Routes of infection and clinical outcome of Mexican women reactive to anti-hepatitis C virus antibodies

Erwin Chiquete a,b, Laura V. Sánchez a,b, Arturo Panduro a,b,*

a Department of Molecular Biology in Medicine, Civil Hospital of Guadalajara “Fray Antonio Alcalde”, Hospital 278, Guadalajara, Postal Code 44280, Jalisco, Mexico
b Department of Physiology, University Center of Health Sciences (CUCS), University of Guadalajara, Guadalajara, Jalisco, Mexico

Received 27 January 2006; received in revised form 12 June 2006; accepted 15 June 2006
Available online 1 August 2006

Abstract

Background: Risk factors for the hepatitis C virus (HCV) infection influence both the frequency and the progression of the liver disease. Routes of transmission and severity of the liver damage may differ by gender. We aimed to describe the risk factors for HCV infection and for the severity of the liver disease among women seroreactive to anti-HCV antibodies. Also, we tested the hypothesis that length of infection influences the levels of anti-HCV, in transfusion-associated hepatitis C.

Methods: Eighty-six interferon-naive women, repeatedly seroreactive to anti-HCV antibodies, aged > 20 years, were studied.

Results: Surgeries (80%) and transfusion before 1993 (58%) were the main risk factors (52% cases had both). The main reason for practicing surgery was obstetric/gynecologic (74%). The main indication for transfusion was also obstetric/gynecologic (68%). Fifty-five (64%) women were positive to HCV RNA in serum, of them, coinfection with the hepatitis B virus (HBV) occurred in three (5%) cases, being occult hepatitis B (i.e., positive to HBV DNA, but negative to hepatitis B surface antigen) in two (4%). In multivariate analysis, determining factors of cirrhosis at histologic examination were age and the antecedent of transfusion before 1993. Anti-HCV levels correlated with the elapsed time from transfusion to diagnosis, but not with age.

Conclusion: An obstetric/gynecologic indication was the most frequent reason for both surgery and transfusion. Hepatitis B coinfection had a low prevalence and did not influence the severity of the liver disease, as age and the antecedent of transfusion certainly did. Infection length influenced the levels of anti-HCV antibodies in transfusion-associated hepatitis C.

© 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: Cirrhosis; Hepatitis B; Hepatitis C; Liver; Serology; Test; Transfusion

1. Introduction

In countries in which the use of illicit intravenous drugs is not frequent, the recipients of transfusion before the systematic screening for the hepatitis C virus (HCV) in blood banks represent the largest group of persons with the infection [1–5]. Several risk factors for HCV infection and for the progression of the liver disease may differ by gender [6–10]. Compared with men, in some countries women have transfusion of blood products as the main route of acquisition of HCV [1,9]. Nevertheless, little is known about the indication for blood transfusion in women who underwent this procedure before the screening programs [10] and the relation of this antecedent with the progression of the liver disease.

The severity of the liver fibrosis in HCV infection has been associated with some clinical antecedents, like mode of transmission [6,11–13], length of infection [6,7,13], age, gender, alcohol consumption, body mass index [6,7], hepatitis B virus (HBV) coinfection [14], and other factors. Also, different levels of anti-HCV antibodies are observed among people with distinct clinical outcomes [5,15]; however, the exact significance of this difference is scarcely known.

* Corresponding author. Tel.: +52 33 3614 7743; fax: +52 33 3614 7743.
E-mail address: apanduro@prodigy.net.mx (A. Panduro).
In some populations, women appear to be not only the most affected gender with HCV infection [5,10,16–18], but also a homogeneous group with respect to risk factors and clinical outcome [10,19,20]. We aimed to describe the risk factors associated with HCV infection and events related to blood transfusion prior to the screening program for HCV in Mexican blood banks, in women currently seroreactive to anti-HCV antibodies. Also, we sought to test the hypothesis that the mode of transmission and the length of infection influence the clinical outcome and anti-HCV antibodies levels.

2. Methods

This study was performed from March 2002 to June 2004 at the Department of Molecular Biology in Medicine, Hospital Civil de Guadalajara “Fray Antonio Alcalde”. The internal Committee of Ethics of our hospital approved the present study. Informed consent was obtained from all patients.

2.1. Study population

Ambulatory patients were referred after our request from blood bank and departments of infectology, gastroenterology, general internal medicine and hematology to confirm HCV infection after at least two screening tests for anti-HCV antibodies yielding reactive results. None of the patients were referred to our research facility by the departments of obstetrics or gynecology. All patients had known their screening test status before arrival to our department. Consecutive women older than 20 years and never treated for HCV infection were eligible for the study. In cases with transfusion of blood products, only those occurring before 1993 were considered at significant risk for HCV infection, since it was the year in which blood bank screening for anti-HCV antibodies was introduced in Mexico [21,22]. In women who received blood products in more than one occasion, the earliest transfusion was considered as the putative source of the infection. Decompensated cirrhosis was considered to occur in a patient if jaundice, ascitis or collateral superficial veins were present. Also, the antecedent of upper gastrointestinal bleeding, hepatic encephalopathy, tense ascitis, and spontaneous peritonitis was considered as being part of the liver failure syndrome. Patients were excluded if a new assay for anti-HCV performed in our laboratory tested negative or if complete history and clinical data were not available.

2.2. Detection of anti-HCV antibodies

A new determination of anti-HCV was practiced in our laboratory for all patients, in order to confirm the screening tests performed prior to the arrival to our department and to assess the quantity of anti-HCV antibodies. A semi-quantitative automated third-generation microparticle enzyme immunoassay (MEIA, IMx HCV Version 3.0 Abbott Diagnostics, Chicago, IL, USA) was used in all sera samples, first stored at −70°C. Immunoassay signal strength of the sample to cut-off rate (S/CO) is the numeric value of the assay, which is directly proportional to the quantity of antibodies against HCV [15,23–26]. S/CO ratio > 1 is considered a reactive (i.e., positive) result, according to the manufacturer.

2.3. Detection of HCV RNA in serum

A home-made, qualitative nested reverse-transcription polymerase chain reaction (RT-PCR) was used to detect HCV RNA in all sera samples, first stored at −70°C. Total RNA was extracted from each serum without pooling, using QIAamp Viral RNA Mini Kit (QIAGEN, Chatsworth, CA) as indicated the manufacturer. Afterwards, RT was carried out to obtain complementary DNA (cDNA) using M-MLV RT kit (MMLV, GIBCO/BRL). PCR amplification of cDNA and later a nested-PCR were performed with two pairs of primers that hybridize in a segment of the 5′ non-coding region of HCV genome, as is described elsewhere [27]. Appropriate measures were taken to minimize the risk of sample cross-contamination. These measures comprised the inclusion of serum samples from normal subjects and aliquots of water as negative controls. Qualitative nested RT-PCR was performed per duplicate in all samples. Cases with detectable HCV RNA in serum were referred to an external party to undergo liver biopsy, if the patient accepted this procedure.

2.4. Assessment of HBV coinfection

Infection with HBV was investigated by detection of the hepatitis B surface antigen (HBsAg) and anti-hepatitis B core antigen (anti-HBc) antibodies, using an automated third-generation microparticle enzyme immunoassay (MEIA, IMx Version 3.0 Abbott Diagnostics, Chicago, IL, USA), as well as by detection of HBV DNA using a home-made qualitative nested PCR. For HBV DNA assessment, an aliquot of 200 μl of sera samples were obtained to isolate viral DNA using the QIAamp Blood Kit (Qiagen, Chatsworth, CA). HBV DNA was amplified by standardized first-round and nested PCR of S-gene fragment using the primers and conditions described previously [28]. PCR assay was practiced per duplicate in all patients. The criterion for adjudication of a confirmed diagnosis and the measures taken to minimize the risk of cross-contamination were the same as in the assessment of HCV RNA. Reactive sera to HBV DNA undergone direct sequencing to determine HBV genotypes, as is described elsewhere [28].

2.5. Data analysis

Chi-square or Fisher exact test was used to assess nominal variables in bivariate analyses, as corresponded. To compare quantitative variables between two groups, Student t-test and Mann–Whitney U-test were performed for parametric and non-parametric variables, respectively. Pearson correlation
Table 1
Demographic and clinical characteristics of the 86 women analyzed

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>HCV RNA-positive (n = 55)</th>
<th>HCV RNA-negative (n = 31)</th>
<th>P-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>53 (21–82)</td>
<td>54 (21–79)</td>
<td>51 (22–82)</td>
<td>0.18</td>
</tr>
<tr>
<td>School education</td>
<td>68 (79)</td>
<td>42 (76)</td>
<td>26 (84)</td>
<td>0.58</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married, n (%)</td>
<td>64 (74)</td>
<td>41 (74)</td>
<td>23 (74)</td>
<td>0.98</td>
</tr>
<tr>
<td>Single, n (%)</td>
<td>10 (12)</td>
<td>4 (7)</td>
<td>6 (19)</td>
<td>0.11</td>
</tr>
<tr>
<td>Divorced, n (%)</td>
<td>7 (8)</td>
<td>6 (11)</td>
<td>1 (3)</td>
<td>0.23</td>
</tr>
<tr>
<td>Widower, n (%)</td>
<td>5 (6)</td>
<td>4 (7)</td>
<td>1 (3)</td>
<td>0.45</td>
</tr>
<tr>
<td>Occupation</td>
<td>43 (50)</td>
<td>31 (56)</td>
<td>12 (39)</td>
<td>0.17</td>
</tr>
<tr>
<td>Liver enzymes activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT, mean (S.D.)</td>
<td>53.1 (48.8)</td>
<td>64.2 (63.6)</td>
<td>39.8 (15.7)</td>
<td>0.25</td>
</tr>
<tr>
<td>AST, mean (S.D.)</td>
<td>49.2 (39.3)</td>
<td>59.4 (47.9)</td>
<td>35.9 (18.9)</td>
<td>0.16</td>
</tr>
<tr>
<td>Risk factors for HCV infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major surgeries, n (%)b</td>
<td>69 (80)</td>
<td>46 (84)</td>
<td>23 (74)</td>
<td>0.40</td>
</tr>
<tr>
<td>Blood transfusion, n (%)c</td>
<td>50 (58)</td>
<td>34 (62)</td>
<td>16 (52)</td>
<td>0.37</td>
</tr>
<tr>
<td>None identified, n (%)</td>
<td>12 (14)</td>
<td>6 (11)</td>
<td>6 (19)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

ALT: alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; NA, not applicable; S.D., standard deviation.

a P-value for differences between HCV RNA-positive and HCV RNA-negative women; Chi-square, Fisher exact test, t-test or Mann–Whitney U-test, as corresponded.
b Any major surgical procedure before the first screening test for HCV infection yielded a reactive result.
c Transfusion of blood products before 1993.

was used in continuous distributions. To find independent predictors of the presence of cirrhosis at histologic assessment, we did a multivariate analysis by a binary logistic regression model. Independent variables were chosen if \( P < 0.1 \) in selection step by bivariate analysis, subsequently a forward-stepwise method was performed. Adjusted odds ratios (OR) with 95% confidence intervals (CI) resulting in final step of the model are provided. Corrected OR were calculated for categorical predictors in order to approximate to the exact relative risk in a small sample with a high frequency of a research outcome, with the formula proposed by Zhang and Yu [29] as follows: corrected OR = multivariate OR/(1 – incidence of the outcome in the nonexposed group) + (incidence of the outcome in the nonexposed group \( \times \) multivariate OR). The fitness of the regression model was evaluated by using the Hosmer–Lemeshow goodness-of-fit test, which was considered as reliable if \( P > 0.20 \). Final statistical analyses were regarded as significant when \( P < 0.05 \). All \( P \)-values reported are two-sided. SPSS v12.0 statistical package was used for all calculations.

3. Results

In the period comprised by this study, 103 women aged > 20 years who tested reactive to anti-HCV antibodies were studied. Of them, nine cases were excluded because a new test for anti-HCV tested negative and eight cases because complete data on medical records were not available. Thus, 86 interferon-naive women, older than 20 years and

Table 2
Characteristics of the earliest transfusion in the 50 women who were recipients of blood products before 1993

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>HCV RNA-positive (n = 34)</th>
<th>HCV RNA-negative (n = 16)</th>
<th>P-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indication for transfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstetric/gynecologic, n (%)</td>
<td>34 (68)</td>
<td>24 (71)</td>
<td>10 (62)</td>
<td>0.75</td>
</tr>
<tr>
<td>Anemia, n (%)</td>
<td>7 (14)</td>
<td>3 (9)</td>
<td>4 (25)</td>
<td>0.19</td>
</tr>
<tr>
<td>Major surgery, n (%)b</td>
<td>5 (10)</td>
<td>4 (12)</td>
<td>1 (6)</td>
<td>0.98</td>
</tr>
<tr>
<td>Injury, n (%)c</td>
<td>4 (8)</td>
<td>3 (9)</td>
<td>1 (6)</td>
<td>0.75</td>
</tr>
<tr>
<td>Time from transfusion to diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range) in years</td>
<td>21 (7–39)</td>
<td>23 (7–39)</td>
<td>20 (9–33)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

HCV, hepatitis C virus.

a P-value for differences between HCV RNA-positive and HCV RNA-negative women; Chi-square, Fisher exact test or Mann–Whitney U-test, as corresponded.
b Major surgeries related to blood loss that required transfusion, before the first screening test for HCV infection yielded a reactive result.
c Injuries related to blood loss that required transfusion.
repeatedly reactive to anti-HCV antibodies were analyzed for the purposes of this study (Table 1). Mean age was 50.4 years (median 53, range 21–82 years). Of the 86 women studied, 55 (64%) had detectable HCV RNA in serum (Table 1).

3.1. Risk factors for HCV infection

Any major surgical procedure and transfusion of blood products before 1993 were the most frequent risk factors, with 74 (86%) cases having one or another, and 45 (52%) having both (Table 1). The main reason for practicing surgery was obstetric/gynecologic (74%). There were no patients who declared past or current illicit intravenous drug use, the antecedent of a sex partner infected with HCV or sexual promiscuity. Twelve (14%) women had no identifiable risk factor for HCV infection.

3.1.1. The antecedent of transfusion

Median age of the 50 (58%) women with the antecedent of transfusion was 54.5 years (range 21–82), which was different from the median of 46 years (range 22–72) in the 36 women without this antecedent (P = 0.009). The most frequent indication for transfusion was obstetric/gynecologic complication (e.g., miscarriage, abruptio placentae, dysfunctional uterine bleeding, and other conditions) in 34 (68%) cases, followed by anemia in 7 (14%) (Table 2). The median year of the earliest transfusion was 1980 (range 1968–1992). The median time from the earliest transfusion to the diagnosis of HCV infection by a reactive screening test was 21 years (Table 2).

3.2. Coinfection with HBV

Three (3%) cases were positive to HBV DNA, two corresponding to genotype H and one to genotype A. No HCV RNA-negative cases with a positive result to HBV DNA in serum were identified. Hence, confirmed coinfection (i.e., the presence of both HCV and HBV genomes in serum) occurred in 3 (5%) of the 55 women who tested positive to HCV RNA. Occult hepatitis B (i.e., seronegative to HBsAg, but positive to HBV DNA) occurred in two (4%) cases. One patient with coinfection had transfusion of blood products and surgeries as risk factors, the other two had only surgeries.

3.3. Factors influencing liver disease

Patients declaring significant alcohol intake (i.e., >10 g per day) were not identified. Thirty-six (42%) women had decompensated liver cirrhosis at inclusion in the study; 30 (83%) of them with detectable HCV RNA in serum. Among the 50 patients with the antecedent of transfusion, decompensated cirrhosis was more frequent in those who had >20 years of age at the moment of the transfusion (22 of 24, 92%) than in younger women (17 of 26, 65%) (P = 0.02). Among the 55 women with detectable HCV RNA in serum, 36 (65%) underwent liver biopsy and histologic examination, with 13 (36%) cases having chronic liver inflammation with fibrosis and 23 (64%) cirrhosis. There were no cases with hepatocellular carcinoma. In a multivariate analysis adjusted for diabetes mellitus, HBV coinfection, HBV genotypes and for anti-HCV antibodies levels; determining factors of cirrhosis at histologic assessment were age and the antecedent of transfusion (Table 3).

Table 4
Median (minimum and maximum) of anti-HCV antibodies levels, expressed as sample-to-cut-off ratio (S/CO), in relation to several characteristics

<table>
<thead>
<tr>
<th>HCV RNAa (n = 86)</th>
<th>Transfusionb (n = 86)</th>
<th>Surgeriesc (n = 86)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (n = 55)</td>
<td>Negative (n = 31)</td>
<td>Positive (n = 50)</td>
</tr>
<tr>
<td>43.3 (1.6–146.5)</td>
<td>2.0 (1.65–7.0)</td>
<td>41.1 (1–146.5)</td>
</tr>
<tr>
<td>Negative (n = 36)</td>
<td>Positive (n = 69)</td>
<td></td>
</tr>
<tr>
<td>34.9 (1.1–67.4)</td>
<td>40.0 (1–146.5)</td>
<td></td>
</tr>
<tr>
<td>Negative (n = 17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38.0 (1.1–58.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a P < 0.001, for differences between HCV RNA-positive and HCV RNA-negative women; Mann–Whitney U-test.
b P = 0.08, for differences between women with and without transfusion of blood products before 1993; Mann–Whitney U-test.
c P = 0.36, for differences between women with and without surgeries; Mann–Whitney U-test.
3.4. Levels of anti-HCV antibodies

Anti-HCV antibodies levels were higher in patients with decompensated cirrhosis than in asymptomatic women (median S/CO value 41.2 versus 36.2, respectively; \(P = 0.03\)). However, after considering only HCV RNA-positive cases (\(n = 55\)), anti-HCV levels were not different between patients with or without decompensated cirrhosis (median S/CO value 41.2 versus 45.1, respectively; \(P = 0.28\)), and did not differ between women who were or were not recipients of transfusion (Table 4; Fig. 1A). Nevertheless, HCV RNA-positive patients had higher anti-HCV levels than women without detectable HCV RNA (Table 4; Fig. 1B), and this difference remained regardless the antecedent of transfusion (Fig. 1C and D). Anti-HCV levels directly correlated with the elapsed time from transfusion to diagnosis of HCV infection (Fig. 2A), but not with age (Fig. 2B). After subgroup analysis, this correlation was higher in HCV RNA-positive (Fig. 2C) than in negative women (Fig. 2D).

4. Discussion

In Mexico, the proportion of women with HCV infection might be higher [5,16] than that reported in other countries [30–32], as is observed in several populations [10,17,18]. We found that the antecedent of surgeries was the most prevalent risk factor for HCV infection, followed by transfusion of blood products before 1993. However, a great proportion of patients who had the antecedent of surgeries also had transfusion, in most cases with an obstetric or gynecologic indication. This finding had only been reported in a female population from Ireland [10]. That most women with surgeries or transfusion as the main risk factors for HCV infection had an obstetric/gynecologic indication for these procedures, may help to explain the higher frequency of women with HCV infection, as compared with that of men [5,16]; however, this issue needs further investigation.

The levels of anti-HCV were higher in HCV RNA-positive than HCV RNA-negative women. Furthermore, the time elapsed from the acquisition of HCV to the diagnosis correlated with the levels of anti-HCV antibodies, in women with transfusion as the putative route of infection. Hence, as it is shown by the determination coefficient \((r^2)\) obtained from the correlation of anti-HCV levels and duration of the infection in HCV RNA-positive women, it is expected that about 14% of the variation in anti-HCV levels is determined by the state of chronicity. This is in agreement with a previous study that analyzed 43 recovered and 34 chronically infected women who were recipients of human Rhesus immunoglobulin contaminated with HCV genotype 1b [15]. This report demonstrated that in women with chronic hepatitis C,
anti-HCV levels were higher whereas cellular and interferon response were weaker than in patients with resolved infection [15]. Thus, on one hand, there is a pattern favoring humoral response in the chronic state; and on the other hand, a strong cellular immune response [15,33] and an initial low viral load [34] are associated with further resolved infection. It is possible that a long duration of the infection enables a continuous reinforcement of the humoral response, thus affecting the levels of anti-HCV antibodies. Nonetheless, certainly other factors as the cellular immune competence, viral replication reservoirs, and other complex characteristics inherent to HCV may determine the rest of the variation of anti-HCV levels. Our findings confirmed previous observations [15] and add that, even in transfusion-associated hepatitis C, the duration of HCV infection influences the levels of anti-HCV antibodies.

The frequency of occult HBV infection was lower than that reported in other studies [35,36], and HBV coinfection was not associated with the severity of the liver damage. The clinical importance of occult HBV infection has been a very debated topic, with studies that report a significant correlation with the severity of the liver damage [14,37] and other studies reporting no association at all [35,36], even in populations with a high prevalence of occult HBV infection [35]. Here, independent predictors of cirrhosis at histologic level were age and the antecedent of transfusion, a finding previously reported [6]. Both age and transfusion may have a pathophysiological connection with the chronic state, since age is associated with a longer persistence of the virus and a high vulnerability to oxidative stress [38] and the antecedent of transfusion before blood bank screening for HCV to a large inoculum that may imply a high number of viral genetic variants [39], than other sources of infection [40].

In conclusion, the source of HCV infection influences the severity of the liver disease, and the duration of the infection affects the levels of anti-HCV antibodies, in transfusion-associated hepatitis C. In most cases, surgery and transfusion had an obstetric or gynecologic indication, a finding that may explain why more women than men are observed with HCV infection in Mexico. Further studies are necessary for confirmation of these results.

Acknowledgements

We thank to Dr. Jorge Segura, Dr. Miguel A Jiménez, Dr. Guadalupe Becerra, Dr. Angeles Quintero and Dr. Benjamín Cárdenas for their clinical support and kind attention given to this work. We are also indebted to Montserrat Maldonado and Daniel Quezada for their friendship and assistance in laboratory. CONACYT, Salud-2004-C01-025 to A.P.

References


