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Hepatitis C Virus Core Mutations Reduce the Sensitivity of a Fluorescence Enzyme Immunoassay

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Four of 107 samples obtained from hepatitis C virus (HCV) carriers showed lower HCV core antigen levels in a fluorescence enzyme immunoassay (FEIA) than expected from corresponding HCV RNA levels. Nucleotide sequencing revealed a mutation in the HCV core region (Thr49Pro) that appears to have reduced the FEIA sensitivity.

Hepatitis C virus (HCV) infection causes considerable morbidity and mortality (15). The quantitation of plasma HCV viral load has become a routine clinical assessment for HCV-infected patients receiving antiviral therapy (4, 10). Assays to determine HCV viral load include a quantitative reverse transcriptase PCR assay (Amplicor monitor assay) and a branched-DNA (bDNA) probe assay. Alternatively, a fluorescence enzyme immunoassay (FEIA) that determines HCV core antigen (HCVcAg) levels, using the high-affinity monoclonal antibodies (MAbs) 5E3 and 5F11, which are specific for amino acids 21 to 40 and 41 to 60 of the HCV core protein c11 (2, 9, 13), has become available. The HCV core region, in particular the area between amino acids 21 and 60, is highly conserved, showing identical nucleotide sequences for all HCV genotypes (1, 14). The HCVcAg assay may have several advantages over PCR-based techniques. First, it does not require amplification of the viral genome, rendering the assay less susceptible to contamination. Second, the test is simple and inexpensive, because no sophisticated laboratory equipment is needed. A number of studies demonstrated a significant association between serum HCVcAg and HCV RNA levels, using this FEIA method and either the Amplicor monitor or bDNA probe assay (3, 8, 10, 13).

In this study, HCVcAg and HCV RNA levels in 107 serum samples of treatment-naïve subjects with chronic HCV infection who attended the National Tokyo Hospital from 12 to 1999 April 20 were determined. Chronic HCV infection was diagnosed by a qualitative HCV RNA assay (Amplicor HCV kit; Nippon Roche, Tokyo, Japan) testing positive on at least two occasions 6 months apart. HCV RNA levels were measured by the Amplicor monitor assay (Amplicor HCV Monitor assay version 1.0; Nippon Roche) and the bDNA probe assay (Quantiplex HCV-RNA version 1.0; Chiron, Emeryville, Calif.). The cutoff values were set at 1.0 kilocopies/ml and 0.5 million genome equivalents/ml, respectively. An FEIA was used for the quantitation of HCVcAg levels (Imucheck F-HCV Ag Core Kokusai; International Reagents Corp., Kobe, Japan). The cutoff value was set at 8.0 pg/ml. HCV genotypes were determined by a PCR method as reported by Okamoto et al. (7). Nucleotide sequences of the HCV core genome were

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FIG. 2. Amino acid sequences of the HCV core regions of four strains (HC-S1 to HC-S4) isolated from samples S1 to S4 with discrepant HCVcAg and HCV RNA levels and eight strains (HC-N1 to HC-N8) isolated from randomly selected control samples (N1 to N8). HCV core amino acid positions are given at the top. The recognition sites of MAbs 5E3 and 5F11, reported by Kashiwakuma et al. (2), are shown in the two boxes.

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Legend:
- 5E3 recognition site
- 5F11 recognition site

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determined as previously reported by Ohno et al. (6). Amino acids were numbered starting at the core of the HCV genome of genotype 1b (HC-J4/83 [accession no. D01217]). A Spearman rank correlation test and a Mann-Whitney U test were used for the statistical analysis.

All 107 samples tested positive in the qualitative HCV RNA assay, while 72 samples (67%) had the samples (1.245 versus 26.632; low, ranging from 0.609 to 2.482 and from 0.017 to 0.042, and HCVcAg-to-Amplicor monitor ratio (CA ratio) were very low. These samples the HCVcAg-to-bDNA probe ratio (CP ratio) in the samples showed relatively low HCVcAg levels with regard to their corresponding HCV RNA values. In these samples the HCVcAg-to-bDNA probe ratio (CP ratio) and HCVcAg-to-Amplicor monitor ratio (CA ratio) were very low, ranging from 0.609 to 2.482 and from 0.017 to 0.042, respectively. The median CP ratio was significantly lower in these four discrepant samples than in those in the remaining samples (1.245 versus 26.632; P < 0.001). Similarly, the median CA ratio was lower (0.024 versus 0.453; P < 0.005). These observations led us to determine nucleotide sequences of the HCV core region (amino acid positions 4 to 106) for the four discrepant samples (S1 to S4) and for eight randomly selected control samples in the same cohort (N1 to N8) (Fig. 2). Both groups showed similar characteristics with respect to serum transaminase levels, age, sex, and liver disease. S1 to S4 and N1 to N6 were HCV genotype 1b, whereas N7 was genotype 2a and N8 was genotype 2b. All HCV strains isolated from discrepant samples S1 to S4, however, showed a point mutation at codon 49, where threonine had been replaced by proline (Thr49Pro). This mutation was not found in control samples (N1 to N8). Moreover, one discrepant sample (S1) also demonstrated a mutation at codon 23, where lysine had been replaced by arginine (Lys23Arg). This sample also showed the lowest HCVcAg level and lowest CP and CA ratios. However, in one control sample (N3), arginine had been replaced by threonine at codon 43 (Arg43Thr), which did not appear to affect the HCVcAg level or CP and CA ratios (16.973 and 0.431, respectively).

To evaluate the prevalence of HCV core mutations, an HCV database was consulted (http://s2ad02.genes.nig.ac.jp). Thr49Pro was found in two of 215 isolates (1%; accession no. M58335 and L38333), which were genotype 1b (12) and 4f (11). The Lys23Arg mutation was not found in the database.

Our findings suggest that HCV core mutations may affect the affinity of MAbs 5E3 and 5F11, used by the FEIA, to HCV core proteins. It is conceivable that the replacement of hydrophilic threonine by hydrophobic proline may have led to a change in polarity of the HCV core protein. Although we cannot completely exclude other reasons for the discrepancy between HCVcAg and HCV RNA levels, alternative explanations appear to be highly unlikely. The manufacturer warns about the possibility of a nonspecific inhibition of the FEIA by chylomicrons, which was not observed in these samples. Several investigators reported significant differences in HCV RNA levels in individuals with distinct HCV genotypes, using the Amplicor monitor or bDNA probe assay (5, 8). In this study, all four discrepant samples and six out of eight control samples were genotype 1b, which is the most prevalent strain in Japan (7). Our findings therefore strongly suggest that HCV core mutations may affect the sensitivity of the FEIA. Clinicians need to be aware that this assay may provide inaccurate results in a small proportion of subjects with mutations in the HCV core region.

Nucleotide sequence accession numbers. The GenBank/EMBL/DDJB accession numbers of the sequences reported in this study are AB039866 to AB039877.

We express our gratitude to International Reagents Corporation (Kobe, Japan) for the measurement of HCVcAg in this study.

REFERENCES


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