DISTRIBUTION OF HEPATITIS C VIRUS GENOTYPES IN PATIENTS WITH CHRONIC HEPATITIS C IN NORTHERN ESTONIA

T. TALLO[1]*, M. LAPPALAINEN[2], V. TEFANOVA[1], L. PRIIMÄGI[1]

[1]Department of Virology, Estonian Institute of Experimental and Clinical Medicine, Hiiu 42, 11619 Tallinn, Estonia;
[2]Department of Virology, Helsinki University Central Hospital/Haartman Institute, Helsinki, Finland

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Summary. – Distribution of hepatitis C virus (HCV) genotypes among 30 patients with chronic liver diseases and antibodies to HCV (anti-HCV) was investigated. Sera of these patients were analyzed for HCV genotype by reverse transcription–polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP) analysis of the 5′-non-coding region (5′-NCR) of the virus genome and for HCV serotype by detecting antibodies to HCV NS4 peptides by enzyme-linked immunosorbent assay (ELISA). The following distribution of genotypes was found: genotypes 1b in 32.0%, 3a in 20.0%, 2a in 12.0% and 1a/b (double infection) in 28.0%. The results of serotyping were interpretable in 92.0% and concordant with those of genotyping in 80.0% of the patients. In Northern Estonia, the genotypes 1b and 3a seem to be most common in chronically infected patients. Serotyping is an generally available and cheap assay and can be performed in most diagnostic laboratories in comparison to genotyping. However, genotyping is a more sensitive and more specific assay.

Key words: hepatitis C virus; genotyping; serotyping

Introduction

HCV is at present recognized as the leading cause of chronic hepatitis and cirrhosis and the most common risk factor associated with development of hepatocellular carcinoma in many developed countries (Alter, 1997; Hoofnagle, 1997). It is a positive-strand RNA virus containing about 9400 nucleotides which belongs to the *Flaviviridae* family (Choo *et al.*, 1991). Based on sequence variation within the highly conserved 5′-NCR and the relatively well-conserved core and 5′-non-structural regions, the majority of isolates can be classified into 6 major genotypes and at least 50 subgenotypes (Simmonds *et al.*, 1993; Purcell, 1997). Variable number of subgenotypes show distinct geographic and ethnic variations (Bukh *et al.*, 1995). In Europe, the genotypes 1a, 1b, 2a, 2b and 3a occur, while genotype 3a is particularly prevalent in north-western Europe including Finland (Viazov *et al.*, 1994; Pohjanpelto *et al.*, 1996). In the USA, genotype 1a is prevalent, while genotype 3a is rare (Lau *et al.*, 1994). In Japan and Taiwan, genotypes 1b and 2a are most common (Kinoshita *et al.*, 1993). In Central Africa and Egypt, genotype 4 predominates (Stuyver *et al.*, 1994).

HCV genotyping is now widely used for clinical purposes in chronically infected patients as a predictive marker of response to interferon therapy. It may indicate severity of the liver disease and is useful for prognosis.

HCV is a serious problem in Estonia and especially in its northern part. In the general population, the incidence rate of new HCV infection per 100,000 inhabitants increased 5.6 times in last 4 years and was 25.2 (total of 367 cases) in 1998 in Estonia (Tefanova *et al.*, 1998). The incidence rate

*E-mail: virologia@hot.ee; fax: +3726-706814.

Abbreviations: anti-HCV = antibodies to HCV; EIA = enzyme immunoassay; ELISA = enzyme-linked immunosorbent assay; HCV = hepatitis C virus; 5′-NCR = 5′-non-coding region; RFLP = restriction fragment length polymorphism; RT-PCR = reverse transcription–polymerase chain reaction

Table 1. HCV genotypes and serotypes in 25 anti-HCV-positive Estonian patients with chronic liver diseases

Genotype[a]	No. (%)	Serotype[b] 1	2	3	4	5	6	NTS	NR	Concordance[c] (%)
1b	8 (32.0)	6						1	1	75.0
1a/b	7 (28.0)	7								100.0
1*	2 (8.0)	2								100.0
2a	3 (12.0)		2					1		66.7
3a	5 (20.0)			3				1	1	60.0
Total	25 (100)							3	2	80.0

[a]Determined by RFLP analysis of 5′-NCR sequences.
[b]Determined by ELISA.
[c]Concordance of genotyping and serotyping.
*Subgenotype other than a or b.
NTS = non-type-specific antibody reactivity with NS-4 peptides.

of HCV increased 6.9 times and was 54.4 (total of 221 cases) in 1998 in northern Estonia including Tallinn – the capital of the country. The increase of morbidity rate of HCV concerns two age groups, 15–19 and 20–29 years, and the intravenous drug use is the main cause of acquiring this infection (Tefanova *et al.*, 1999). Distribution of HCV genotypes was not yet studied in northern Estonia. Genotyping of circulating HCV isolates could be very important not only for the prognosis and treatment of chronically infected patients, but also for an epidemiological study of Estonia and of the whole Europe.

In the present study we determined HCV genotypes by RT-PCR assay and RFLP analysis and HCV serotypes by ELISA in patients from Tallinn and its surroundings. This region is located in the northern part of the country and includes up to 500,000 inhabitants or 1/3 of the population of the country.

Materials and Methods

Sera of 342 patients were sent to our laboratory for viral hepatitis diagnosis by gastroenterologists from hospitals and outpatient clinics during the period of October 1997–May 1998. These patients had chronic liver diseases, were multiply transfused and had no history of intravenous drug abuse. After screening for anti-HCV, a group of 30 anti-HCV-positive patients (12 males and 18 females at the age of 25 to 57 years, mean age 38) was formed. HCV viremia, geno- and serotypes of HCV were determined in this group of patients.

Enzyme immunoassay (EIA). The sera were screened for anti-HCV by an EIA (Ortho HCV 3.0, Ortho Diagnostics Systems, Germany; Murex a-HCV, version III, Murex Diagnostics, France; Monolisa HCV, Sanofi Diagnostics Pasteur, France) according to xthe instructions of manufacturers of the kits used. All anti-HCV-positive sera were confirmed by immunoblot analysis (Deciscan HCV, Sanofi Diagnostics Pasteur); the reactivities with structural and non-structural proteins (C1,C2, NS3, and NS4) were determined. The presence of at least 2 visible HCV antigen bands was regarded as a positive result.

Total RNA was extracted from a serum with a commercial reagent TriPure™ (Isolation Reagent, Boehringer Mannheim) essentially according to the manufacturer's instructions.

RT-PCR assay of HCV was performed according to Pohjanpelto *et al.* (1996). All steps from cDNA synthesis to the first and second PCR were carried out in a single tube under oil to prevent a carry-over contamination. The detection limit was 16 gene quivalents per ml for both the genotypes 1 and 3. The PCR products were vizualized by running 15 µl out of the total of 60 µl in horizontal electrophoresis in 3.5% agarose gel (MetaPhor, FMC) at 100 V for 50 mins. After ethidium bromide staining, a single 174 bp product specific for HCV was visible in the gel under UV light.

HCV genotyping. The remaining volume (45µl) of the PCR product was used for HCV genotyping by RFLP analysis (Pohjanpelto *et al.*, 1996).

HCV serotyping was performed with a commercial ELISA kit (Murex HCV serotyping 1–6 assay HCO2, Murex Diagnostics, France) using NS4 peptides to identify type-specific anti-HCV.

Results

Analysis of the sera of 30 anti-HCV-positive patients gave the following results. Twenty-five (83.3%) of these sera were also found positive for HCV RNA. These 25 HCV-RNA positive sera were further analyzed for HCV genotype and serotype (Table 1) . Genotype 1b was most predominant (found in 8 (32.0%) patients), genotype 3a in 5 (20.0%) and genotype 2a was found in 3 (12%) patients. Double-infection with two different genotypes (1a/1b) was found in (28.0%) 7 patients.

Among 25 HCV-RNA-positive patients, 23 were reactive in the serotyping assay. In 20 of them the serotype corresponded to the genotype. The remaining 3 patients gave discrepant results: the serotypes 2, 1+6 and 1 corresponded to the genotypes 1b, 2a and 3a, respectively. Thus, the

sensitivity, specificity and concordance for HCV geno- and serotyping were 92.0, 87.0 and 80.0%, respectively.

Discussion

The prevalence of anti-HCV was found in 8.8% of 342 patients with chronic liver diseases.

Using a RFLP-based typing system for 25 HCV RNA-positive patients we found three major HCV genotypes, 1b, 3a and 2a in northern Estonia. The most common genotypes were 1b and 3a which have also been found to be prevalent in Germany (Viazov *et al.*, 1994), France (Castelain *et al.*, 1997), various regions of the former USSR (Lvov *et al.*, 1996), and Lithuania (Ambrozaitis *et al.*, 1995). Genotyping based on genotype-specific primers for PCR (Okamoto *et al.*, 1992) revealed a high percentage of mixed infections. Most of them were of subtype 1b plus other one. We found that the double infection 1a/1b made up to 1/3 from 25 cases of HCV infection. The clinical significance of mixed infections is uncertain. However, in some patients (Kao *et al.*, 1994) mixed infections may be associated with acute exacerbations of chronic HCV.

Using the RFLP analysis as a reference method of genotyping we determined serotypes of HCV in 25 HCV RNA-positive sera. Out of 23 interpretable results (92.0% sensitivity) 20 gave identical results with those of genotyping. We found an 80.0% (20/25) concordance between the results of serotyping and genotyping. Castelain *et al.* (1997) have compared results of serotyping carried out by the Murex anti-HCV serotyping assay and those of genotyping performed by reverse hybridization assay. They have found a 64% concordance between these results in chronically HCV-infected patients. Bell *et al.* (1996) have found an almost complete concordance (98.2%) between the results of serotyping by an EIA with NS4 peptides and those of serotyping by RFLP analysis in patients with chronic hepatitis C.

We conclude that in northern Estonia the genotypes 1b and 3a seem to be most common in chronically infected individuals without the history of intravenous drug abuse. Serotyping is an generally available and cheap assay and can be performed in most diagnostic laboratories in comparison to genotyping. However, the latter is more sensitive and more specific.

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