Comparison of HCV core antigen and anti-HCV with HCV RNA results

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Abstract

Background: The measurement of anti-HCV antibodies using immunological methods and the confirmation of viral nuclear acid based on molecular methods is important in diagnosis and follow-up of the HCV infection.

Objectives: In this study, we aimed to analyse HCV core Antigen positivity among anti-HCV antibody positive sera to determine the significance of testing of HCV core Ag for the laboratory diagnosis of HCV infection, by considering the correlation between serum HCV core Ag and HCV RNA levels.

Methods: 115 patients suspected of having hepatitis C and who were positive for anti-HCV antibody were investigated using chemiluminescent and molecular methods. Anti-HCV antibody, HCV core Ag and HCV RNA levels were detected by the Vitros ECiQ immunodiagnostic system, Architect i2000 system and RT-PCR, respectively.

Results: The sensitivity, specificity, positive and negative predictive values and accuracy rate of HCV core Antigen assay were detected as 86.5%(83/96), 100%(19/19), 100%(83/83), 59.4%(19/32), 88.7%(102/115) respectively.

Conclusion: HCV core Ag assay could be used for diagnosis of HCV infection as it is easy to perform, cost-effective, has high specificity and positive predictive value. However, it should be kept in mind that it may have lack of sensitivity and negative predictive value.

Key Words: HCV, anti-HCV antibody, HCV core Ag, HCV RNA

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Introduction

The measurement of antibodies against hepatitis c virus (HCV) using immunological methods and the confirmation of viral nuclear acid based on molecular methods is important in diagnosis and follow-up of HCV infection[1]. The most widely used virological test for the diagnosis of HCV infection is the measurement of anti-HCV antibody in serum, by using chemiluminescent immunoassay(CLIA) or enzyme immunoassay(EIA) method[1]. Sometimes there is a long seronegative period in the course of HCV infection before an anti-HCV antibody can be found in the serum[2]. It has been reported that immunosuppression can also be a reason for an insufficient antibody response in a large number of patients[3]. Thus, anti-HCV assay results that show values under the critical value designated by EIA or CLIA must be confirmed by an additional confirmatory test, such as the HCV ribonucleic acid (RNA) test, or with the preconfirmatory HCV core antigen(Ag) assay[4]. Nucleic acid testing (NAT) for the detection of HCV RNA remains the gold standard for diagnosing active HCV infections.

However, in comparison with HCV core Ag and anti-HCV antibody tests, the need for experienced staff, special laboratory conditions and equipment and the need for standardisation are drawbacks of HCV RNA assays[1, 6]. Furthermore, depending on exposure to the virus, detection of HCV RNA shows differences in patients with no antibody found[2].

In the last decade, several HCV core Ag assays have been developed, due to the problems associated with HCV RNA assays[4, 6]. The results of recent studies indicated that measurements of HCV core Ag in serum or plasma can be used as indirect markers of HCV replication[7, 8, 9, 10].

The majority of the previously used enzyme-linked immunosorbent assays (ELISAs) or EIAs detecting HCV core Ag may have required time and skill to conduct. However, a fully automated CLIA with higher sensitivity has been developed to overcome the shortcomings of the conventional core Ag assays[6].

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In this study, we aimed to determine the significance of testing of HCV core Ag in laboratory diagnosis of HCV infection, to compare HCV core Ag, anti-HCV antibody and HCV RNA levels, and to investigate the correlation between serum HCV core Ag levels and HCV RNA levels for the diagnosis of HCV infection.

Materials and methods Serum samples

The study was carried out at Clinical Microbiology Laboratory of Suleyman Demirel University Medical Faculty between September 2011 and June 2012. Serum samples which have been detected to be positive for anti-HCV antibody of 115 patients who had a prediagnosis of HCV infection were investigated for the presence of HCV core Ag and HCV RNA using chemiluminescent and molecular methods. HCV RNA results were accepted as the gold standard in performing the comparisons.

Ethical approval

All patients had given informed consent about the study. Ethical approval was provided by the Ethics Committee of Medical School, Suleyman Demirel University (Isparta, Turkey).

Serological tests

Anti-HCV antibody, HCV core Ag and HCV RNA levels were detected by the Vitros ECiQ immunodiagnostic system (Ortho-Clinical Diagnostics, Raritan, NJ, USA), Architect i2000 system (Abbott Laboratories, Abbott Park, IL, USA) and real time polimerase chain reaction (RT-PCR) (Anatolia Diagnostics and Biotechnology Products Inc.), respectively.

Interpretation of the tests

Anti-HCV antibody test results of ≥ 1.00 signal-to-cut-off (s/co) were considered reactive, while results of <0.90 s/co were considered non-reactive and results of ≥ 0.90 s/co and <1.00 s/co were considered borderline according to the manufacturers' instructions. HCV core Ag test results of <3.00 femtomole/liter (fmol/L) were considered nonreactive, and results of ≥3.00 fmol/L were considered reactive according to the manufacturers' instructions. Values between ≥3.00 fmol/L and <10.00 fmol/L were retested in duplicate. If one or both results were ≥3.00 fmol/L, this was considered as repeatedly reactive. According to the manufacturers' instructions, HCV RNA measures of $<10^1$ International Unit/mililiter (IU/ml) were considered as low-level viremia and values of $>10^1$ IU/ml were considered as positive.

For the calculation of sensitivity and specificity, low viremia group was included in the positive group.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 15.0 (SPSS Inc., Chicago, IL, United States). Descriptive variables were presented as numbers and percentages.

Sensitivity was accepted as the probability of being test positive with the presence of the disease and calculated as (true positive) / (true positive + false negative). Specificity was accepted as the probability of being test negative with the absence of the disease and calculated as (true negative) / (true negative + false positive). Positive predictive value was accepted as the probability having disease when test is positive and calculated as (true positive) / (true positive + false positive). Negative predictive value was accepted as the probability of not having disease when test is negative and calculated as (true negative) /(false negative + true negative).

A receiver operating characteristic (ROC) curve analysis was performed to determine a cut-off HCV Ag value in order to justify the cut-off of the manufacturer. A $p < 0.05$ was taken (considered) to indicate statistical significance.

Results

Serum samples were provided from a total of 115 patients 65 (56.5 %) women and 50 (43.5 %) men). The patients' ages ranged from 16 to 86 years (57.9 ± 14.5 years).Of the 115 patients with anti-HCV antibody positivity, 83 were determined as positive, 32 were negative for HCV core Ag, and 84 were positive for HCV RNA. In addition, low viremia levels were detected among 12 samples and 19 samples were detected as negative (95% CI 5.7-17.2 s/co). HCV core Ag and HCV RNA results of the 115 samples with anti-HCV antibody positivity are summarized in table 1.

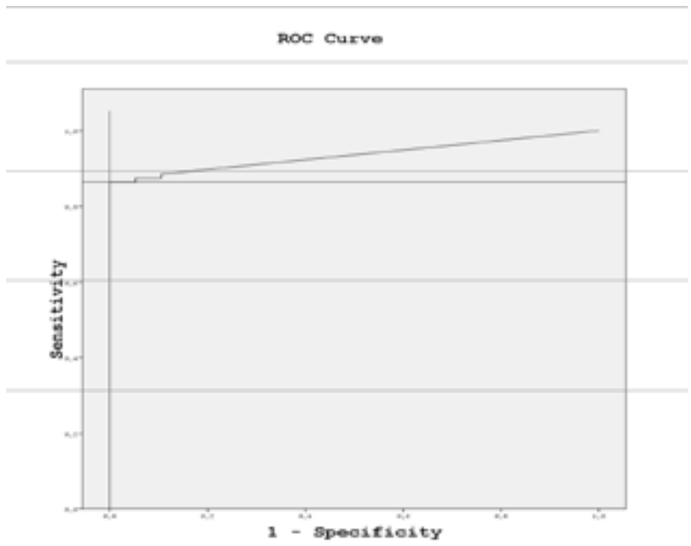
Table 1: The comparison of HCV Ag and HCV RNA results in 115 patients with positive Anti HCV.

	HCV RNA					
	In terms of positivity and negativity				In terms of viral load	
HCV Ag	Positive**	Non determined	Total		<10[1] IU/ml	>10[1] IU/ml
Reactive	83	0	83	PPV*: 100%	3	80
Non-reactive	13	19	32	NPV*: 59.4%	9	4
Total	96	19	115	Accuracy*: 88.7%	12	84
	Sensitivity*: 86.5%	Specificity*: 100%				

NPV: Negative predictive value, PPV: positive predictive value, *The analysis was performed accepting HCV RNA results as the reference method. **Low viremia grup was added into the positive group.

Comparing the total of 115 anti-HCV antibody positive serum samples with the test results of the HCV core Ag and HCV RNA assays, the sensitivity, specificity and positive and negative predictive values and accuracy rate of HCV core Ag assay were detected as 86.5%(83/96), 100%(19/19), 100%(83/83), 59.4%(19/32) and 88.7% (102/115) respectively.

ROC analysis indicated that HCV core Ag level ≥ 5.445 fmol/l had a sensitivity of 86.5%, specificity of 100%, positive predictive value of 100%, negative predictive value of 59.4%, and accuracy of 88.7% (Area under curve: 0.935, P < 0.001; Lower bound: 0.892, Upper bound: 0.978)Figure 1.



Discussion

This study focused mainly on the evaluation of the correlation between HCV core Ag and HCV RNA. We used a test for detection of HCV core antigens developed by Abbott.

HCV core Ag assay was evaluated to determine its intrinsic analytical performance characteristics and potential utility in the clinical management of HCV infection suspected patients. Our data showed that HCV core Ag assay results displayed good correlation with HCV RNA assay results in spite of the fact that sensitivity and negative predictive value of HCV core Ag assay was not as high as we expected.

In recent years, HCV core Ag tests have been developed for the monitoring of antiviral treatment and the identification of active HCV infection. Although these tests are relatively simple and fast, they have not been widely adopted, which has primarily been due to the shortcomings of HCV core Ag sensitivity. Recently developed tests have shown improved sensitivity and may be used as an alternative or in addition to NAT HCV assays. Automatic HCV core Ag results showed good correlation with HCV-RNA viral load tests and the advantages of the latter are that they provide easy and fast reporting[11].

The sensitivity of the test used in our study was approximately 0.06 pg/ml. The sensitivity of the HCV core Ag assay was 3.00 fmol/l (i.e. 0.06 pg/ml), based on the c11 recombinant Ag.

This assay (Architect HCV core antigen assay) is therefore approximately [16–25]-fold more sensitive than similar assays utilised in previous studies[8]. In addition, the ROC curve analysis showed exactly the same sensitivity and specificity rates with our results if HCV core Ag ≥5.455 fmol/l was accepted as a cut-off value. This finding was close to the manufacturer's cut-off, however we considered that the small difference might be due to the low number of negative patients.

Previous studies have shown that detection of HCV core Ag assay in serum or plasma is useful as an indirect marker of HCV replication due to the good correlation between HCV core Ag and HCV RNA levels[7,12].

Our specificity and positive predictive values were found as 100% and similar results were obtained in comparison with the other studies showing that there were no false-positive results[4,6,13]. However, our sensitivity (86,5%) and negative predictive value (59.4%) were a little bit lower than those of the other studies[4,6,13]. This was due to our higher rate of false negative results.

Leary et al.[14] demonstrated that the HCV core Ag was detected prior to the appearance of anti-HCV antibody in the patients' sera and this phenomenon may have resulted in a reduction of the window period by 23 days or even longer. However, since we conducted this study with anti-HCV antibody positive serum samples, we didn't have any sample with a result like HCV core Ag-reactive and anti-HCV antibody negative, so we were not able to consider whether the early HCV infection without antibodies could be detected using the HCV core Ag assay.

In a study using 152 serum samples to compare HCV RNA with HCV core Ag, Koroglu et al.[13] found that sensitivity, specificity and positive and negative predictive values were 96.9%, 100%, 100% and 99.1%, respectively. Furthermore, in a similar comparison of 212 serum samples with anti-HCV antibody positivity, Kesli et al[4] found that sensitivity, specificity and positive and negative predictive values were 96.3%, 100%, 100% and 89.7%, respectively. In addition, Park et al.[6] obtained similar results comparing HCV RNA with HCV core Ag in 282 serum samples; sensitivity, specificity and positive and negative predictive values were determined to be 90.2%, 100%, 100% and 86,4%, respectively.

Consequently, all positive results found by the HCV Ag assay were also positive with the HCV RNA assay. However, all negative results found by the HCV core Ag assay were not negative with the HCV RNA assay. Thus, it can be concluded that the positive results of the HCV core Ag assay can be reported as positive. However, when there is a serum sampleshowing anti-HCV antibody positivity, the negative results found by the HCV core Ag assay should be also confirmed by a HCV RNA assay.

Conclusion

Our data showed that HCV core Ag assay could be used for diagnosis of HCV infection due to its easy to perform, cost-effective, high specificity and positive predictive value. However, it should be kept in mind that it may have lack of sensitivity and negative predictive value. Future studies are needed to address these issues.

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