COMPARISON OF HCV RNA AND HCV CORE ANTIGEN IN GENOTYPE 1 CHRONIC HEPATITIS C PATIENTS

OGUZ KARABAY[1], AZIZ ÖĞÜTLÜ[1], HANDAN ANKARALI[2], FATMA ÖZDEMIR[3], MELTEM KARABAY[4], HASAN TAHSIN GOZDAS[1]
[1]Department of Infectious Diseases and Clinical Microbiology, Sakarya University Faculty of Medicine, Sakarya - [2]Department of Biostatistics and Medical Education, Düzce University Faculty of Medicine, Düzce - [3]Department of Biochemistry, Sakarya University Faculty of Medicine, Sakarya - [4]Department of Pediatrics, Sakarya University Faculty of Medicine, Sakarya, Turkey

ABSTRACT

***Background**: Anti-HCV test is currently used in the diagnosis of hepatitis C, while hepatitis C virus ribonucleic acid (HCV RNA) is used in the determination of viral load. Recently developed hepatitis C virus core antigen (HCV Ag) test is also used in the diagnosis of hepatitis C. The aim of this study was to compare HCV Ag and quantitative HCV RNA in terms of sensitivity and specificity as well as pharmacoeconomic footprint.*

***Patients and methods**: Sixty patients with positive anti-HCV were enrolled to this study. Quantitative HCV RNA was studied by ready commercial kits (Qiagen, lower limit of <12 IU/ml). HCV Ag test was studied with microparticule chemiluminiscent immunoassay method using ready commercial kits (Architect HCV Ag, Abbott).*

***Conclusions**: HCV Ag and HCV RNA levels were measured in the sera of chronic hepatitis C patients. Quite high correlation was found between the two tests (r = 0.83). According to our results, HCV Ag test may be an alternative to quantitative reverse transcription polymerase chain reaction (PCR) test. Thus, use of HCV Ag in place of HCV RNA will be more cost-effective for countries with limited sources.*

***Key words**: HCV Ag, HCV RNA, hepatitis C, diagnosis, follow-up, cost-effectiveness.*

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Introduction

Hepatitis C virus (HCV) is one of the important agents that cause chronic viral hepatitis.

Chronic HCV infection may cause a variety of conditions from mild liver damage to cirrhosis and hepatocellular carcinoma. The prevalence of HCV infection in Turkey is about 1%[(1)].

Anti-HCV tests, recombinant immunoblotting and hepatitis C virus ribonucleic acid (HCV RNA) are used in the diagnosis and follow-up of HCV infection[(2)]. Although the second and third generation anti-HCV tests have been developed, these tests have limitations due to low sensitivity in the early window period between day 45 and 68[(3)].

On the last decade, hepatitis C virus core antigen (HCV Ag) test entered the literature in adjunction to anti-HCV and real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) test[(4,5)]. These tests can either be used in the diagnosis of HCV infection or monitoring antiviral therapy[(6,7)]. HCV Ag tests can also be used in monitoring of immunocompromised patients and patients undergoing hemodialysis[(8)]. But in the past, many of the tests used in the determination of HCV Ag were enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) tests that requires time and skill[(9-12)]. In recent years, full automatic chemiluminescence immunoassay (CLIA), which eliminates the disadvantages of the old core Ag tests and yields high sensitivity, has been developed[(13)]. The aim of this study was to evaluate the usefulness of the newly developed HCV Ag test in comparison to HCV RNA qRT-PCR.

Methods

This study was conducted at the Department of Infectious Diseases and Clinical Microbiology in Sakarya University Medical Faculty between

November 2009 - March 2010. Ethical consent was approved by the the local ethical committee of Düzce University.

Patients: Patients with chronic HCV infection who presented to our clinic between December 2009 - March 2010 were subjected to this study. Some of the patients were under treatment, while some of them were on follow-up. In total, 60 patients among whom 29 are male were enrolled in the study.

Inclusion criteria were

1. Patients between 18-65 years of age and diagnosed with HCV genotype 1 (confirmed by standard tests) were included in the study. All patients were required to show the below findings of chronic HCV:

• to have a detectable HCV RNA in serum previously.

• Histology Activity Index (HAI) > 4 or Fibrosis> 1 in liver biopsy with Modified Knodell scoring

2. Neutrophil count:> 1200/mm^3

3. Platelet count: > 90.000/mm^3

4. Hemoglobin > 11g/dl

5. Hepatitis B surface antigen (HBsAg): Negative

6. Human immunodeficiency virus (HIV)1 and 2 antibodies: Negative

7. Female patients not to be pregnant

8. Not the concurrent use of any drug or nutrient that may affect test results

9. Informed consent form to be read and signed by all the patients with respect to accept the study.

Exclusion criteria were

1. Antinuclear antibody (ANA) titer of 1:320 and above

2. HIV or hepatitis B virus (HBV) positivity

3. Cirrhosis, ascites or have a history of or variceal bleeding

4. Suspected hepatocellular carcinoma [alpha-fetoprotein (AFP) > 100 ng / mL, or mass in ultrasound]

5. HAI>12 or fibrosis> 4 in liver biopsy.

6. Determination of alcoholism

All patients diagnosed with chronic HCV were tested for liver enzymes [Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), bilirubin, albumin, total protein, prothrombin time). Abdominal ultrasound was performed to patients. Blood samples of the patients were obtained and stored at -20 ° C until the study day. Quantitative HCV RNA were measured by ready commercial kits (Qiagen, lower limit of <12 IU / ml). HCV core antigen test was studied with microparticule chemiluminiscent immunoassay method using ready commercial kits (Architect HCV Ag, Abbott).

Descriptive values were given as mean Standard deviation. Receiver operating characteristic (ROC) analysis was used to assess the diagnostic achivement of HCV Ag, Spearman's rank correlation analysis to examine the relationship between the results of HCV RNA and HCV Ag, paired t-test to compare ALT levels before and after treatment. For the statistical tests, PASW (SPSS, ver. 18.0) statistical packet programme was used and $p<0.05$ was accepted statistically significant.

Results

There were 60 patients in our study among whom 29 were male.

The mean age was 54.9 (16-76). The mean ALT: 38 IU / l, mean GGT :44, mean hemoglobin: 12.4 g / dl. There were 12 cases of renal failure undergoing hemodialysis. Evaluation of the efficacy and sensitivity of the test was presented in Table 1.

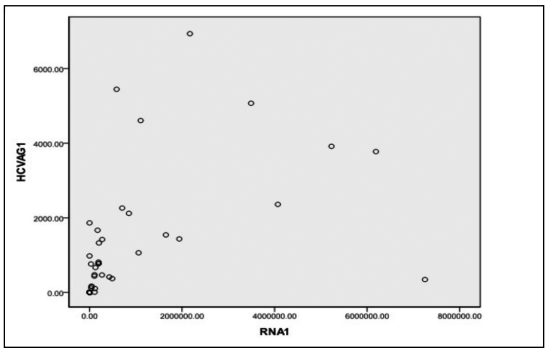

Figure 1: Sensitivity of HCV Ag test according to gold standart HCV RNA.

HCV Ag	Result	HCV RNA >2000 IU/ml
Sensitivity	94.10%	100%
Specificity	84.60%	86.70%
Positive predictive value	94.10%	94.10%
Negative predictive value	84.60%	100%
Correlation	r=0.830	r =0.703
	P=0.0001	P=0.0001
Overall success of diagnostic accuracy	91.30%	

Table 1: Comparison of HCV Ag levels with respect to HCV RNA levels.

It was seen that HCV RNA level increased in parallel to HCV Ag level (Figure 1) especially when HCV RNA levels were more than 2000 IU/ml. All of the 12 patients undergoing hemodialysis showed meaningful results in accordance with HCV RNA. Therefore, positive diagnosic achivement based on HCV Ag (sensitivity) was found 100% in this group (Table 1 and Figure 2).

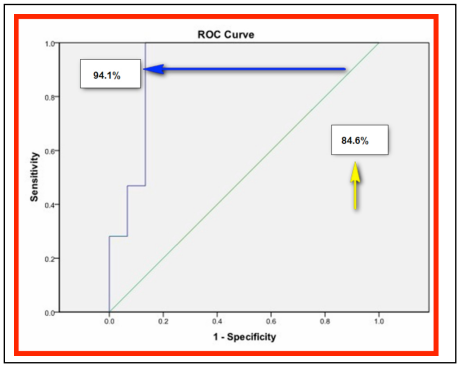

Figure 2: Sensitivity and specifity according to ROC analysis.

Discussion

Today, without doubt the most important way in the treatment and follow-up of hepatitis C is monitoring quantitative HCV RNA test[14]. However; HCV RNA test is not cheap, requires qualified staff and expensive equipment. In addition, HCV Ag test is studied with ELISA device and was found to be quite successful in monitoring viral load. Real-time quantitative reverse transcription-polimerase chain reaction (qRT-PCR) tests are used for the determination of viral load, but have some disadvantages. The possibility of contamination and false positivity could not be discarded in these techniques. In addition, some polimerase chain reaction (PCR) techniques are expensive and requires technical skills and intensive labor.

Lorenzo et al. studied HCV RNA and HCV Ag simultaneously in patients with chronic HCV infection[15]. They studied 86 sera in total and found a linear correlation between the two tests especially in patients with genotype 1a. They concluded that HCV Ag might be useful in the early diagnosis of HCV infection.

Miedouge et al. investigated the performance and usefulness of HCV Ag in hemodialysis patients with chronic hepatitis C[16]. They compared HCV Ag and HCV RNA in 98 samples and found that HCV Ag is correlated with HCV RNA. They reported that HCV Ag is a reliable test for screening acute HCV infections in hemodialysis units.

Dialysis units are one of the most risky places for transmission of hepatitis C. Therefore, HCV Ag can be used to monitor viral load especially in hemodialysis patients due to its rapid and reliable working principle[16, 17]. Use of HCV Ag for transaminase elevations of these patients will be a more economical approach.

The cost of one HCV Ag test is approximately 10 €, while the same for HCV RNA is 73.3 € (7-fold)[18]. If HCV RNA is tested 4 times annually for one patient's follow-up, the annual cost for 1000 patients' follow-up will be 293.162 €. If HCV RNA is tested once and HCV Ag is tested for the remaining, the annual cost will be 103.300 €. Therefore, use of HCV Ag in place of HCV RNA will help to decrease the cost of diagnosis and follow-up in certain patient groups.

Before any conclusion, we should declare study limitations. HCV RNA levels of most patients in our study were more than 2000 IU/ml. This is an important limitation of our study. Also, we could not classify the patients periodically during the treatment course.

In conclusion, HCV Ag test was found to be highly correlated with and less expensive than HCV RNA. Our study showed that sensitivity and specificity of the test increases when HCV RNA level is more than 2000 IU/ml. So, it can be an alternative to HCV RNA for the diagnosis of acute HCV infection in patients undergoing hemodialysis. It will also be useful in monitoring of chronic hepatitis C patients after treatment and in whom therapy is contraindicated. The fact that this test can be studied with macroelisa device, this is especially important for countries with limited sources. To our knowledge, use of HCV Ag as an adjunctive to HCV RNA will be cost-effective.

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Corresponding Author
DR. HASAN TAHSIN GOZDAS
Department of Infectious Diseases and Clinical Microbiology,
Sakarya University Faculty of Medicine
Sakarya
(Turkey)