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Hepatitis C virus molecular epidemiology in Latvia

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Abstract

The aim of this study was to identify the hepatitis C virus (HCV) genotypes distributed in Latvia and to estimate their prevalence in various risk groups. HCV genotypes of 65 isolates were estimated using amplification and direct sequencing of PCR fragments from the core region of HCV genome. Phylogenetic analysis of the genotypes was conducted. Genotype 1b was identified in about 85 % of cases, genotype 3a in about 10 %, and HCV genotypes 1a and 2c were present only in some isolates. A similar epidemiological distribution is typical for the former Soviet Union Republics. Our study suggests that contaminated blood products may be main route of HCV infection in Latvia.

Key words: genotypes, HCV, prevalence.

Introduction

Hepatitis C virus (HCV) is the major agent of parenterally transmitted non-A, non-B hepatitis worldwide. Although representative prevalence data are not available from many countries, it is known that about 3 % of the world's population are infected with HCV. It is estimated that 170 million people worldwide are at risk of liver cirrhosis and hepatocellular carcinoma due to chronic infection with HCV (Cohen 1999). HCV causes 20 % of acute hepatitis cases, 70 % of all chronic hepatitis cases, 40 % of all cases of cirrhosis of the liver, 60 % of hepatocellular carcinomas, and 30 % of liver transplants in Europe (European Association for the Study of the Liver 1999).

Molecular characterization of HCV revealed the existence of a positive sense RNA genome of approximately 9400 bases in length. The complete genome sequence has been determined in different HCV isolates worldwide, which indicated substantial nucleotide sequence variability throughout the viral genome (Choo et al. 1991). HCV exhibits enormous genetic diversity: the comparison of published sequences of HCV has led to the identification of distinct HCV genotypes that may differ from each other by as much as 33% over the entire viral genome (Okamoto et al. 1992). The variability within the HCV genome has formed the basis for several genotyping systems. The current, most commonly used classification system has been proposed by Simmonds et al. (1994). HCV is classified into six major types (genotypes 1 to 6) and numerous subtypes (e.g., genotype 1a, 1b), which differ in diversity, geographical distribution, and transmission routes. Genotypes 1 to 3 are distributed widely around the world, while others have a more restricted distribution. For example, types 5 and 6 are only found in specific geographical (Simmonds 1999).

The genotypes of HCV appear to differ in serological reactivity and in treatment, although their role in variation of disease progression remains unclear (Poynard et al. 2003). Any successful HCV vaccination or control strategy, therefore, requires an understanding of the nature and variability of the epidemic behavior among subtypes.

The aim of this study was to identify hepatitis C virus genotypes distributed in Latvia and to estimate their prevalence in various risk groups.

Materials and methods

Patients

In total, 65 anti-HCV positive sera were included in this work: 23 sera from patients with chronic HCV infection, 18 sera from patients after kidney transplantation, 5 sera from patients undergoing dialysis and 19 sera from patients from the pediatric oncology ward. All sera were tested for the presence of anti-HCV antibodies using a second generation ELISA kit (Abbott Laboratories, Chicago, IL).

Amplification of fragments of HCV and HBV genomes

HCV-RNA was extracted from 100 μl of serum with a commercially available DNA/RNA isolation kit based on phenol/chloroform extraction (“Litech“, Moscow, Russia). Amplification of HCV core region 476-725 nt fragment (numbering according to Takamizawa et al. 1991) was performed by "in house" nested RT-PCR. cDNA synthesis for amplification of core sequences was carried out using primer AS1 (5'ATGTACCCC ATGAGGTCGGC3'). Primers 2S (5'TAGATTGGGTGTGCGCGCGA3',) and 1AS were used for the first round PCR, whilst primers 3S (5'CGCGCGACTAGGAAGACTTC3'), 4S (5' TGTGTGCGCGACGCGTAAA3') and 5AS (5'GCAYGTRAGGGTATCGATGA CYT3') for the second.

Sequencing of the PCR fragments

Products of the PCR were excised from the agarose gel and purified using the DNA Extraction Kit (MBI Fermentas, Vilnius, Lithuania). Purified fragments were subjected to direct sequencing in both directions using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction (Applied Biosystems, Foster City, USA), and electrophoregrams were obtained using an ABI Prism 377 sequencer (Applied Biosystems). PCR primers 3S and 5AS served as sequencing primers.

The sequences were edited manually by BioEdit Sequence Alignment (Hall, 1999) and subsequently aligned in the FASTA format (http://ngfnblast.gbf.de/docs/fasta.html). The phylogenetic tree was constructed using the DNA-distance algorithm and the neighbor-joining method in the PHYLIP package (Felsenstein 1989).

Results

Phylogenetic analysis of HCV core region nucleotide sequences showed that 57 isolates belonged to genotype 1b, six isolates to genotype 3a, one isolate to genotype 1a and one isolate to genotype 2c (Fig. 1). Distribution of HCV genotypes in isolates from different patient groups are shown in Table 1.

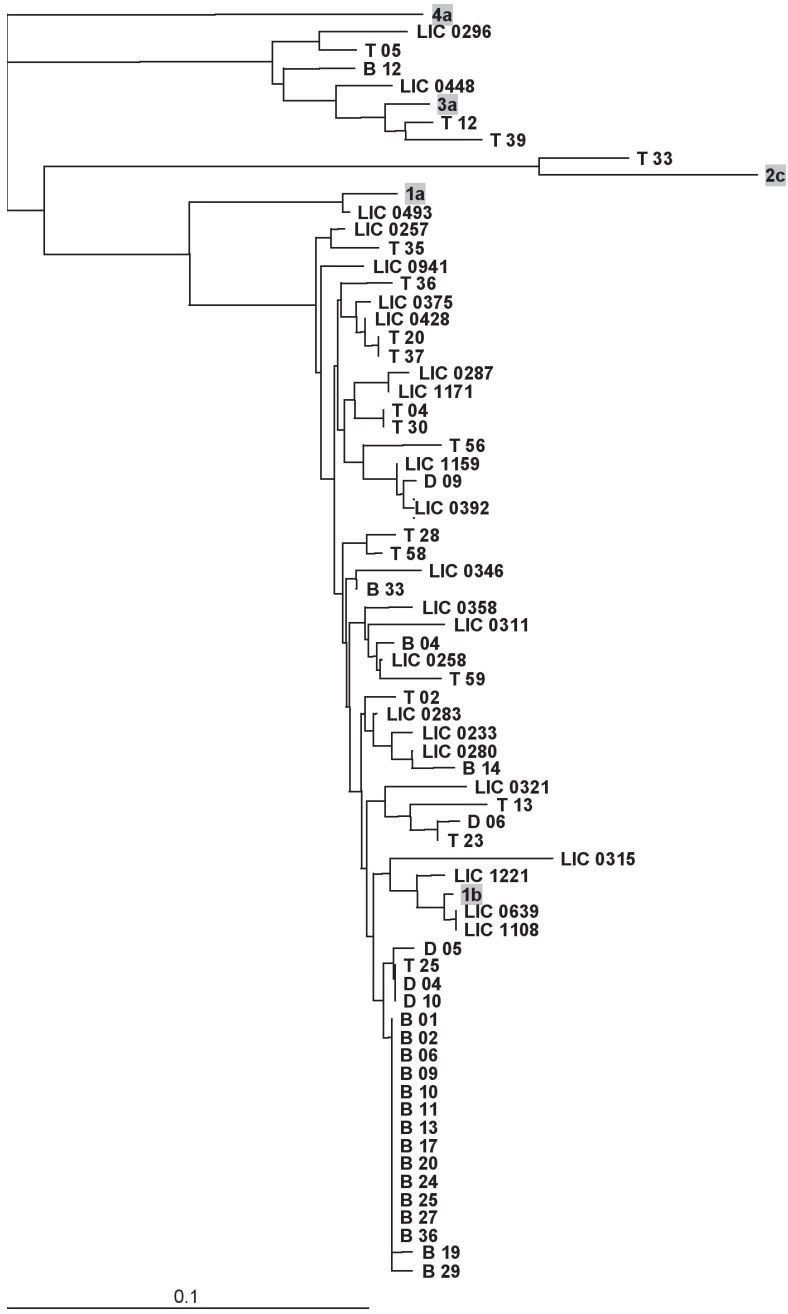

Fig. 1. Phylogenetic tree of 70 HCV core region sequenced fragments. Sequences from patients with chronic HCV infection have the prefix LIC, sequences from patients after kidney transplantation have the prefix T, sequences from patients ongoing dialysis have the prefix D and sequences from patients from the pediatric oncology ward have the prefix B. Reference sequences representing published genotypes 1a, 1b, 2c, 3a and 4a are included.

Table 1. Distribution of HCV genotypes in different groups of patients

Group of patients	1b	1a	2c	3a
Patients with chronic HCV infection	20	1	–	2
Patients after kidney transplantation	14	–	1	3
Patients undergoing dialysis	5	–	–	–
Patients from the pediatric oncology ward	18	–	–	1
Total	57	1	1	6

Discussion

The obtained results showed a strong predomination of genotype 1b in all groups of patients: 87 % in cases of chronic HCV infection, 78 % in posttransplantation, 100 % during dialysis treatment and in 95 % of cases in patients from the pediatric oncology ward. Not all of the analyzed groups are equally representative. As it was shown earlier (Dumpis et al. 2003), most of the examined patients from the pediatric oncology ward were infected in an outbreak. According to phylogenetic analysis, 13 of the 19 patients were infected with the same HCV genotype 1b isolate. On the other hand, the rate of evolution to chronicity after acute exposure to HCV was 92 % in patients exposed to HCV genotype 1b infection, compared with 33 % to 50 % in patients exposed to other genotypes (Zein 2000). The distribution of the HCV genotypes in patients undergoing dialysis and after kidney transplantation is similar to the distribution among non-renal patients in the same country (Fabrizi et al. 2001). Taking all of these obstacles into consideration, about 85 % of HCV isolates present in Latvia may belong to genotype 1b. Genotype 1b is seen more often in patients who acquired HCV through blood transfusion of unscreened blood products and medical procedures (Zein 2000), and unfortunately it shows relatively poor response to treatment by traditional anti-viral drugs (Poynard et al. 2003).

The genotype 3a may be present in about 10% of the Latvian patients infected by HCV. According to published data, this genotype is most common among intravenous drug users and shows a good response to interferon therapy (Zein 2000).

In a contrast with West-Europe countries (Zein 2000), HCV genotypes 1a and 2 are not common in Latvia. A similar epidemiological distribution was also described in other former Soviet Union Republics and some of Asian countries (Lvov et al. 1996; Viazov et al. 1997; Kurbanov et al. 2003). We believe that, at present in eastern neighbour countries, the main transmission pattern of HCV epidemic in Latvia is 1b genotype infection that spreads through blood transfusion and medical procedures.

Thereby, complete and proper screening of blood products, improving conditions needed for sterilisation of medical instruments, and using disposable syringes on the one hand, and management of measures targeted to users on the other, are two main directions that are important for limiting HCV transmission and the prevention of new outbreaks in Latvia.

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Hepatīta C vīrusa molekulārā epidemioloģija Latvijā

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Kopsavilkums

Darba mērķis bija identificēt Latvijā izplatītus hepatīta C vīrusa genotipus, kā arī novērtēt to izplatību dažādās riska grupās. Pielietojot HCV genoma *core* reģiona fragmenta PCR amplifikāciju, sekvinēšanu un iegūto sekvenču filoģenētisko analīzi, HCV genotipu noteica 65 paraugiem. 1b genotipu atrada apmēram 85 % gadījumu, 3a genotipu – apmēram 10 % gadījumu, HCV genotipus 1a un 2c atrada tikai dažos paraugos. Līdzīga epidemioloģiskā situācija ir raksturīga bijušajām PSRS valstīm. Mūsu pētījumi ļauj secināt, ka Latvijā HCV izplatās ar inficētiem asins materiāliem.