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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 Transcriptiase-Polymerase Chain Reaction; Genotype; Alanine Transaminase*

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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 biposy 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 (antinuclear antibody, anti-smooth muscle antibody, and antimitochondrial antibody), or history of alcohol abuse or taking a herbal medicine or plant alkaloids or clinical (ascites and variceal bleeding) hematologic (leukopenia and thrombocytopenia) or biochemical (hypoalbuminemia, hyperbilirubinemia, and prolonged prothrombin time) evidence of portal hypertension or hepatic failure by liver cirrhosis were excluded from the study.

Table 1. Clinical characteristics of patients with chronic hepatitis subjected in this study

No. of Patients	56
Sex (M/F)	36/20
Mean age (yr) (range)	46 (18-73)
ALT (IU/L) (range)*	129.4±82.2 (11-438)
Albumin (g/dL)*	3.8±0.5
Total bilirubin (mg/dL)*	1.6±0.7
WBC (/μL)*	5,200±1030
Platelet (/μL)*	185,000±24,500
Prothrombin index (%)	93.5±7
Anti-HCV positivity (%)	100
HCV RNA positivity (%)	100
HCV RNA titer (range)†	5.9 (4.6-7.6)
HCV genotype (number of subjects)	
HCV 1b	34
HCV 2a	20
Undetermined	2
Histologic Activity Index	
Mean grading	1.5±0.9
Mean staging	2.1±1.2

*Mean±SD, †log copies/mL

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) 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 are were as follows: universal primer, sense-5′-TGG GGA TCC CGT ATG ATA CCC GCT GCT; universal primer, antisense-5′-GGC GGA ATT CCT GGT CAT AGC CTC CGT GAA-3′; genotype 1a, sense-5′-CGA CAT CCGT ACG GAG GAGG-3′; genotype 1a, antisense-5′-CAG GCT GCC CGG GCC TTG AT-3′; genotype 1b, sense-5′-TGA CAT CCG TGT TGA GGA GT-3′; genotype 1b, antisense-5′-CGG GCC GCA GAG GCC TTC AA-3′; genotype 2a, sense-5′-TAT GTT CAA CAG CAA GGG CCA GA-3′; genotype 2a, antisense-5′-CCT GGT 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 describes 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 PCRs were performed. The sequences of primers used were: HCV QC1, sense-5′-CCA CCA TAG ATC ACT CCC CTGT -3′ and HCVL70, antisense-5′-TTG AGG TTT AGG ATT CGT GCT CAT-3′ for the first PCR; HCVQC2, sense-5′-CTG TGA GGA ACT ACT GTC TTCA-3′ and HCVQC3, antisense-5′-ACT CGC AAG CAC CCT ATC AGGC-3′ for second PCR. A 10 μL of second PCR products was analyzed by electrophoresis on 2.5% agarose gel containing

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

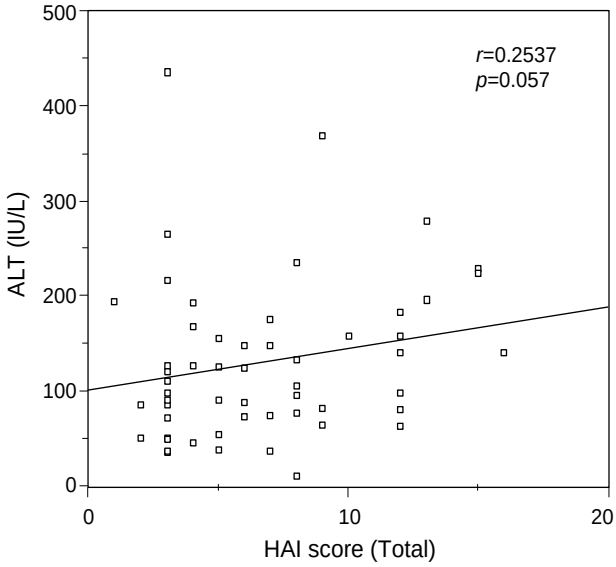

Fig. 1. The relationship between serum ALT levels and total HAI score in patients with chronic hepatitis C. The correlation is not significant (r=0.2537, p>0.05).

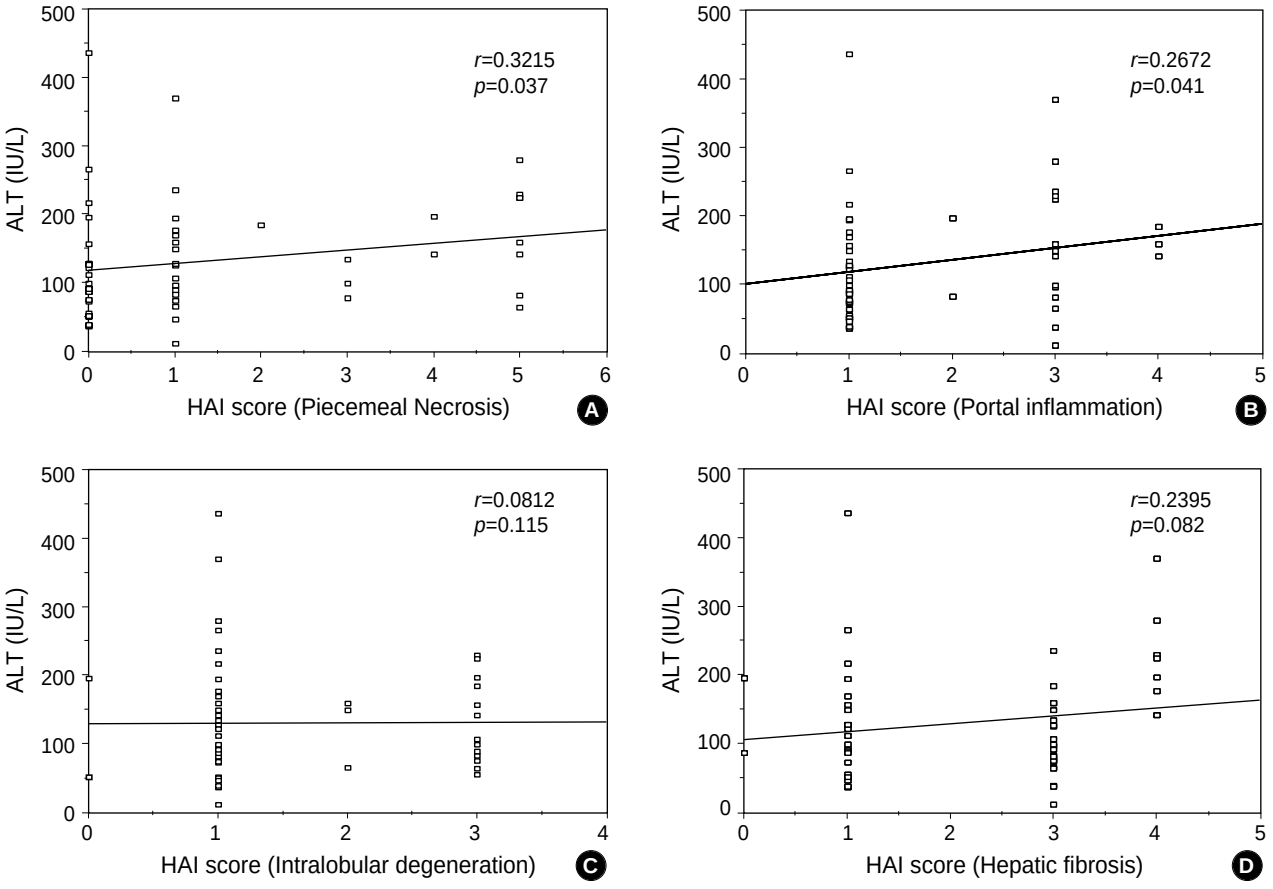

Fig. 2. The relationship between serum ALT levels and individual component of HAI score : periportal inflammation (A), portal inflammation (B), intralobular degeneration (C) and fibrosis (D). Good correlations of periportal inflammation (r=0.3215, p<0.05) and portal inflammation (r=0.2672, p<0.05) to 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_s=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_s= 0.3215, p=0.037) and portal inflammation (r_s=0.2672, p= 0.041) significantly correlated with ALT level. However, no significant correlation between the degree of intralobular degeneration (r_s=0.0812, p=0.115) or fibrosis (r_s=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.

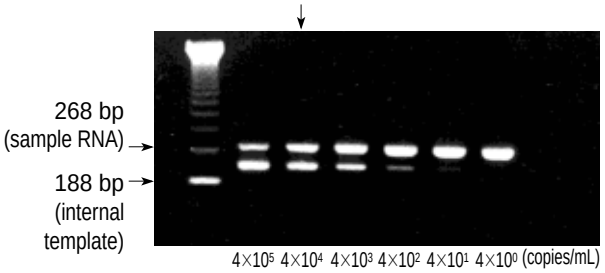

Fig. 3. Competitive RT-PCR assay for measuring HCV RNA titers. Determination of HCV RNA levels in sera. Amplification products were visualized by ethidium bromide staining after gel electrophoresis. 268 and 315 bp DNA bands represent amplification products derived from native HCV RNA and internal control RNA, respectively. Position(↓) of equal intensities of both bands is the HCV RNA titer deduced from sera (4×10^4 copies/mL).

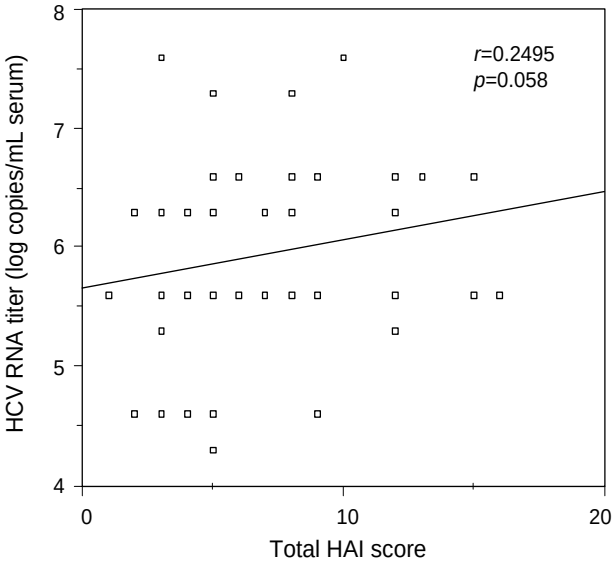

Fig. 4. The relationship between HCV RNA titer and total HAI score. No significant correlation is found between two parameters.

Table 2. Range of HCV RNA titers according to histologic activity index (HAI) by Knodell scoring system

HAI (degree of hepatitis)	Biopsies (n)	Range of HCV RNA titers (log copies/mL)
1-5 (mild)	27	4.6-7.6 (mean:5.7)
6-10 (moderate)	19	4.6-7.6 (mean:6.1)
11-22 (severe)	10	5.3-6.6 (mean:6.0)

Table 3. Histological scores as measured by Knodell scoring systems comparing genotype 1b with genotype 2a. For each category, results are listed as median score (range of score) and finally mean rank for each genotype group

	Periportal inflammation	Portal inflammation	Intralobular degeneration	Fibrosis
Genotype 1b	0 (0,5) 27.94	1 (0,3) 26.93	1 (1,4) 28.07	1 (0,4) 26.85
Genotype 2a	0 (0,5) 25.32	1 (0,3) 27.13	1 (1,3) 25.08	3 (0,4) 27.26
p value	0.523	0.956	0.409	0.92

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_s=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

virus-specific responses to inhibit infection before the virus can devise strategies to evade immune surveillance (23). However, little is known about the precise role played by host or viral factors in the pathogenesis of liver damage due to HCV.

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 (11, 13, 24-30).

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 correlation 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 previous 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 predict the presence of liver damage, although it seems to correlate 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 circulating 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 components 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 according 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 serum HCV load fluctuates and that is not a stable parameter, it can not reflect the degree of liver damage in a given subject (31). Thirdly, HCV replicates in extra-hepatic sites as well as within the liver (32). Thus a high amount of circulating HCV do not always imply a more active state of viral replication in the liver, nor a more severe degree of liver disease (33). Lastly, the discrepancy may result from the time interval between tests of ALT and HCV RNA and performing liver biopsy. Furthermore, the determination of enzyme level on a single serum sample might not be related to the ALT profile over time (34). However, in this study both ALT test and HCV RNA titer were drawn on the same day with the liver biopsy.

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 subjects 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 little 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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