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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.

About half of all patients with acute hepatitis C virus (HCV) infection progress to chronic disease, and many of them develop hepatocellular carcinoma in later life (1). HCV has been classified into six major genotypes, many of which contain a number of more closely related subtypes (21). Higher HCV RNA levels have been reported for patients infected with genotype 1 strains than for patients infected with other genotypes (2), but possible viral load differences between genotypes have not been extensively studied. Similarly, although multiple infection with different HCV genotypes has been reported (8), it is not clear whether such patients have higher virus loads than do patients infected with a single genotype, or whether there is virus interference. In some studies, higher levels of liver transaminases correspond with higher HCV RNA levels, suggesting that the latter are associated with liver damage (7, 9, 22). However, a direct relationship between the level of viremia and either the severity of liver disease or transaminase levels is not universally accepted (10, 11). Besides, the roles of HCV genotype, human immunodeficiency virus (HIV) coinfection, age, race, and sex have been investigated in this respect, with various results (1, 2, 15–17, 22).

In the present study, we investigated HCV viral load in relation to genotype in monoinfected patients but also in patients coinfected with different HCV genotypes. In the latter case, an attempt was made to establish whether these patients had higher viral loads than did patients infected with a single genotype, and if so, whether this could be correlated with other demographic characteristics such as age and sex or was dependent on specific genotypes and/or subtypes coinfecting the patient.

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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

TABLE 1. HCV loads in male and female patients

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)
HCV positive[a]	245	52	344,000	123	55	341,000	122	48	345,000
Genotype 1a	32	47	360,000	20	50	356,000	12	45	372,000
Genotype 1b	116	54	352,000	57	56	352,000	59	48	348,000
Genotype 2[b]	71	55	320,000	39	55	300,000	32	52	320,000
Genotype 3a	22	39	332,000	6	44	300,000	16	38	340,000
Genotype 4	4	56	316,000	1[c]	54	356,000	3	59	272,000

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

[b] Sixty-nine patients were infected with genotype 2a or 2c. The assay used did not permit a clear assignment to either of these subtypes. Two patients were infected with genotype 2b.

[c] Only one patient was infected with this genotype.

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

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[a]	12	42	356,000	4	48	348,000	8	37	368,000
Coinfection with genotypes 1a and 1b	10	44	364,000	4	48	312,000	6	37	372,000

[a] 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.

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; 360,000 ± 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).

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

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

[a] Normal range, 21 to 40 IU/liter.

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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