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by Eti Yerizel

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Research Article

Correlations of Viral Load Toward HCV Core Antigen and Aminotransferase in Hepatitis C Virus Genotype 1 Infection

¹Eti Yerizel, ²Almurdi and ³Gusti Revilla

¹Department of Biochemistry, Faculty of Medicine, Andalas University, Indonesia

²Department of Clinical Pathology, Faculty of Medicine, Andalas University, Indonesia

³Department of Anatomy, Faculty of Medicine, Andalas University, Indonesia

Abstract

Background and Objective: Hepatitis C becomes a problem in liver disease over the world. The diagnosis of hepatitis C infection is carried out by detecting of antibodies against the virus using an immunoassay but unreliable in the seroconversion phase of infection, due to the long window period. Real time PCR examination and hepatitis C virus core antigen (HCVcAg) detection by ELISA offer an alternative method to detect the virus infection. The aim of this study was to identify the viral load correlation with the number of HCV core antigen and aminotransferase levels in HCV infection. **Materials and Methods:** This study combined the molecular examination for determining the genotype of HCV and viral load with q-PCR and ELISA method for investigating the HCVcAg. The correlation between number of viral load with HCVcAg and Alanine aminotransferase (ALT) and aspartate transaminase (AST) were analyzed with Pearson's correlation test. **Results:** There was a significant correlation between the number of viral load with HCVcAg levels in hepatitis C patients but levels of ALT (49.71 U mL⁻¹) and AST (31.83 U mL⁻¹) serum were not correlated with the number of viral load. **Conclusion:** HCVcAg can be applied as an alternative examination to detected viral load in hepatitis C infection to prevent the infection of HCV lead to cirrhosis and even hepatocellular carcinoma.

Key words: Viral load, hepatitis C virus core antigen, alanine aminotransferase, aspartate transaminase, genotype-1, hepatitis C virus

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Corresponding Author: Almurdi, Department of Clinical Pathology, Faculty of Medicine, University of Andalas Padang, Jl. Perintis Kemerdekaan No. 94, P.O. BOX 49, Padang, 25127 West Sumatra, Indonesia Tel: +6275131746 Fax: +6275132838

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

Ethics Committee of Medical Faculty, Andalas University, Padang, Indonesia.

Viral RNA extraction and RT-PCR: Serum samples stored at -80°C were retrieved for analysis. The HCV RNA was extracted from 140 μL serum or plasma using QIAamp viral RNA mini kit (Qiagen, German) according to the manufacturer's protocol and stored at -80°C until further analysis.

RT-PCR was performed using one-step RT-PCR kit (Invitrogen, Inc). The NS5B region was amplified by PCR to identify the HCV genotype with forward primers NS5B-1: 5'-TATGAYACCCGYTGCTTGAC-3' and reverse primers NS5B-2: 5'-GAGGAGCAAGATGTTATCAGCTC-3'¹⁹. DNA amplification was performed for 40 cycles each consisting of 94°C for 15 sec, 55°C for 30 sec and 72°C for 1 min in a thermal cycler (Biorad, USA). The last cycle was followed by a 5 min extension step at 72°C . One microliter of amplicons was used for second-round PCR using Go TaqGreen (Promega, USA). The following cycling parameters were used for 35 cycles.

Quantification of HCV RNA was performed using the Bio-Rad HCV RT-PCR kit according to the manufacturer instructions.

DNA purification and sequencing: Amplicons were purified by using the QIAquick PCR purification kit (Qiagen, German) and analyzed by ethidium bromide 2% agarose gel electrophoresis. Samples showing a band of the appropriate size (449 bp) were further analyze by DNA sequencing in Macrogen, Republic of Korea. The HCV genotyping was based on NS5B, the reference sequences were retrieved from the NCBI GeneBank.

Alignment of sequence target: The sequence of DNA was edited using Geneious's version R 7.

HCV core antigen assay: Sera samples were assayed for HCV core antigen according to the manufacturer's instructions using an EIA kit (Eitest-Biolabs, USA). Briefly, 100 μL of samples and controls to anti-HCVc Ag antibody were coated in plate, incubated for 2 h and wash five times with 250 μL wash buffer. One hundred microliters of FITC-Conjugated anti HCVcAg were added to each well, incubated and washed, 100 μL HRP-conjugated anti-FITC monoclonal antibody was added to all well. A substrate solution was added and stopped the enzyme reaction by adding stop solution. The absorbance of each well was measured using ELISA reader at 450 nm.

Biochemical tests: Liver function tests, including alanin aminotransferase (ALT) and aspartate aminotransferase (AST)

were performed on an autoanalyzer (Selectra E-Mrk, Germany) by manufacture' protocol.

Statistical analysis: Statistical analysis were performed using SPSS 15.0, the correlation between parameters was analyzed by Pearson's correlation test. Pooled data are presented as means. Data were compared using Student t-test. p-values <0.05 were considered statistically significant.

RESULTS

In this study, 75 samples were obtained from anti-HCV positive blood donors and from clinical laboratory, 53 samples (70.67%) with HCV specific positive signal by PCR, consist of 39 genotype-1 (78.0%), 2 genotype-2 (4.0%), 9 genotype-3 (18.0%) and three samples were undetermined.

The number of viral load of patients infected with hepatitis genotype-1 virus (6.82 log copy/mL) was significantly different ($p = 0.000$) compared with other genotypes (6.26 log copy mL⁻¹). The number of HCV core antigens (HCVcAg) of patients infected with hepatitis C virus genotype 1 did not differ significantly with other HCVcAg ($p > 0.169$) (Table 1). ALT serum levels of patients infected with hepatitis C virus were slightly above normal, while AST serum levels were within normal limits.

A significant correlation was showed in viral load of patients infected by genotype-1 hepatitis C virus with HCVcAg ($p = 0.000$) with $r = 0.773$. This correlation indicated positive linear, the higher the viral load was followed by the elevation of HCV core antigen levels.

A significant correlation ($p = 0.001$) between viral load of all genotype hepatitis C-infected patients with HCVcAg levels presents moderate correlation ($r = 0.474$) as shown in Fig. 1a and b.

There was no significant correlation ($p = 0.10$) between hepatitis C viral load with serum ALT level, the strength of correlation was moderate ($r = 0.426$) and no significant correlation ($p = 0.126$) between hepatitis C viral load with AST serum levels and the strength of correlation was less ($r = 0.259$) (Fig. 2a, b).

Table 1: Average of several parameters on HCV infection

Parameters	Mean (SD)	p-value
Viral load (log copy mL⁻¹)		
Genotype 1	6.82 \pm 6.85	0.000
Other genotype (2/3)	6.26 \pm 6.30	
HCVcAg (log pg mL⁻¹)		
Genotype 1	5.78 \pm 5.09	0.169
Other genotype (2/3)	5.74 \pm 5.27	
ALT (U mL ⁻¹)	49.71 \pm 28.8	
AST (U mL ⁻¹)	31.83 \pm 18.1	

ALT: Alanine aminotransferase and AST: Aspartate transaminase

standard. The persistence of HCV after acute infection is often undiagnosed causing chronic hepatitis, liver cirrhosis and even death. Spontaneous clearance of HCV infection is only found in about 17% of untreated patients found to be anti-HCV reactive with negative HCV RNA. The benefit of HCV protein cores test is to use the immunology format, requiring no sample processing such as molecular examination, no carry-over risk and relatively cheap checking rates.

In this study, there was a significant difference between the numbers of viral load of patients infected with genotype-1 hepatitis virus with other genotypes. Gremion and Cerny²¹ reported that genotype-1 HCV is often associated with severe liver disease, while the genotype-2 viral infection is found in asymptomatic carriers. Low number of viral load can also be found in patients undergoing antiviral therapy or in patients with hemodialysis; because the particles of virus can be broken as they pass through the dialysis membrane²².

There was a significant correlation between the numbers of viral load of hepatitis C-infected patients with HCVcAg levels, because HCVcAg can be found in serum of HCV-infected patients in virion and also in core free RNA protein form. Several studies have indicated that HCVcAg kinetics is proportional to HCV RNA in various infections and HCVcAg concentrations with HCV RNA has a good correlation. HCVcAg examination performance is similar to HCV RNA examination which can decrease window period, besides it can also be used to monitor antiviral therapy in patients with chronic infection²³.

The detection and quantification of HCV RNA has become the standard method for diagnosis and monitoring of chronic HCV infection and for assessing the response of antiviral treatment to HCV infection²⁴. In addition, RNA viral is also used as an acute infection marker before anti-HCV seroconversion. HCVcAg examination is recommended as an alternative examination to determine the viral load in patients with hepatitis C virus infection²⁵.

The liver is an organ that contains many aminotransferase enzymes, this enzyme will be released in large quantities to the circulation during liver damage due to inflammation. High level of ALT serum in blood indicates the occurrence of necro-inflammation. Liver damage appears in the process of immune clearance, long response of immune processes will increase the risk of liver cirrhosis. The examination of ALT enzymes provides important information about the state of liver function. According to Fabris *et al.*²⁶ that the levels of ALT enzyme increase after 10-14 weeks of initial infection and the elevation reaches up to 20 times of the normal limit level but a slight increase in ALT levels is often found mainly in chronic infections, as found in this study.

In this study, there was no significant correlation between viral load and aminotransferase levels, both ALT and AST levels. This could be due to inadequate of immune response, especially in chronic infection. According to Maheshwari *et al.*²⁷ the failure to produce an effective immune response during acute infection is a key factor in the development of HCV infection becomes chronic hepatitis.

The average levels of ALT was higher compared with AST levels in the study, it is caused by ALT as a cytosolic enzyme that is found with high concentrations and more specific in the liver. AST enzymes are found in the liver, also in heart muscle, skeletal muscle, kidney, brain, pancreas and lung. This enzyme is less specific for the liver.

This study is very useful for determining the number of viruses in the beginning of infection and for follow-up therapy. When quantitative viral load testing is not available, HCV core antigen examination can be used as an alternative assay.

CONCLUSION

Based on the results of the study, it can be concluded that a significant correlation between hepatitis C viral load and HCV core antigen levels indicates that HCVcAg examination can be used as an alternative assay to determine viral load in hepatitis C viral infection, in order to follow up therapy.

SIGNIFICANCE STATEMENT

The examination of HCV RNA by reverse transcriptase PCR is a very sensitive method but consumes much time, cost and complete equipment requirements. In contrast, the ELISA examination for the detection of HCV core antigens is simpler and inexpensive. By combining the molecular examination for determining the genotype of hepatitis C virus and viral load with q-PCR and ELISA method for investigating the core HCV (HCVcAg) antigen, this study can be the novel alternative to prevent the infection of HCV lead to cirrhosis and even hepatocellular carcinoma.

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