

THE RELATIONSHIP OF SERUM AMINOTRANSFERASE LEVELS TO VIRAL LOAD AND GENOTYPE IN CHRONIC HEPATITIS C

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Summary: Chronic hepatitis C is a slowly progressing inflammatory disease of the liver and the main cause of cirrhosis and hepatocellular carcinoma worldwide. Different factors have been proposed to determine the clinical outcome of HCV infection. The precise mechanism by which hepatitis C virus damages the liver remains poorly understood. Many studies observed that HCV RNA levels and aminotransferase levels as markers of liver damage and many worldwide studies provide evidence that patients with a lower viral load have better response rates to antiviral therapy compared to those with higher levels. Moreover, a direct association has been observed between serum titers of HCV and transmission rates of the virus. The aim of this study was to determine the existence of any correlation between HCV viral load, genotype of virus and serum aminotransferase levels in our group of previously untreated chronically infected patients. Dominant genotype among our study group was 1b and we observed that patients with this genotype have significantly higher both ALT levels and viral load in comparison with other genotypes. We found a significant inverse correlation between serum HCV RNA viral load and ALT levels in our group of patients, but no correlation between viral load and AST levels. Our results suggest that HCV RNA viral load is the most sensitive marker of disease activity and all patients with high viral load must be treated, including patients with low or normal serum aminotransferase levels.

Key words: chronic hepatitis C, serum aminotransferase levels, HCV RNA viral load HCV genotype

Introduction

Hepatitis C virus (HCV) is an enveloped positive stranded RNA virus that preferentially replicates in hepatocytes (1). HCV is a bloodborne pathogen that is endemic in most parts of the world, with an estimated overall prevalence of nearly 3% (2) Prevalence of chronic hepatitis C in our country (Serbia and Montenegro) is estimated at about 1% in whole population (3). Approximately 80% of patients with hepatitis C virus develop chronic infection (CHC), and progression to cirrhosis occurs in nearly 20% of these subjects (4).

Moreover, patients with HCV-related cirrhosis are at increased risk of developing hepatocellular carcinoma, which is estimated to occur at the rate of 1.5% to 4% per year (5). Many attempts to identify the natural history, progression, and treatment of HCV infection have been made, but several aspects remain to be elucidated. In chronically infected individuals, viral load, genotype, and elevated serum alanine aminotransferase (ALT) levels may have clinical relevance (5).

Several studies have assessed the correlation between serum HCV viral titers and different clinical and laboratory parameters. The most important knowledge is that HCV titers have been found to be associated with responses to antiviral treatment. Patients with a baseline HCV viral load of $\leq 2 \times 10^6$ copies/mL have significantly better responses to antiviral therapy compared to those with higher viral titers (6). Patients with HCV genotype 1 have been found in some studies to have higher viral loads than those with HCV genotype 2, 3, 4 or mixed (7, 8). Previous

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attempts to assess the effect of viral titers on the severity of liver disease have produced conflicting results and the present study was designed to examine this issue in more detail.

Serum aminotransferases levels are elevated in the majority of patients with chronic HCV infection. Whether there is any correlation between aminotransferase level, viral load and genotype is still unclear. Additional potential correlates of HCV load with the patient's age, sex, hepatitis duration of infection and mode of infection have been investigated, with various results (9–13).

In the present study, we investigated the relationship between HCV genotypes, viral loads and aminotransferase activity, and the correlation between these parameters in chronic hepatitis C.

Materials and Methods

Patients eligible for the study were adults of Caucasian origin, previously untreated with interferon or other antiviral therapy (treatment-naïve) and with the following characteristics: positive anti-HCV antibody for more than 6 months; chronic hepatitis C virus infection diagnosed on the basis of a positive anti-HCV antibodies enzyme-linked immunoblot assay (*ELISA*, *Ortho Diagnostics, Germany*) and confirmed with liver biopsy result which showed histopathological evidence of chronic hepatitis. Also, patients were between 18 and 65 years of age and all patients were negative for other causes of chronic liver disease including hepatitis B virus infection. Patients were interviewed with respect to alcohol and drug abuse, and in those with a positive history the periods of abstinence were longer than 12 months before the study. The following exclusion criteria were applied: clinical and biochemical evidence of advanced disease, such as decompensate cirrhosis; serum albumin < 35 g/L; platelet count < 100 000/ μ L; white cell count < 3500/ μ L; anemia (haemoglobin concentration of < 120 g/L in women and 130 g/L in men); psychiatric conditions; uncontrolled diabetes; autoimmune diseases; concurrent hepatitis B virus or human immunodeficiency virus infection; alcohol intake; current intravenous drug use; pregnancy; or concomitant significant medical illnesses.

Quantitative HCV analysis

Serological blood examination evidenced anti-HCV positivity in two samples obtained in a one-month interval. Serum HCV RNA level was determined using PCR method (*Amplicor, Roche Diagnostics*). The results of viral RNA titers in clinical samples are expressed as number of viral copies per mL (copies/mL). Viral genotype was determined using Line probe assay (*LiPa, Innogenetics, Ghent, Belgium*). The HCV genotypes were designed according to the

nomenclature proposed by Simmonds classification (14), which distinguishes five HCV RNK genotypes: 1b, 2a, 3, 4 and mixed genotype.

Biochemical analysis

Serum ALT and AST activity was measured with a multichannel analyser type Monarch plus IL at 37°C, according to the manufacturer's protocol (commercial reagents, *Dialab Diagnostic, GmbH, Vienna, Austria*). Reagent kit for the determination of the ALT and AST activity in serum based upon IFCC (*International Federation of Clinical Chemistry and Laboratory Medicine*) recommendations.

Statistical analysis

Descriptive statistical analyses were performed, and the results are presented as mean \pm SD. Baseline data were descriptively summarized and assessment of differences was completed using Students t-test. The Students t-test was used to assess the difference in HCV RNA levels between different genotypes (using Microsoft Excel). Linear regression analysis was employed to examine the presence of any correlation between serum HCV RNA levels and different laboratory parameters. Also, statistical analysis was carried out using GraphPad Prism/Instat 1.1 (GraphPad Software, California, USA) using one-way analysis of variance (ANOVA) followed by Dunnett's post-significance testing. A *P* value of less than 0.05 was considered to indicate significance (NS = non significant).

Results

The demographic and clinical characteristics of all patients included in the study are shown in *Table 1*.

Females included in the study were significantly older than males (43.61 \pm 13.52 vs. 36.93 \pm 10.98 years; *t*=2.613, *DF*=92, *p*=0.0105; *P*<0.05). Also, an unknown way of disease transmission was dominant among females in comparison with males (63% vs. 35%) while IVDU was the dominant route of disease transmission among male patients. There was no correlation between HCV RNA levels and the age of the patients (*r*=0.1803, *p*=0.08; *P*=*N.S.*).

We further speculate that HCV genotype differences may potentially contribute to higher HCV RNA levels among our patients.

Dominant genotype in our study group was genotype 1b (40/93; 43%) which is significantly higher than incidence of other genotypes (χ^2 =9.502, *p*=0.0021; *P*<0.01). Also, in the group of patients with genotype 1b, there was a significantly higher number of patients with HCV RNA level higher than 2 million copies/ml in comparison with other genotypes (χ^2 =9.713, *p*=0.0045; *P*<0.05). Furthermore, pati-

Table I Characteristics of the patients with chronic hepatitis in this study

Patient's characteristics	Total patients (n = 93)
Age (yr) (range)	38.3 ± 12.1 ^a (18-65)
Gender (male/female) (%)	57/36 (61%/39%)
<i>Possible way of transmission of disease</i>	
History of blood transfusion	17 (18%)
IVDU	36 (39%)
Professional exposure	1 (1%)
Risk sexual behavior	2 (2%)
Unknown	37 (40%)
<i>Genotype n (%)</i>	
1b	40 (43%)
2	6 (6%)
3	25 (27%)
4	12 (13%)
Mixed	10 (11%)
ALT (IU/L) (range)	70.29 ± 37.23 ^a (30-214)
AST (IU/L) (range)	42.94 ± 19.45 ^a (21-115)
HCV RNA titer (copies/mL)	2862570.38 ± 825897.318
HCV RNA titer (log ₁₀ copies/mL)	5.9 ± 0.65
Abbreviations: ^a Mean ± SD, IVDU, intravenous drug addictions	

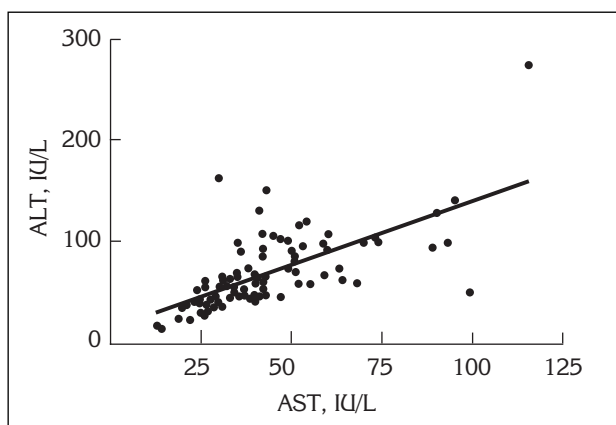


Figure 1 Correlation between alanine and aspartate aminotransferase levels in a study group of patients, $r=0.657, P<0.001$

ents with genotype 1b have significantly higher levels of ALT in comparison with other genotypes (1b vs. others: 78.88 ± 41.32 vs. 58.84 ± 19.06 ; $t=2.285, p=0.0247; P<0.05$), but not significantly higher levels of AST (1b vs. others: 45.75 ± 19.86 vs. 43.46 ± 19.26 ; $t=0.5912, p=0.5558; P=N.S.$). Patients in group 1b also have a significantly higher level of HCV RNA in comparison with other genotypes: $6\,314\,000 \pm 1\,845\,000$ vs. $1\,566\,000 \pm 339\,800$, $t=2.882, p=0.0049; P<0.01$

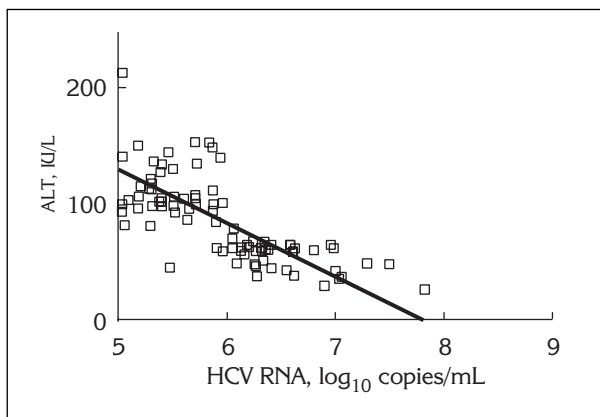


Figure 2 Correlation between ALT levels and HCV RNA viral load in patients with chronic hepatitis C, $r=-0.8, P<0.01$

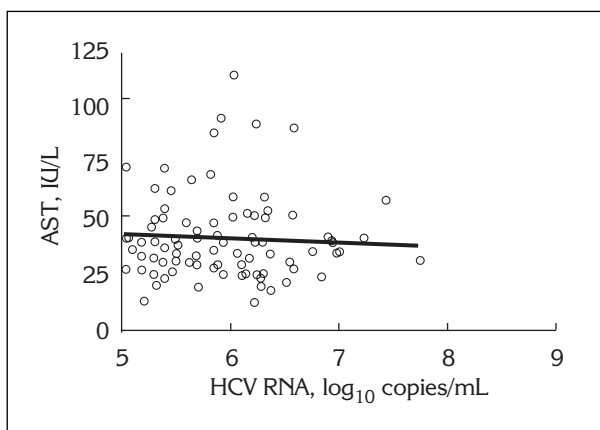


Figure 3 Correlation between AST levels and HCV RNA viral load in patients with chronic hepatitis C, $r=-0.05904, P=0.5740$

There was, as we expected, significant correlation between serum aminotransferase ALT and AST levels ($r=0.657, P<0.001$) (Figure 1).

In our study group we also observed that patients with high viral load have almost normal serum aminotransferase levels, while patients with relatively small viral load have very high serum aminotransferase level, especially ALT.

Values of HCV RNA viral load were significantly inversely correlated with ALT levels ($r=-0.8, P<0.01$) (Figure 2).

At the same time we observed that inverse correlation exists when we compare viral load and AST levels, but this correlation is not statistically significant ($r=-0.05904, p=0.5740; P=N.S.$) (Figure 3).

Discussion

Chronic hepatitis C virus infection affects approximately 3% of the population worldwide (2, 4). The

clinical outcome of the CHC depends mostly on the balance between the rate of virus replication and the capacity of the immune system to mount rapid, multispecific and efficient virus-specific responses to inhibit infection before the virus devises strategies to evade immune surveillance (13, 15).

In general, chronic hepatitis C patients with elevated ALT levels and high HCV RNA titers in sera are considered to have active CHC and at the same time these two parameters are recognized as a marker reflecting the degree of histological damage and have served as parameters for starting therapy or judging the response to antiviral treatment in chronic hepatitis C. Beside serum aminotransferase levels, HCV RNA viral load and HCV genotype in CHC, several other factors have also been implicated in predicting the rate of progression of HCV-related chronic liver disease. These include the age at acquisition of HCV infection, gender of the patient, alcohol abuse and co-infection with HBV and HIV infections (5, 11, 15).

Cross-sectional studies on the correlation between serum HCV RNA viral load and serum aminotransferase (ALT and AST) levels in patients with chronic hepatitis C have yielded conflicting results. Some found no correlation between HCV viral load and serum ALT levels and at the same time the extent of histological damage (8,16,17). On the other hand, Kato et al. (18) observed significantly higher HCV RNA titers in patients with chronic active hepatitis and cirrhosis compared to those with milder histological abnormalities such as persistent chronic hepatitis.

We conducted a longitudinal study to examine the correlation between HCV RNA viral load and serum aminotransferase levels in individual patients.

Our patients were categorized into two groups according to the genotype of virus: first group had dominant genotype 1b (40/93 patients; 43%) and second group had other genotypes: 2, 3, 4 and mixed (53/93 patients; 57%). We compared these two groups and found that the serum viral load and ALT levels were significantly higher in the first group ($P < 0.01$ and $P < 0.05$, respectively).

Bozdayia et al. (19) got similar results in their study and they suggest that hepatitis C patients infected with genotype 1b may show a relatively weaker immune response resulting in lower ALT and higher viremia levels, while patients with higher ALT levels and lower viral load have more active immune response to chronic viral infection.

Viral load in our group of patients showed significant inverse correlation with ALT levels. In addition, viral load was significantly higher in patients with normal ALT levels than in those with a high level of ALT. Also, at the same time we observed no significant correlation between HCV RNA viral load and AST levels, while these two aminotransferase (ALT/AST) levels were in positive correlation in our study

group. These findings suggest that immune response to hepatitis C virus infection plays an important role in chronic HCV infection.

Kurasaki et al. (20) showed that the ALT in the HCV RNA high level group was much higher than that in the HCV RNA low level group and indicated that HCV replication is related to the progress of chronic liver disease, and supported the theory that HCV may have cytopathogenic effect.

Ghany et al. (21) found in their investigation significant correlation between serum HCV RNA viral load and ALT levels in the patients who received therapy (interferon), but no correlation was observed in the untreated and immunosuppressed patients. Among the untreated and the immunosuppressed patients they found significantly higher viral load and significantly lower ALT levels when compared to the immunocompetent and previously treated patients. Ghany et al. (21) also suggest that immunosuppression results in higher HCV RNA but lower ALT levels in chronically HCV infected patients.

Zechini et al. (22) in their recent study found significant correlation among viral load, ALT and AST levels. Also, their study results demonstrated a statistically significant correlation of aminotransferase levels with the histological parameters, and an even stronger correlation with the AST levels and suggested that aminotransferase values, especially AST, may correlate with the degree of liver damage. Considerable changes in viral load are sometimes observed during the normal course of HCV infection. Ito et al. (23) observed that changes of serum aminotransferase correspond to the changes of viral load of HCV and vice versa and that they have been frequently observed in chronic hepatitis B infection.

However, some authors have reported findings different from ours on the correlations between ALT levels and viral load – that patients with low or normal levels of ALT showed small viral load (24). Dincer et al. (25) observed that there was no significant difference in viral load between patients with abnormal ALT levels and those with normal ALT levels.

The precise mechanism by which hepatitis C virus damages the liver remains poorly understood. Our results indicate that the severity of liver disease is independent from serum levels of hepatitis C virus and that HCV RNA viral load significantly correlates inversely with ALT levels. Thus, we suggested that viral load is partly affected by inflammatory activity signified by ALT levels, and the inverse correlation of viral load with ALT levels suggests that viral load is in suppression by liver inflammation.

Our results suggest that viral load in chronically infected individuals is the most sensitive marker of disease activity and all patients with high HCV RNA viral load must be treated including patients with low or normal ALT levels.

ODNOS IZMEĐU AKTIVNOSTI SERUMSKIH TRANSAMINAZA, NIVOA VIREMIJE I GENOTIPA KOD HRONIČNOG HEPATITISA C

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Kratak sadržaj: Hronični hepatitis C je sporo progredirajuća inflamatorna bolest jetre i vodeći uzrok razvoja ciroze i hepatocelularnog karcinoma u svetu. Različiti faktori se smatraju odgovornim za klinički ishod ove infekcije. Cilj ove studije bio je da ispita povezanost nivoa viremije, genotipa virusa i aktivnosti serumskih transaminaza kod hronično inficiranih predhodno nelečenih bolesnika. Dominantni genotip u ispitanoj grupi bolesnika bio je 1b i bolesnici inficirani sa ovim genotipom imali su statistički značajno veću aktivnost ALT i veću viremiju u poređenju sa drugim genotipovima. Ispitivanje je pokazalo da u ispitanoj grupi ispitanika postoji statistički značajna inverzna korelacija između nivoa viremije i aktivnosti alanin aminotransferase (ALT). Istovremeno, nije dokazana povezanost viremije i aktivnosti aspartat aminotransferaze (AST). Dobijeni rezultati ukazuju da je vrednost viremije najosetljiviji pokazatelj aktivnosti bolesti, mnogo osetljiviji od aktivnosti ALT.

Ključne reči: hronični hepatitis C, aktivnost serumskih transaminaza, HCV RNK genotip HCV

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