Natural clearance of hepatitis C virus in hemophilia patients

Raimonds Simanis, Sandra Lejniece, Arturs Sochnev, Jelena Eglite, Gunta Chernevska, Zhanna Kovalova, Dace Gardovska, Agita Jeruma, Velga Kuse, Ludmila Viksna

Department of Traditional Infectology, Tuberculosis and AIDS, Institute of Immunology, Riga Stradins University, University Children’s Hospital, Latvia, Department of Pediatrics, Riga Stradins University, Latvia

Key words: hepatitis C virus; hemophilia; natural clearance; human leukocyte antigen.

Summary. Objective. The objective of this study was to investigate the prevalence of HCV infection in hemophilia patients in Latvia and to analyze association between natural clearance of HCV and human leukocyte antigen (HLA) class II genes.

Material and methods. From 61 hemophilic patients participating in this study, 38 were adults and 23 were pediatric patients younger than 18 years. To analyze association between HLA class II alleles and natural clearance of HCV, the gene frequency was compared in hemophilia patients group and the control group of 60 healthy subjects, all men. Serum HCV RNA was qualitatively determined and HLA class II alleles were identified by polymerase chain reaction (PCR) method.

Results. HCV infection is common among hemophilia patients in Latvia. Antibodies to HCV were found in 45 of 61 (74%) hemophilia patients. In 41% of hemophilia patients (18 of 44), HCV infection resolved spontaneously. Children cleared HCV more frequently than adults (7 of 11 comparing to 11 of 33, respectively; OR=3.50; P<0.05). The frequency difference was found to be statistically significant when comparing HLA alleles distribution in the sample of hemophilia patients who naturally cleared HCV (n=18) and in the control group (n=60) (corresponding frequency of HLA-DRB1*07 allele – 4 (11.11%) and 9 (1.67%); OR=7.38; P<0.05).

Conclusions. Natural clearance of HCV infection is frequently found in hemophilia patients in Latvia. Children are more likely to clear virus naturally than adults. There is an association between natural clearance of HCV and HLA allele DRB1*07 in hemophilia patients.

Introduction

Chronic hepatitis is an important public health problem affecting more than 500 million people worldwide (1) and 80–100 thousand in Latvia (2). According to literature data, hepatitis C virus (HCV) is the major cause of chronic liver disease in adults and children with hemophilia (3). Before the introduction of HCV screening of human blood products, most of children with hemophilia were infected with HCV. In Latvia screening of donated blood for presence of anti-HCV was introduced in November 1993. Cryoprecipitate and fresh-frozen plasma for treatment of hemophilia patients were used until June 2000. Approximately 10% (4) to 50% (5, 6) of patients who were infected with HCV have been shown to clear the infection naturally. Higher rates of spontaneous resolution of HCV infection have been observed in some patient subgroups such as young women after a common-source outbreak of contaminated anti-D immunoglobulin, children infected with HCV while undergoing cardiac surgery or cancer chemotherapy, or in healthcare workers after a needle stick injury (7). The mechanisms underlying the spontaneous viral clearance or development of chronic HCV infection have not been clearly identified yet (8). Apart from viral characteristics (viral genotype, quasispecies distribution, and viral load), it is generally accepted that cellular immune responses play an important role in viral clearance and disease resolution (5).

The outcome of HCV infection is determined by complex host-virus interactions (9) and based on studies involving hemophilia patients; it seems that genetic factors have rather modest influence on the outcome of hepatitis C (7). Nevertheless, immune system, including human leukocyte antigen (HLA) complex, plays an important role in natural clearance of HCV (10) and is worth examining in different populations.

Hemophilia patients who received unsterilized blood products such cryoprecipitate have extremely
high prevalence of HCV infection reaching 90% in many countries (7, 11). The preliminary analysis showed that Latvia is not an exception in terms of HCV infection prevalence in hemophiliacs. Since modern treatment options are available now, it was important to determine the prevalence of HCV infection among hemophilia patients in Latvia in order to determine those who need treatment and those who cleared virus naturally. By analyzing the group of hemophilia patients who have cleared HCV naturally, possible reasons of this phenomenon were found and described. The study of HCV infection in hemophiliacs has been performed in Latvia for the first time. There are also not so many studies of this kind performed in different countries around the world. The most important study so far, trying to find answers for reasons of natural clearance of HCV and including 257 sibling pairs from 3993 hemophilic patients, has been published recently (7). Our study focused more on one internal factor, namely, some HLA II class alleles, that could contribute to spontaneous resolution of HCV infection in population in Latvia. The results of different studies confirm the negative association between chronic HCV infection and DQB1*0301 allele (12). Association of DQB1*0301 allele with natural clearance of HCV is mentioned by many authors in original studies and reviews (10, 12–23). The other allele described as being important in patients who clear HCV spontaneously is DRB1*1101 (13, 16, 18, 20–24).

The objective of this study was to investigate the prevalence of HCV infection in hemophilia patients in Latvia and to analyze the association between natural clearance of HCV and HLA class II genes.

The another objective was to determine distribution of HCV genotypes in children and adults with hemophilia in Latvia.

Material and methods

Subjects. Serum samples were obtained from 61 patients with hemophilia. Thirty-eight of them were adults and 23 were pediatric patients younger than 18 years. The study was performed in accordance with the principles of Helsinki Declaration and approved by Ethics Committee. All hemophilia patients or their parents (for the group of pediatric patients) signed informed patient consent forms.

To analyze association between HLA class II alleles and natural clearance of HCV, the gene frequency was compared in hemophilia patient group (n=44) and the control group of healthy subjects (n=60, all men). The data on HLA class II alleles of the control group were obtained from the database of the Laboratory of Immunology of the Riga Stradins University.

Laboratory methods. Blood samples were analyzed to detect anti-HCV, HCV RNA, and HCV genotypes. Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to detect antibodies to HCV (AxSYM system HCV version 3.0, Abbott, USA; ORTHO HCV version 3.0, Ortho-Clinical Diagnostics Ltd., USA; INNOTEST HCV Ab IV, Innogenetic, Belgium; MONOLISA anti-HCV PLUS version 2, BIO-RAD, France). Serum HCV RNA was qualitatively determined by a commercially available reverse transcription polymerase chain reaction (PCR) method (AMPLICOR Hepatitis C Virus Test, version 2.0, Roche Molecular Systems, Inc., NJ, USA). HCV genotype was determined using a method of reverse hybridization LiPA (The VERSANT HCV Genotype Amplification Kit (LiPA), Bayer Corporation, Germany). HLA class II alleles were determined by PCR method. DNA isolation was performed as follows: genomic DNA was extracted from proteinase K-treated peripheral blood leukocytes with the routine method of salt off. The DNA was stored in TE buffer (10 mM Tris-HCl, pH 7.5, and 2 mM 0.5 M Na2 EDTA/L d-H2O). We used DNA obtained stamps for genotyping or storing in −20°C. DNA concentration was determined by fluorescent method with DNA fluorimeter and was 100–200 µg/mL in the middle. Dry DNA and resuspend in TE buffer (25). HLA-DR and -DQ Genotyping by PCR: low-resolution HLA-DR typing for DRB1*01 through 18 as well as for DQB1*0201-202, *0301-305, *0401-402, *0501-504, *0601-608 was performed by PCR method with amplification with sequence-specific primers (PCR-SSP). The reaction mixture (15 ūL) contained: 1 μL DNA, 1.5 μL PCR buffer [50 mM KCl, 1.5 mM MgCl2, 10 mM Tris-Cl, (pH 8.3)], 0.6 μL dNTPs (25 mmol/L), 1.0 μL specific primers (0.2 mol/L), and 0.5 U of the Taq DNA polymerase (Promega). In addition, the internal positive control primer pair, C3 and C5, was included in all reaction mixtures at a concentration of 5-fold lower than the allele- and group-specific primers. The reaction mixture was subjected to 35 amplification cycles (Program the Thermocycler DNA-technology, Russia), each consisting of denaturation at 94°C (60 s), followed by one cycle, annealing at 94°C (20 s), 67°C (2 s) followed by seven cycles and extension at 93°C (5 s), 65°C (4s) with a final extension step 28 cycles. PCR products were visualized by agarose gel electrophoresis. After addition of 2 M loading buffer, the PCR reaction mixtures were loaded in agarose gels pre-stained with ethidium bromide (0.5 μk/mL gel). Gels were run for
15 min at 10 V/cm gel in 0.5 mM TBE buffer, then examined under UV illumination, and documented (25, 26).

Statistical analysis. The distribution of HLA-DRB1*, HLA-DQA1*, and HLA-DQB1* alleles in hemophilia patients and control group subjects was compared using Pearson’s chi-square ($\chi^2$) test with Mantel-Haenszel correction or Fisher’s exact probability test when expected value was less than 5. P values less than or equal to 0.05 were regarded as statistically significant. Odds ratios (ORs) were used in place of relative risk. SPSS statistical software version 14 and Microsoft Excel 2003 were used.

Results

HCV infection in hemophilia patients. HCV infection is common among hemophilia patients in Latvia. Antibodies to HCV were found in majority of hemophilia patients (Table 1). HCV infection is more frequently found in adults (89% or 34 of 38) than in pediatric patients (48% or 11 of 23). Twenty-three children, born between 1987 and 2004, were included in the study. All children who cleared virus spontaneously were born during the period from 1990 to 1994. From all hemophilia patients with positive antibodies to HCV, persistent hepatitis C was determined in 26 (58%) patients. In one patient, sustained viral response was achieved by monotherapy with interferon-alpha before this study. This patient was excluded from calculations when assessing the frequency of natural clearance of HCV.

HCV genotype distribution. Hepatitis C virus genotypes 1b, 3a, and 2 were found in 16 (61%), 8 (31%), and 2 (8%) patients, respectively. Since there are no precise data on distribution of HCV genotypes in Latvian population, comparison with different patient groups (27, 28) was made (Table 2).

Natural clearance of HCV. In 41% (18 of 44) of hemophilia patients, HCV infection resolved spontaneously. Children cleared HCV more frequently than adults (7 of 11 (63.63%) comparing to 11 of 33 (33.33%), respectively; OR=3.50; P<0.05). Adults older than 40 years cleared HCV more often as compared to those younger than 40 years (5 of 10 (50%) comparing to 6 of 24 (25%), respectively; OR=3.00; P<0.23). Differences between frequencies of natural clearance did not reach statistical significance due to a small number of hemophilia patients.

HLA class II alleles in hemophilia patients in Latvia. The frequency difference was found to be statistically significant when comparing the distribution of HLA class II alleles in hemophilia patient group (n=60) and control group (n=60): HLA-DRB1*05 (corresponding number of alleles – 32 (26.67%) and 17 (14.17%)), HLA-DRB1*07 (corresponding number of alleles – 16 (13.33%) and 2 (1.67%)) and HLA-DQB1*0502-4 (corresponding number of alleles – 14 (11.67%) and 4 (3.33%)), Table 3.

Table 1. Prevalence of HCV infection in patients with hemophilia in Latvia

<table>
<thead>
<tr>
<th>Category</th>
<th>Adults n (%)</th>
<th>Children n (%)</th>
<th>All patients n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive anti-HCV</td>
<td>34 (89)</td>
<td>11 (48)</td>
<td>45 (74)</td>
</tr>
<tr>
<td>Negative anti-HCV</td>
<td>4 (11)</td>
<td>12 (52)</td>
<td>16 (26)</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>23</td>
<td>61</td>
</tr>
</tbody>
</table>

Anti-HCV – antibodies to hepatitis C virus.

Table 2. Distribution of HCV genotype in different patient groups

<table>
<thead>
<tr>
<th>HCV genotype</th>
<th>Hemophilia patients in USA n (%) (n=31)</th>
<th>Hemophilia patients in Latvia n (%) (n=26)</th>
<th>Chronic hepatitis C patients in Lithuania n (%) (n=1097)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>10 (32)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1b</td>
<td>4 (13)</td>
<td>16 (61.5)</td>
<td>678 (61.8)</td>
</tr>
<tr>
<td>2</td>
<td>2 (6)</td>
<td>2 (7.7)</td>
<td>100 (9.1)</td>
</tr>
<tr>
<td>3</td>
<td>13 (43)</td>
<td>8 (30.8)</td>
<td>319 (29.1)</td>
</tr>
<tr>
<td>4a</td>
<td>1 (3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mixed</td>
<td>1 (3)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Medicina (Kaunas) 2008; 44(1)
There are reports about association of HLA class II alleles with inhibitor formation to coagulation factor VIII (29). In our study, there was no statistically significant difference between groups of alleles related to inhibiting antibody formation to coagulation factor VIII: HLA-DQA1*0102, HLA-DQB1*0602-8, and HLA-DRB1*01.

**HLA class II alleles in hemophilia patients with natural clearance of HCV.** The frequency difference was found to be statistically significant when comparing the distribution of HLA alleles in the sample of hemophilia patients who naturally cleared HCV (n=18) and in the control group (n=60): HLA-DRB1*07 (corresponding frequency of allele – 4 (11.11%) and 9 (1.67%), Table 4. There was no statistically significant difference in frequency distribution of allele DQB1*0301 between hemophilia patients and control group.

**HLA class II alleles in pediatric hemophilia patients with natural clearance of HCV.** Because of the very high number of children, who have cleared HCV naturally, we undertook comparison of HLA II class genes of these children (n=7) with the control group of 60 healthy subjects. The frequency difference was found to be statistically significant when comparing HLA allele distribution in the sample of hemophilia patients – children who naturally cleared HCV (n=7) and in the control group (n=60): HLA-DRB1*03 (frequency of the allele correspondingly 21.43% and 5%) and DQB1*0401-2 (21.43% and 3.3%), Table 5.

In hemophilia – children with persistent hepatitis C – HLA alleles DRB1*07 (25% and 1.67%; OR= 19.67; P=<0.0003) and DQA1*0401 (12.50% and 1.67%; OR=8.43; NS) were found more frequently when comparing with the control group.

**Discussion**

In comparison to the literature data, where approximately 20% of patients have been shown to clear HCV infection naturally, in our report it reaches 64% in pediatric group. In adult hemophilia patients in Latvia, natural clearance of HCV virus shown in our study is 33%.

It is possible and has been also confirmed by other

<table>
<thead>
<tr>
<th>Table 3. Frequencies of HLA alleles in hemophilia patients and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HLA allele</strong></td>
</tr>
<tr>
<td>DRB1*05</td>
</tr>
<tr>
<td>DRB1*07</td>
</tr>
<tr>
<td>DQB1*0502-4</td>
</tr>
</tbody>
</table>

Only statistically significant results are displayed.

<table>
<thead>
<tr>
<th>Table 4. Frequencies of HLA allele in hemophilia patients with natural clearance of HCV and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HLA allele</strong></td>
</tr>
<tr>
<td>DRB1*07</td>
</tr>
</tbody>
</table>

*Fisher exact two-sided test. Only statistically significant results are displayed.

<table>
<thead>
<tr>
<th>Table 5. Frequencies of HLA alleles in pediatric hemophilia patients with natural clearance of HCV and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HLA allele</strong></td>
</tr>
<tr>
<td>DRB1*03</td>
</tr>
<tr>
<td>DQB1*0401-2</td>
</tr>
</tbody>
</table>

*Fisher exact two-sided test. Only statistically significant results are displayed.
Natural clearance of hepatitis C virus in hemophilia patients

Raimonds Simanis, Sandra Lejniece, Arturs Sochnevs, Zhanna Kovalova, Dace Gardovska, Agita Jeruma, Velga Kuse, Ludmila Viksna

Rygos Stradins universiteto Tradicinės infektologijos, tuberkuliozės ir AIDS katedra, Vidų ligų klinika, Immunologijos institutas, Latvijos universiteto vaikų ligoninė, Rygos Stradins universiteto Pediatrijos skyrius, Latvija

Raktažodžiai: hepatitis C virus, hemofilia, natūralus išnykimas, Žmogaus leukocitų antigenai.

Natural clearance of hepatitis C virus is frequently found in hemophilia patients. Persons more likely to clear virus naturally are children and those adult hemophilia patients with a long history of treatment with blood products. There is an association between natural clearance of HCV infection and HLA allele DRB1*07 in hemophilia patients in Latvia. In children, there is an association between natural clearance of HCV infection and HLA alleles DRB1*03 and DQB1*0401-2. These alleles could contribute to successful clearance of HCV infection.

Conclusions

Natural clearance of HCV infection is frequently found in hemophilia patients in Latvia. Persons more likely to clear virus naturally are children and those adult hemophilia patients with a long history of treatment with blood products. There is an association between natural clearance of HCV infection and HLA allele DRB1*07 in hemophilia patients in Latvia. In children, there is an association between natural clearance of HCV infection and HLA alleles DRB1*03 and DQB1*0401-2. These alleles could contribute to successful clearance of HCV infection.
Santrauka. Tyrimo tikslas. Ištirti hepatitės B viruso (HCV) infekcijos paplitimą tarp Latvijos ligonų, sergančių hemofilija, ir išanalizuoti natūralaus HVC infekcijos išnykimo ir žmogaus leukocitų II klasės antigenų genų sąsajas.

Medžiaga ir metodai. Tyrimo dalyvavo 61 ligonis, sergantis hemofilija, 38 suaugusiųjų ir 23 vaikai iki 18 metų. Buvo lyginamas genų dažnis tarp ligonų, sergančių hemofilija (n=18), ir sėkmių asmenų kontrolinės grupės (n=60, visi vyrai) sąsajomis tarp žmogaus leukocitų II klasės antigenų alelių ir natūralaus HVC išnykimo nustatytų. HCV RNR kiekis serume ir žmogaus leukocitų II klasės antigenų alelės nustatyti polimerazės grandinės reakcijos metodu.

Rezultatai. HCV infekcija yra paplitusi tarp Latvijos ligonų, sergančių hemofilija. Antikūnų prieš HCV rasta 45 (74 proc.) iš 61 sergančio hemofilija ligonio. Savaimė HVC infekcija išnyko 41 proc. hemofilija sergančiųjų (18 iš 44). HCV dažnai išnyko vaikų organizme nei suaugusiųjų (7 iš 11 palyginus su 11 iš 33; ŠS=3,50; p<0,05). Lyginant žmogaus leukocitų antigenų alelių pasiskirstymą tarp hemofilija sergančiųjų, kurių organizme HVC infekcija natūraliai išnyko (n=18), ir kontrolinės grupės tiriamųjų (n=60), nustatytas statistiškai reikšmingas dažnio skirtumas (DRB1*07 alelės dažnis buvo 4 (11,11 proc.) ir 9 (1,67 proc.), atitinkamai; ŠS=7,38; p<0,05).


Adresas susirašinėti: R. Simanis, Department of Traditional Infectology, Tuberculosis and AIDS, Riga Stradins University, Linezera iela 3, 1006 Riga, Latvia. El. paštas: raimonds.simanis@e-teliamtce.lv

References
Natural clearance of hepatitis C virus in hemophilia patients

Received 18 April 2007, accepted 21 June 2007
Straipsnis gautas 2007 04 18, priimtas 2007 06 21

Medicina (Kaunas) 2008; 44(1)