The Relationship of Histologic Activity to Serum ALT, HCV genotype and HCV RNA titers in Chronic Hepatitis C

It is unclear whether serum ALT levels or virological characteristics of hepatitis C virus (HCV) including HCV genotypes and HCV RNA titers, can reflect the degree of histological injury in chronic hepatitis C. The aim of this study was to investigate the relationships between the levels of histological damage and serum ALT levels, HCV genotypes or circulating HCV RNA titers in chronic hepatitis C. A total of 56 patients underwent liver biopsy and the histological activity index (HAI) was evaluated by Knodell's scoring system. HCV genotype by RT-nested PCR and HCV RNA quantitation by competitive RT-PCR were performed. Thirty-four patients were infected with HCV genotype 1b, 20 patients with genotype 2a, and 2 patients with undetermined type. Serum ALT levels were not positively correlated with total HAI score or HCV RNA titers, but showed a linear correlation with scores of piecemeal necrosis (r=0.32, p<0.05) and portal inflammation (r=0.27, p<0.05). HCV genotype had no significant correlation with RNA titers, HAI score or with serum ALT levels. Also, no statistical relationship was seen between HCV RNA titer and HAI score. These results suggest that liver histology is essential to evaluate the severity of chronic hepatitis C precisely.

Key Words: Hepatitis C, Chronic; Histology; Reverse Transcriptase-Polymerase Chain Reaction; Genotype; Alanine Transaminase

INTRODUCTION

The patients infected with hepatitis C virus (HCV) have different clinical outcomes, ranging from acute resolving hepatitis to chronic liver disease including liver cirrhosis or hepatocellular carcinoma. Approximately 25-30% of individuals with chronic HCV infections have persistently normal alanine aminotransferase (ALT) level (1, 2) and these individuals are usually referred to as “healthy carrier” of HCV (3). However, several studies have demonstrated that the histological features of most healthy carriers showed chronic liver damage of a variable degree, ranging from mild hepatitis to liver cirrhosis (4-8), and thus the existence of the true “healthy carrier” of HCV is still debatable.

Because the relationship of serum ALT level to liver damage or viral replication in chronic HCV carriers remain unclear, liver biopsy is essential to evaluate the degree of liver damage in these subjects. However, it is practically difficult to perform liver biopsy in all asymptomatic healthy carriers with normal ALT level (9, 10), and therefore, non-invasive approach is required to make an accurate diagnosis on such cases.

Recently, many studies have attempted to investigate the relationship between the characteristics of HCV at molecular level and histological liver damage or the clinical outcome of the patients with chronic hepatitis C, especially those receiving interferon therapy (11, 12). However, studies on the correlations between HCV RNA titers or HCV genotype, and the severity of liver damage have shown conflicting results (13-17). In addition, whether HCV RNA titer is a better predictor of underlying liver injury than serum ALT is not known.

For many years, chronic hepatitis has been classified into chronic persistent hepatitis (CPH), chronic active hepatitis (CAH), and chronic lobular hepatitis (CLH). However, this conventional classification has not fully provided the information to predict the natural course of chronic hepatitis. For liver biopsy specimens of chronic hepatitis, Knodell et al. proposed a numerical scoring system, the Histology Activity Index (HAI), which was graded into four categories: periportal necrosis, intralobular necrosis, portal inflammation, and fibrosis (12). Recently, this index is more commonly used for the evaluation of clinical course or therapeutic response of the patients with chronic hepatitis.

The aim of the present study was to determine whether the degree of histological damage correlates with virological features of HCV and serum ALT level in patients with chronic hepatitis C.
MATERIALS AND METHODS

Patients (Table 1)

Fifty-six consecutive subjects with hepatitis C who had undergone liver biopsy were enrolled from Kangnam St. Mary’s Hospital. They consisted of 36 men and 20 women with ages ranging from 18 to 73 yr (mean=46 yr). The diagnosis of chronic hepatitis C was made on the basis of elevated serum ALT level for more than 6 months, positivity for anti-HCV antibody by the second generation enzyme immunoassays (EIA), the confirmation of HCV RNA by reverse transcription-polymerase chain reaction (RT-PCR), and by histology of liver biopsy specimens. Patients with positive serum HBsAg or autoantibodies (antineutrophil antibody, anti-smooth muscle antibody, and antimitochondrial antibody), or history of alcohol abuse or taking a herbal medicine or clinical (ascites and variceal bleeding) hematological (leukopenia and thrombocytopenia) or biochemical (hypalbuminemia, hyperbilirubinemia, and prolonged prothrombin time) evidence of portal hypertension or hepatic failure by liver cirrhosis were excluded from the study.

Biochemical and Serological tests

Anti-HCV assay was determined by second-generation EIA (Abbott Laboratories, Chicago, Ill, U.S.A.). HBsAg was tested with a radioimmunoassay (Abbott Laboratories, Chicago, Ill, U.S.A.). Serum ALT level was determined at the time of liver biopsy. Anti-nuclear, anti-smooth muscle, and antimitochondrial antibodies were determined by immunofluorescence and titers>1/40 were considered positive.

Histological Assessment

All subjects gave their informed consents to liver biopsy. Formalin-fixed, paraffin-embedded specimens were routinely stained with hematoxylin-eosin and histological examination was carried out by one pathologist according to the conventional criteria. Histological scores were determined according to the Knodell’s HAI scoring system which is most widely used. The HAI score (0-22 points) consists of four major elements: 1) portal/periportal bridging necrosis (0-10) 2) intralobular degeneration and focal necrosis (0-4); 3) portal inflammation (0-4), and fibrosis (0-4).

Detection and genotyping of HCV RNA

HCV RNA was detected from sera of the patients by RT-PCR using primers from the 5' non-coding region, as described previously (18). HCV genotypes were determined with type-specific primers on second round PCR following first amplification of the NS5 gene with universal primer pair as described elsewhere (19, 20). The nomenclature of HCV genotype followed to scheme proposed by Simmonds et al. (21). The oligonucleotide primer sequences used were as follows: universal primer, sense-5'-TGG GGA TCC CGT ATG ATA CCC GCT GCT; universal primer, antisense-5'-GGC GAA ATT CCT GGT CAT AGC CTC CGT GAA-3' for the first PCR; HCVQC1, sense-5'-CGA CAT CCGT ACG GAG GAGG-3'; genotype 1a, antisense-5'-CAG GCT GCC CGG GCC TGG AT-3'; genotype 1b, sense-5'-TGA CAT CCG TGT GGA GT-3'; genotype 1b, antisense-5'-CGG GCC GCA GAG GCC TCC AA-3'; genotype 2a, sense-5'-TAT GTT CAA CAG CAA GGG CCA GA-3'; genotype 2a, antisense-5'-CCT GTG CAT AGC CTC CGT GAA-3'.

Quantification of serum HCV RNA

Serum HCV RNA level was quantified by a competitive RT-PCR using a synthetic mutant HCV RNA as a competitive template as described previously (18). Briefly, the synthesis of cDNA following HCV RNA extraction was done by reverse transcription. An equal amount of sample RNA was put into a set of microtubes that already had 10-fold serially diluted mutant RNA plus annealing mixture containing primer KL70. After cDNA synthesis, second round PCR was performed. The sequences of primers used were: PCRs were performed. The sequences of primers used were:

- HCV QC1, sense-5′- GCC CGG GCC TGG AT-3′; genotype 1a, antisense-5′- CAG GCT GCC CGG GCC TGG AT-3′; genotype 1b, sense-5′- TGA CAT CCG TGT GGA GT-3′; genotype 1b, antisense-5′- CGG GCC GCA GAG GCC TCC AA-3′; genotype 2a, sense-5′- TAT GTT CAA CAG CAA GGG CCA GA-3′; genotype 2a, antisense-5′- CCT GTG CAT AGC CTC CGT GAA-3′.

where (19, 20). The nomenclature of HCV genotype followed to scheme proposed by Simmonds et al. (21). The oligonucleotide primer sequences used were as follows:

- universal primer, sense-5′- TGG GGA TCC CGT ATG ATA CCC GCT GCT; universal primer, antisense-5′- GCC GAA ATT CCT GGT CAT AGC CTC CGT GAA-3′ for the first PCR; HCVQC1, sense-5′- CGA CAT CCGT ACG GAG GAGG-3′; genotype 1a, antisense-5′- CAG GCT GCC CGG GCC TGG AT-3′; genotype 1b, sense-5′- TGA CAT CCG TGT GGA GT-3′; genotype 1b, antisense-5′- CGG GCC GCA GAG GCC TCC AA-3′; genotype 2a, sense-5′- TAT GTT CAA CAG CAA GGG CCA GA-3′; genotype 2a, antisense-5′- CCT GTG CAT AGC CTC CGT GAA-3′.
ethidium bromide and visualized by ultraviolet transilluminator. The titer of circulating HCV RNA was defined by log_{10}(copy number of HCV RNA per milliliter of serum).

Statistical Analysis

The correlations among histologic scores, the ALT level, and the HCV RNA titers were analyzed by the Spearman rank-order correlation coefficient. A p value less than 0.05 was considered statistically significant. To determine whether there was any difference in the histological features between the two genotypic groups, the mean ranks by genotypic group of the histological parameters were compared by Mann-Whitney test.

RESULTS

Correlation between HAI score and serum ALT level

Demographic and virological features of the patients are

Fig. 1. The relationship between serum ALT levels and total HAI score in patients with chronic hepatitis C. The correlation is not significant (r=0.2537, p>0.05).

Fig. 2. The relationship between serum ALT levels and individual component of HAI score: periportal inflammation (A), portal inflammation (B), intralobular degeneration (C) and fibrosis (D). Good correlations of periportal inflammation (r=0.3215, p<0.05) and portal inflammation (r=0.2672, p<0.05) to serum ALT levels are seen. However, there is no significant correlation between serum ALT levels and intralobular degeneration or fibrosis.
shown in Table 1. As the serum ALT increased, the total HAI score also increased, but significant correlation between the two groups was not observed (r=0.2537, p=0.057) (Fig. 1). In relationship between separate component of HAI and ALT level (Fig. 2), the degree of piecemeal necrosis (r=0.3215, p=0.037) and portal inflammation (r=0.2672, p=0.041) significantly correlated with ALT level. However, no significant correlation between the degree of intralobular degeneration (r=0.0812, p=0.115) or fibrosis (r=0.2595, p=0.082) and ALT level was seen.

Correlation between HAI score and circulating HCV RNA titer

Circulating HCV RNA levels through competitive RT-PCR assay were determined by comparing the signal intensities of two bands on agarose gel electrophoresis as shown in Fig. 3. Amplified PCR products derived from the target HCV RNA in sera and the mutant HCV RNA as internal template were 268 base pairs (bp) and 188 bp, respectively. Although the patients with worse histology had a trend toward higher HCV RNA titers, there was no significant correlation between circulating HCV RNA titers and the degree of liver injury (r=0.2495, p=0.058). (Table 2, Fig. 4). None of the individual components of the HAI score in relation to circulating HCV RNA levels showed statistically significant value.

Correlation between HAI score and Genotype of HCV

For the determination of HCV genotype, the HCV NS5 region was amplified by second round PCR with type-specific primers. Of the 56 patients, 34 (60.7%) were infected with genotype 1b, 20 (35.7%) with genotype 2a, and 2 (3.6%) with undetermined genotype. Histological differences between genotype 1b and genotype 2a were compared by the Mann-Whitney test and no significant differences were seen (Table 3).

DISCUSSION

Chronic HCV infection affects approximately 3% of the population worldwide and HCV accounts for approximately 20% of cases of acute hepatitis and 70% of cases of chronic hepatitis (1, 22). The clinical outcome of HCV infections is believed to depend mostly on the balance between the rate of replication of the infecting virus and the capacity of the immune system to mount rapid, multi-specific and efficient
Histology and ALT and Virological Characteristics of HCV

had a mild hepatitis (HAI 
Interestingly, 38.5% of 26 patients with high viremic levels 
circulating HCV RNA titers and the degree of liver injury. 
our study, there was no significant correlation between cir-

In general, chronic hepatitis C patients with elevated ALT 
levels and high HCV RNA titers in the sera are considered to 
have active HCV replication in the liver and to be at risk for 
continued liver injury in a clinical basis. Also, the serum 
ALT level is recognized as a marker reflecting the degree of 
the histological damage and has served as a parameter for 
starting therapy or judging response to antiviral treatment in 
chronic hepatitis C. However, a number of recent studies 
showed ambivalent results in the relationships among the 
degree of histological damage, serum ALT level, HCV RNA 
titers and HCV genotype in chronic hepatitis C.

The aim of this study was to address whether there was a 
correlation between the degree of histological damage and 
serum ALT level or virological characteristics including HCV 
RNA titers or HCV genotype in chronic hepatitis C.

The results of this study revealed no significant correla-
tion between serum ALT level and total HAI. But some 
individual components of the HAI score such as piecemeal 
necrosis and portal inflammation correlated with degree of 
ALT elevation. Our observations are in agreement with pre-
vious reports that showed significant hepatic histological 
abnormalities in patients with normal or near-normal serum 
ALT levels (24) and poor correlation between higher serum 
ALT levels and histological abnormalities (28, 30). These 
results suggest that serum ALT levels do not accurately pre-
dict the presence of liver damage, although it seems to cor-
relate with the severity of architectural changes. Thus, it is 
essential to assess the histological activity of liver damage in 
order to reassure the subjects with minimal disease and to 
identify patients with advanced chronic liver disease (4, 9).

Recently many studies regarding HCV RNA titer and its 
correlation to HAI score have shown conflicting results. In 
our study, there was no significant correlation between cir-
culating HCV RNA titers and the degree of liver injury. 
Interestingly, 38.5% of 26 patients with high viremic levels 
had a mild hepatitis (HAI \( \leq 5 \)), while 16.7% of 30 patients 
with low viremic levels showed severe hepatitis (HAI \( \geq 11 \)) 
on liver biopsy. In addition, none of the individual compo-
nents of the HAI score in relation to circulating HCV RNA 
levels should a statistically significant result. Previous data 
showed discrepant results between HCV RNA titers and 
HAI, while some studies revealed no correlation (15, 25, 28).
Still others showed a significant relationship (11, 13, 29).
Many factors may account for these discrepancies. Firstly, 
the test used to quantitate HCV RNA was different accord-
ting to the studies. Gretch et al. indicated the limitations of 
the bDNA assay for quantitation of HCV RNA, especially 
when viremia is very low or very high (13). Secondly, because 

The clinical outcome of HCV infection can be influenced 
by the HCV genotype. Previous data revealed that genotype 
1 was found in a higher percentage of chronic active hepatitis 
and cirrhosis with respect to other genotypes (35), and 
that the rate of response to interferon was higher in patients 
infected with genotypes 2 and 3 (36). The absence of ALT 
elevation despite evidence of chronic hepatitis might be 
related to infection with a specific HCV genotype and/or to a 
lower degree of viral replication (37). Genotype 2 was 
mainly associated with persistently normal or near-normal 
ALT levels, whereas genotype 1b was prevalent among sub-
jects with elevated ALT (37). However, in the present study, 
no statistical relationship was found between liver damage 
and HCV genotype. This observation is in consistent with 
previous report demonstrating that HCV genotype have lit-	le influence on the progression of chronic liver disease (38).

In conclusion, our study shows that viral load or HCV 
genotype does not accurately predict the degree of liver injury 
in chronic HCV carriers, although serum ALT levels weakly 
correlate with portal inflammation and periportal necrosis. 
Thus, the histological evaluation would be the gold standard 
to accurately assess the degree of liver damage and to decide 
therapeutic plan in patients chronically infected with HCV.

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