Hepatitis C Virus Genotypes in Northern Italy: Clinical and Virological Features

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We analyzed the characteristics of subjects from the same area who were infected with hepatitis C virus genotypes 1 through 4 and subtypes 1a and 1b. Our data are consistent with a rapid evolution in the epidemiology of HCV genotypes and argue against different pathogenic potentials for genotypes 1b and 2.

The sequence analysis of hepatitis C virus (HCV) isolates allows the identification of at least six genotypes with distinctive patterns of geographic distribution (11). Previous studies with Italian patients have identified age- and risk-related prevalences of viral genotypes (4, 5, 7, 9, 10). We analyzed the characteristics of HCV genotypes 1 through 4 and of subtypes 1a and 1b in our geographic area of northern Italy.

The study population consisted of 237 subjects (age [mean ± standard deviation], 45.4 ± 15.9 years), including 160 patients with previous diagnoses of chronic HCV infection and 77 subjects occasionally found to be positive for anti-HCV. The latter group included 52 women undergoing pregnancy screening and 25 subjects screened for blood donations.

HCV genotypes 1 through 4 were determined by differential hybridization on microtiter plates (7) of the amplified 5′ untranslated region (6) with genotype-specific probes (Table 1). The viral genotypes could be determined for all samples, indicating that additional genotypes are infrequent in Italy. Genotype 1 was detected in 158 cases (66.7%), genotype 2 was detected in 57 cases (24.1%), genotype 3 was detected in 14 cases (5.9%), and genotype 4 was detected in 8 cases (3.3%). The subtyping procedure was successful in 98 of 110 samples with genotype 1 available for analysis, detecting subtype 1a in 25 cases (25.5%), subtype 1b in 67 cases (68.4%), and mixed 1a-1b infections in 6 cases (6.1%). Typing results were consistent with the sequence analysis of the 5′ untranslated region and/or of genes C and E1, performed on 35 samples (data not shown).

The characteristics of subjects infected with different genotypes and/or subtypes were compared by parametric or non-parametric tests as appropriate (EPIINFO 5.0 statistical package; Centers for Disease Control and Prevention, Atlanta, Ga.). The numbers of males and females were not statistically different among patients infected with each genotype. Subjects infected with genotype 2 were older than those infected with genotypes 1, 3, and 4 (P < 0.01). Furthermore, infections with genotypes 3 (P < 0.01) and 4 (P < 0.05) were associated with lower mean ages than infection with genotype 1, although these results may have been affected by the small sample sizes. Subtype 1a was detected in a younger age group than subtype 1b (P < 0.01).

The analysis of risk factors revealed a similar prevalence of blood transfusions among subjects with genotypes 1, 2, and 4. The absence of transfusions among individuals with type 3 might be linked to the lower mean age. Intravenous (i.v.)-drug users were infected with genotypes 1, 3, and 4, with a significantly increased frequency of genotype 3 (P < 0.01). Among subjects infected with subtype 1a or with mixed 1a-1b infections, i.v.-drug use was more frequent than among subjects infected with subtype 1b only (P < 0.01). Subjects without known parental risk factors were more often infected with genotype 2 or subtype 1b (P < 0.01).

Samples from 176 cases were tested for anti-HCV antibodies by the recombinant immunoblot assay (Ortho Diagnostic Systems, Raritan, N.J.); of these cases, 119 were with genotype 1, 43 were with genotype 2, 9 were with genotype 3, and 5 were with genotype 4. Seventy-seven samples were tested by second-generation assay, and 99 were tested by third-generation assay. Anti-C100/5.1.1 was significantly less frequent in genotype 2 samples (n = 21; 48.8%) than in genotype 1 samples (n = 99; 83.2%) (P < 0.01), consistent with previous data (1, 13). These results had no relationship to the generation of the assay used (data not shown).

The presence of anti-human immunodeficiency virus (HIV) antibodies, for which we tested samples from 178 subjects, was associated with HCV genotypes 1, 2, and 4 with subtype 1a or mixed 1a-1b infections (P < 0.01) (Table 2).

Alanine aminotransferase (ALT) values were available for 176 subjects (120 with genotype 1, 39 with genotype 2, 10 with genotype 3, and 7 with genotype 4). Lower mean levels (P < 0.05) and a higher percentage of normal values (<50 IU/liter) (P < 0.01) were detected in subjects with genotype 2 than in subjects with genotype 1.

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During the last year before sampling, liver biopsies were
FIG. 1. Distribution of HCV RNA titers according to HCV genotype and subtype, ALT level, and the presence or absence of anti-HIV. The horizontal lines represent median HCV RNA levels within the population samples.
performed on 149 subjects, 107 with genotype 1, 36 with genotype 2, 2 with genotype 3, and 4 with genotype 4. Histological analysis detected chronic persistent hepatitis in 14 cases (12 with genotype 1 and 2 with genotype 2), chronic active hepatitis in 89 (63 with genotype 1, 20 with genotype 2, 2 with genotype 3, and 4 with genotype 4), liver cirrhosis in 17 (15 with genotype 1 and 2 with genotype 2), and hepatocellular carcinoma in 29 (17 with genotype 1 and 12 with genotype 2). The severity of histological lesions did not differ significantly between subjects with genotype 1 and those with genotype 2.

Histological activity scores (2) were analyzed for patients with subtype 1b (n = 33) and genotype 2 (n = 21); they show similar epidemiological features but different biochemical and serological features. Statistical analysis failed to show significant differences (data not shown).

The titers of HCV RNA in 110 serum samples adequately stored for quantitative analysis were determined by competitive PCR (8). The viral titers were not related to epidemiological or histological characteristics (data not shown) but were lower in samples with genotype 3 than in samples with genotype 1 (P < 0.01) (Fig. 1). A differential sensitivity of the assay according to the viral type is unlikely, since primers and probe were based on a sequence highly conserved among different genotypes (8). Further studies are needed to assess whether the lower viral load is an intrinsic characteristic of genotype 3 infection or whether it is linked to specific features of the population infected with this genotype.

The presence of anti-HIV was associated with higher median titers of HCV RNA, even though the difference did not reach statistical significance. Subjects with increased ALT levels had higher viral loads than those with normal ALT levels (P < 0.05), suggesting a relationship between the rate of viral replication and the degree of liver cell necrosis.

Altogether, HCV types 1b and 2 were both detected in older subjects and were associated with community-acquired infections. By contrast, genotype 3 and subtype 1a were associated with lower age, i.v.-drug use, and HIV infection. These observations are consistent with a rapid evolution in the prevalence of HCV genotypes in Italy. Our results argue against different pathogenic potentials for genotypes 1b and 2, despite the biochemical silent clinical course associated with genotype 2 in this and other reports (1, 9). However, this issue is still controversial (3, 9) and needs further confirmation by long-term prospective studies. The changing incidence of viral types in younger age groups underlines the need for the reevaluation of the prevalences of HCV genotypes at different times.

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REFERENCES


