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The prevalence of mixed genotype infections in Polish patients with hepatitis C

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SUMMARY

Objectives: Hepatitis C virus (HCV) genotype has been described as an independent predictor of the response to therapy. A mixed infection with two types of HCV is probably an uncommon event. The aim of this study was to determine the occurrence of mixed infection with two different HCV genotypes in adult patients with chronic hepatitis C eligible for treatment.
Methods: Plasma samples and clinical and demographic data were collected from 1159 patients with hepatitis C. The INNO-LiPA HCV assay was used to identify the HCV genotypes.
Results: The dominant genotype was genotype 1, which was found to be responsible for 83.9% of infections, with subtype 1b being the most common. A mixed genotype infection was detected in 26 patients (2.2%). The most common mixed genotype was 1a+1b detected in 17/26 patients (65%). Antiviral therapy led to complete elimination of both genotypes in 50% of patients with 1b+3a infection and in 33% of patients with 1b+4a infection.
Conclusions: The results obtained showed that infection with mixed HCV genotypes in Polish patients with hepatitis C is uncommon. The selective elimination of genotypes 3a and 4a after therapy confirms the greater resistance to treatment of genotype 1b. In the context of new anti-HCV drug development, further investigations are needed to determine the clinical importance of mixed HCV infection.

1. Introduction

Globally, 80–185 million people are infected with the hepatitis C virus (HCV).[1] The majority of infected individuals (75–80%) are unaware of their infection. These patients are at risk of progression of HCV disease, leading to cirrhosis (in 20–30% of them after 10–20 years of infection), end-stage liver disease, and hepatocellular carcinoma (HCC).[2]

There are seven HCV genotypes with whole-genome nucleotide sequences differing by more than 30%.[3,4] According to Robertson et al., genotypes 1, 2, 4, and 5 form separate clades (1, 2, 4, and 5, respectively), genotypes 3 and 10 form clade 3, and genotypes 6, 7, 8, 9, and 11 form clade 6.[5] For the purpose of this article, the term genotype has been used. The heterogeneity within genotypes has resulted in 67 subtypes. Isolates from the same HCV subtype differ in nucleotide sequence by no more than 5–15%.[4,5] Because acute HCV infection is typically asymptomatic, most genotyping is probably conducted on individuals with chronic infection.

The frequency of the different HCV genotypes is different in different parts of the world. Genotype 1 is the most widely dispersed worldwide. In the USA, genotype 1 accounts for 70% of infections and genotypes 2 and 3 account for the majority of the remaining 30%.[1] In Europe the percentage of genotype 2 and 3 infections is greater than in the USA. Genotype 2 is widely

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dispersed in Central Africa.[6,7] Genotype 3 is common among illicit drug users. HCV genotype 4 predominates in the Middle East, genotype 5 in South Africa, and genotype 6 in South China and Southeast Asia. The most common genotypes in England are 1 and 3.[8] The different HCV genotypes do not differ in clinical presentation or severity of the illness.

The standard treatment for chronic hepatitis C consists of pegylated interferon (PEG-IFN) and ribavirin (RBV). The response rate, measured as the sustained virological response (SVR), depends largely on the viral genotype. Many patients drop out of treatment due to the severe side effects of interferon and the length of the therapy. Newer agents – the direct-acting antivirals (DAAs) – raise the prospect of virus elimination, and cure rates reach 90%.[9]

The important question is who should be treated for hepatitis C and when should this treatment be performed? The World Health Organization (WHO) recommends giving therapy to those with advanced disease. Further work is required to determine the response rate and to estimate the response rate and risk of resistance for the different HCV genotypes. HCV genotype has been described as an independent predictor of the response to therapy.

Mixed infection with two HCV genotypes is probably an uncommon event.[10] However, in some populations (e.g., persons who inject drugs (PWID) and men who have sex with men (MSM)) mixed infections occur in 25% to 39% of patients.[11,12] Revealing the occult genotypes might be necessary for the selection of the appropriate antiviral therapy. In the context of the newer drugs, knowledge of the distribution of HCV genotypes may help healthcare systems prepare the resources required to treat the predominant genotypes in their region.

The aim of this study was to determine the occurrence of mixed infection with two types of HCV in a cohort of adult patients with chronic hepatitis C eligible for treatment.

2. Materials and methods

Plasma samples were collected from 1159 adult patients with chronic hepatitis C eligible for treatment. Before genotyping, the detection of HCV RNA was performed to confirm infection. The second-generation test INNO- LiPA HCV II (Innogenetics) for the detection of polymorphisms in the 5′ non-coding region of HCV was used to determine the genotypes. In this procedure, the highly conserved 59-base pair non-coding region of the HCV genome was amplified by nested reverse transcription PCR with two sets of universal biotinylated HCV primers. The amplified products were then hybridized to oligonucleotide probes designed to be specific for the different HCV types and subtypes and were immobilized as parallel bands on nitrocellulose strips. The INNO-LiPA HCV II assay allows the identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, and 4a, 4c, 4d, 4e, 5, or 6. Clinical and demographic data were collected for all patients. A group of patients with mixed genotype was followed after treatment.

Patients were divided into subgroups based on the HCV genotypes detected. The age and sex distributions were compared between the groups. The genotype distribution was evaluated in relation to the age groups 18–40 years, 41–60 years, and >60 years.

3. Results

There were 1159 patients in the study group: 559 women (48.2%) and 600 men (51.8%). All 1159 serum samples tested in this study were HCV RNA-positive by PCR and could thus be genotyped with the INNO-LiPA HCV II assay. For 61 patients with genotype 1 it was not possible to distinguish between 1a and 1b; these patients were diagnosed as having genotype 1. Female patients were significantly older: their mean age was 43.6 years vs. 39.9 years for men (p = 0.0001). Details are presented in Table 1.

Table 1
Genotype distribution

Genotype	Number	Female n = 559	Male n = 600	p-Value	Mean age (years)
1	61	43	18	0.0004	42
1+3a	4	0	4	0.0531	43
1a	101	51	50	0.6337	28
1a+1b	17	10	7	0.3786	47
1b	810	399	411	0.2594	43
1b+3a	2	1	1	0.96	46.5
1b+4	3	0	3	0.3342	33
2b	1	0	1	0.3342	53
3a	111	38	73	0.0019	40
4	14	7	7	0.894	43
4a/4c/4d	34	10	24	0.0181	34
4e	1	0	1	0.3342	43
Total	1159	559	600		

The dominant genotype in the population studied was genotype 1, which was responsible for 83.9% (972/1159) of infections. Subtype 1b was the most common single genotype in all age groups and was detected in almost 70% of patients. Genotypes 5 and 6 were not detected.

3.1. Single genotypes

The comparison of patients infected with a single genotype showed significant age and sex differences between the groups. The distribution of genotypes was different in the different age groups (details are presented in Figure 1). The youngest were patients infected with genotype 1a (mean age 28 years) and the oldest with genotype 2b. Female patients with a single infection were significantly older than male patients. Genotype 3a was more common among men (p = 0.0024), while genotype 1 was more common among women (p = 0.0003). The difference was of statistical significance and was greatest in the 41–60 years age group (details are presented in Table 2).

3.2. Mixed genotypes

A mixed genotype infection was detected in 26 patients (2.2%): 15 men (2.5%) and 11 women (1.9%). The difference was not of statistical significance (p = 0.5519). The mean age of these patients was 46.9 years. The age differences among patients with particular mixed genotype infections were not statistically significant. The most common mixed genotype was 1a+1b, which was detected in 17/26 patients (65%). Genotype 1a+1b was detected in 10 of 11 women with an infection caused by mixed genotype.

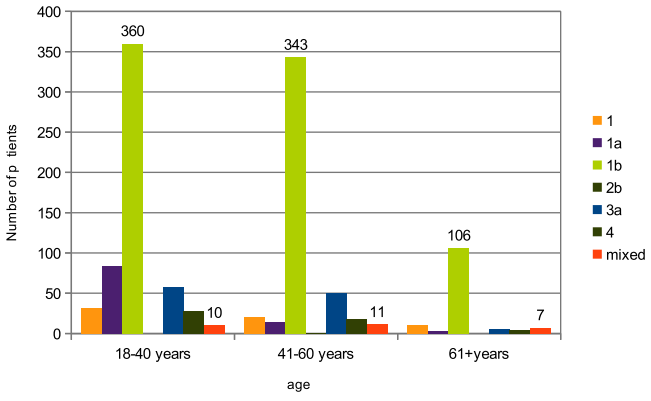

Figure 1. The distribution of genotypes in different age groups.