitis (Reherman et al. 2000). Despite mandatory blood testing for anti-HCV in most countries nowadays, HCV is still the major cause of liver disease throughout the world (Busek & Oliveira 2003). The World Health Organization (WHO) estimates that the global prevalence of HCV infection averages 3%, equivalent to about 170-175 million HCV-infected persons worldwide (Yen et al. 2003, Poynard et al. 2003). Hemodialysis patients are at high risk of infection by HCV due mainly to blood transfusion, partial immunosuppression and frequent parenteral interventions (Olmer et al. 1996, Carneiro et al. 2001, Fabrizi et al. 2003a, Fabrizi et al. 2003b).
HCV-related infection may result in cirrhosis in 10-20% of dialysis patients and it is worsened by transplantation because of the immunosuppressive therapy for prevention of graft rejection (Pol et al. 2002). In the vast majority of hemodialysis units, HCV infection is routinely tested by serology (anti-HCV), although it has already been shown that HCV seronegativity in hemodialysis patients does not exclude the presence of viremia (Carneiro et al. 2001, Neng 2001, Fabrizi et al. 2003a, Fabrizi et al. 2003b, Moreira et al. 2003, Seef & Hoofnagle 2003). The most effective control of HCV infection in hemodialysis units would be the combination of serological and molecular follow-up of patients (Bacon 2004, Seef & Hoofnagle 2003, Kamar et al. 2004, Souza et al. 2003, Moreira et al. 2003). Patients attending the HemoDiaIsis Unit of São Lucas Hospital (HUSLH) – PUCRS (Porto Alegre, Brazil) are monitored monthly with serological and biochemical markers, including anti-HCV and alanine aminotransferase (ALT) levels. In August and September 2001, an HCV molecular survey was carried out at the HUSLH. Among 70 HCV-seronegative patients tested, 4 (5.7%) were positive by RT-nested PCR for HCV. We report here the case of an anti-HCV negative hemodialysis patient who has undergone seroconversion after HCV infection with a different HCV genotype.

MATERIAL AND METHODS

Patient.
A male chronic renal patient, 39 years old, attending the HUSLH/PUCRS for about 10 years, tested seronegative for HCV and positive for HCV genotype 1 in August 2001. In February 2002, a slight increase in alanine amino transaminase (ALT) level was observed, which persisted for three months, increased in the following three months, then began to fall gradually (Table 1). In July 2002, when an important increase on ALT level was observed, the ALT testing was repeated and also aspartate aminotransferase (AST) and gamma glutamyl transferase (γ-GT) levels were tested. In September 2002, an RT-nested PCR for HCV was performed.

Methods.
All serum samples from the study patient were tested for anti-HCV by a third generation ELISA (Ortho) according to manufacturer’s instructions.

Table 1. Relevant data from the study patient follow up.

<table>
<thead>
<tr>
<th>Blood collection date</th>
<th>Anti-HCV</th>
<th>ALT(^1) (U/L)</th>
<th>AST(^1) (U/L)</th>
<th>GammaGT(^1) (U/L)</th>
<th>HCV genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sep 2001</td>
<td>Neg</td>
<td>11</td>
<td>NT</td>
<td>NT</td>
<td>1</td>
</tr>
<tr>
<td>Jan 2002</td>
<td>Neg</td>
<td>8</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Feb 2002</td>
<td>Neg</td>
<td>17</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Mar 2002</td>
<td>Neg</td>
<td>18</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Apr 2002</td>
<td>Neg</td>
<td>16</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>May 2002</td>
<td>Neg</td>
<td>41</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Jun 2002</td>
<td>Neg</td>
<td>1(^\text{st}) 206</td>
<td>134</td>
<td>334</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2(^\text{nd}) 561</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jul 2002</td>
<td>Pos</td>
<td>200</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Aug 2002</td>
<td>Pos</td>
<td>10</td>
<td>NT</td>
<td>NT</td>
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<td>NT</td>
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</tr>
<tr>
<td>Jan 2003</td>
<td>Pos</td>
<td>11</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

\(^1\) U/L: Units per litre; Neg: negative; Pos: positive; NT: not tested
\(^2\) Reference values for male 5-23 U/L
\(^3\) Reference values for male 5-17 U/L
\(^4\) Reference values for male 6-28 U/L
The ALT, AST and a-GT testing were performed using the specific enzymatic UV test for ADVIA Bayer 1650, following manufacturer’s instructions.

Samples for molecular testing consisted of 200μL aliquots of plasma, frozen at most one hour after blood collection. HCV RNA was extracted with Trizol® LS (Invitrogen) according to manufacturer’s instructions, and resuspended in 20μL of DEPC-treated MilliQ water. Reverse transcription (RT) followed by a nested polymerase chain reaction (PCR) for HCV and the restriction fragment length polymorphism (RFLP) for genotyping were performed according to Okamoto et al. (1990), with minor modifications. In brief, the reverse transcription reaction was performed at 42°C and the reaction mix consisted of 200U Superscript® II (Invitrogen), 10pmol reverse primer 1A (5'-GAT GCA CGG TCT ACG AGA CCT C-3') , 10mM DTT, 10U RNasin (Invitrogen), 200mM dNTP mix (Invitrogen), 50mM Tris-HCl (pH 8.3), 75mM KCl, 3mM MgCl₂, and 5μL of extracted RNA. Both nested PCR reaction mixtures contained 20mM Tris-HCl (pH 8.4), 50mM KCl, 2mM MgCl₂, 200mM dNTP mix (Invitrogen), 0.5U Tag DNA polymerase (Cenbiot), 50 pmol of each reverse and forward primers. For the first PCR reaction, 5μL of cDNA and primers 1A (5'-GAT GCA CGG TCT ACG AGA CCT C-3') and 1B (5'-AC TAC TGT CTTCAC GCA GAA-3') were used. For the second PCR, primers were 2A (5'-GCC ACC CAA CAC TAC TCG GCT-3') and 2B (5'-ATG GCG TTA GTA TGA GTG-3'), and 5μL of the amplicon of the first PCR was used as template. Amplification conditions for both PCR amplification rounds included initial denaturation (94°C for 5 minutes) and final extension (72°C for 5 minutes) steps, and cycles (30 for the first and 40 for the second round) of 94°C/1 min, 45°C/1 min, 72°C/1 min. PCR products were electrophoresed on a 2% agarose gel containing 0.5μg/mL of ethidium bromide in TAE buffer and analysed under UV light. All RT and PCR reactions were performed in duplicate. For HCV genotyping, the nested PCR amplicons (187 bp) were double digested with 10U each Rsal (Invitrogen)/HaeIII (Invitrogen) and HinfI (Invitrogen)/BstNI (New England Biolabs). Restriction fragments were electrophoresed on 15% polyacrilamide gel in TBE buffer and restriction patterns were examined under UV light after gel staining with ethidium bromide (McOmish et al. 1994).

RESULTS

The relevant results of ALT, AST, a-GT, anti-HCV and nested RT-PCR tests for the study patient during follow-up are presented in Table 1.

DISCUSSION

A high prevalence of anti-HCV antibodies is reported among chronic hemodialysis patients worldwide (Kamar et al. 2003, Medeiros et al. 2004, Fissel et al. 2004, Jadoul et al. 2004). Studies conducted in different hemodialysis units in Brazil reported seroprevalence rates for HCV ranging from 6% up to 72% (Medeiros et al. 2004, Busek et al. 2002). HCV seronegativity in hemodialysis patients does not exclude the presence of viremia. In fact, in a previous study conducted with patients attending the HUSLH, the seroprevalence for HCV was 11.8% and the prevalence of HCV positive patients was 12.5% (Kupski et al. 2001). There are many reports of patients positive for HCV-RNA who remain seronegative for a period of time. Many factors can contribute to this event, including the patient’s relative immunodepression and frequent hemodialysis sessions (de Lamballerie et al. 1996, Carneiro et al. 2001, Fabrizzi et al. 2003a, Fabrizzi et al. 2003b, Alavian et al. 2003).

Coinfection with different HCV genotypes is difficult to demonstrate, but there are some reports of superinfection or reinfection with different HCV genotypes (Koppe & Adler 1998, Toyoda et al. 1999, Hafion et al. 1999, Kao et al. 2001, Accapezzato et al. 2002). These reports also suggest that there is no effective immune-mediated cross-protection against different HCV genotypes.

In the case of the patient described here, it was not possible to determine the precise moment of the first infection with HCV genotype 1, because he was already positive at the first molecular testing for HCV. But it is clear that this infection was not sufficient to induce a detectable immune response, since the patient remained seronegative and with normal ALT levels for a long period of time. When a new infection occurred, this time with HCV genotype 3, biochemical and immune responses typical of acute hepatitis were observed, with elevation of transaminases and a-GT levels, followed by seroconversion. However no clinical manifestation was observed.
In conclusion, after analysing all the data presented above, we believe the patient had a chronic infection by HCV genotype 1 with normal ALT levels and no or undetectable immune response. When the infection with HCV genotype 3 occurred (superinfection or reinfection), a consistent immune response was induced, detected by anti-HCV seroconversion, with liver damage, reflected by the elevation of transaminases and y-GT levels.

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REFERENCES


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