Comparison of Hepatitis C Viral Loads in Patients with or without Coinfection with Different Genotypes

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Hepatitis C virus genotyping was assessed for 257 chronic hepatitis C patients with viral loads above 1,000 IU/ml. Twelve patients were coinfected with more than one genotype. Their median viral loads did not differ significantly from those observed for monoinfected patients, which in turn did not vary significantly among different genotypes.

TABLE 1. HCV loads in male and female patients

| Group       | No. of samples | Median age (yr) | Median HCV load (IU/ml) |
|-------------|----------------|-----------------|-------------------------|
| All patients|                |                 |                         |
| Male        | 245            | 52              | 344,000                 |
| Female      | 123            | 55              | 341,000                 |
| Male        | 122            | 48              | 345,000                 |
| Female      | 48             | 48              | 345,000                 |

a) HCV-infected patients, excluding 12 coinfected patients, and patients with viral loads of <1,000 IU.

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To test our hypothesis, a total of 396 chronically infected patients from Argentina (n = 305), Uruguay (n = 66), Russia (n = 12), and India (n = 13), seen from March 2000 to June 2001, were initially investigated. None of these patients had markers of HIV infection or received antiviral treatment. All samples were stored at −20°C before being used for HCV RNA extraction as previously described (3). For viral load determination, the Amplicor HCV Monitor test version 2.0 was used, according to the instructions of the manufacturer (Roche Diagnostics, Geneva, Switzerland). From the 396 patients originally enrolled in our study, 139 patients with HCV RNA levels below 1,000 IU/ml were excluded from further analysis in order to achieve reproducible findings. The genotype and subtype of the remaining 257 patients’ HCV isolates were determined by the InnoLipa HCV II assay (Innogenetics, Ghent, Belgium) or by phylogenetic analysis of nucleotide sequences from the 5’ noncoding region (4, 5, 19, 23), following PCR amplification, as previously described (3, 6). To avoid
false-positive results, the recommendations of Kwok and Higuchi were strictly adhered to (13). From these studies it was possible to establish that 12 out of the 245 patients investigated were coinfected by two different genotypes, as determined by the InnoLipa assay (20). These patients were from Argentina and had an age range from 29 to 67 years.

We next compared the median HCV RNA levels among the patient groups shown in Table 1. The median HCV RNA level did not differ significantly between different genotypes in monoinfected patients. Similarly, median HCV RNA levels for the coinfected patients (356,000 ± 56,000 [standard deviation (SD)] IU/ml) were not significantly higher than those for the patients infected with only one genotype (344,000 ± 52,000 [SD] IU/ml) (P > 0.05) (Table 2). Comparisons of the median HCV RNA levels for patients coinfected by specific genotypes in the same age group (i.e., genotype 1a plus 1b; 364,000 ± 60,000 [SD] IU/ml) with median HCV RNA levels for patients infected with only one genotype (i.e., either 1a or 1b; 300,000 ± 56,000 [SD] and 352,000 ± 48,000 [SD] IU/ml, respectively) did not show a statistically significant difference between the two groups (P > 0.05) (Table 1).

Most of the coinfected patients were coinfected by genotypes 1a and 1b. Only two patients were coinfected by genotype 1b plus 2a/c or 2a/c plus 2b. The viral loads obtained in these last two cases were similar to the ones obtained from patients coinfected with 1a plus 1b (Table 2). This indicates that the viral load in coinfected patients is independent of the combination of genotypes infecting the patient, even though a more detailed analysis will be needed to draw definitive conclusions.

We also analyzed the data for potential correlates of coinfection such as patient age and sex and alanine aminotransferase (ALT) levels. No significant correlation was found among HCV RNA level, age, and sex in the HCV-coinfected group (Table 3). Higher levels of ALT were found in male than in female coinfected patients (Table 3). There is no clear explanation for this difference. We believe that fluctuations in ALT level during the natural course of infection with HCV in different patients may be responsible for this finding. This discrepancy may also be related to the undefined genetic and immunologic factors that may lead to differences in control of HCV replication among different groups of people (16).

The results of this study also confirm previous findings that HCV RNA levels in patients do not correlate with age or sex (16). Patients aged 29 to 39, 40 to 49, 50 to 59, and 60 to 69 years from our study did not show any significant differences in viral loads (data not shown). In addition, our results show that chronic patients coinfected by two different HCV genotypes have viral loads similar to those of patients infected by a single genotype (Tables 1 and 2). This suggests that there is no additive effect in coinfected patients. Whether the two genotypes replicate with equal efficiency is not clear, as this was not within the scope of the study. However, interference between two infecting genotypes is possible, as suggested by others (12, 14, 18). A better understanding of the effect of coinfection with different genotypes of HCV, the determinants of increased HCV RNA level, and the significance of high HCV RNA levels during the natural course of HCV infection is needed, in order to identify patients who would benefit most from treatment with antiviral agents.

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| Group | All patients | | Female | | Male |
|-------|-------------|---|--------|---|--------|
|       | No. of samples | Median age (yr) | Median HCV load (IU/ml) | No. of samples | Median age (yr) | Median HCV load (IU/ml) | No. of samples | Median age (yr) | Median HCV load (IU/ml) |
| All coinfections | 12 | 42 | 356,000 | 4 | 48 | 348,000 | 6 | 37 | 368,000 |
| Coinfection with genotypes 1a and 1b | 10 | 44 | 364,000 | 4 | 48 | 312,000 | 8 | 37 | 372,000 |

* All coinfections were in patients within the age range of 29 to 67 years. This group includes 10 patients coinfected with genotypes 1a and 1b, one patient coinfected with genotypes 1b and 2a/c, and one patient coinfected with genotypes 2a/c and 2b.

**TABLE 2.** HCV loads in male and female patients coinfected by more than one genotype

**TABLE 3.** Demographic data and clinical features of patients coinfected with HCV

| Group | No. of samples | Mean age (yr) | Mean ALT level (IU/liter)* | Mean HCV level (IU/ml) |
|-------|---------------|--------------|---------------------------|----------------------|
| Male  | 8             | 41.5         | 211.0                     | 316,000              |
| Female| 4             | 48           | 95.8                      | 352,000              |

* Normal range, 21 to 40 IU/liter.
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