

Full Length Research Paper

Correlation between architect hepatitis C virus (HCV) core antigen and HCV Ribonucleic acid levels in Anti-HCV reactive patients in Turkey

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Quantitative tests for the detection of hepatitis C virus ribonucleic acid (HCV-RNA) levels and HCV core antigen have been mainly used for the management of patients with HCV infection. The aim of this study was to evaluate the correlation between HCV core antigen and HCV RNA levels in patients reactive for anti-HCV antibodies. Three hundred and twenty-five anti-HCV reactive sera samples were included in the study. HCV core antigen and HCV RNA levels were determined using the Architect HCV Ag test and Abbott RealTime™ HCV RNA test (RT-PCR), respectively. The correlation coefficient between the levels of HCV core antigen and HCV RNA test results was calculated using Spearman's rank test, and linear regression analysis was applied. One hundred and sixteen of the 325 samples were detected positive by both methods. Three additional samples by RT-PCR, and 4 samples by Architect HCV Ag, the negative samples were found positive by the other method. All of these contradictory results were obtained from the low level HCV RNA or HCV core antigen including samples. A correlation coefficient (r) was determined as 0.899 between the levels of HCV core antigen and HCV RNA ($p < 0.0001$). The sensitivity, specificity, positive predictive value and negative predictive value of the HCV core antigen test were 97.48, 98.06, 96.67 and 98.54%, respectively, using the HCV RNA test as a reference. The Architect HCV core antigen test exhibits a good correlation with the HCV RNA test. It can be used as an alternative method, especially when the HCV RNA test is unavailable.

Key words: Anti-hepatitis C virus, Architect hepatitis C virus Ag test, hepatitis C virus RNA.

INTRODUCTION

The most frequently used screening method in the diagnosis of hepatitis C virus (HCV) infections is the detection of anti-HCV antibodies in serum or plasma. Nevertheless, anti-HCV antibodies reached detectable levels in the serum after a long window period of HCV infection. Additionally, even if the virus is eliminated from the blood its

presence may persist for many years, for which reason this method cannot be used for the differentiation of active or past infection or for monitoring antiviral therapy. At the same time, tests used for determining anti-HCV antibodies can give false positive results for various reasons (Chevaliez, 2011; Richter 2002; Alter et al., 2003).

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Table 1. Comparison of HCV RNA and HCV core antigen tests' results.

HCV Core Ag	HCV RNA		
	Positive (%)	Negative (%)	Total (%)
Positive	116 (35.69)	4 (1.23)	120 (36.92)
Negative	3 (0.92)	202 (62.16)	205 (63.08)
Total	119 (36.61)	206 (63.39)	325 (100)

The recombinant immunoblot test is used in order to determine true positive results. It has disadvantages, such as giving a large number of indeterminate results and being a time-consuming procedure (Makuria et al., 2012). For these reasons, HCV RNA tests are the most commonly used technique in the confirmation of HCV infections and in the monitoring of antiviral therapy. Tests used for the detection of HCV RNA have high sensitivity and specificity, and low detection limits such as 15 to 30 IU/ml and can give quantitative results. However, these tests also require special technical and laboratory facilities, carry a risk of contamination and also impose high costs on users (Chevaliez, 2011; Richter 2002; Alter et al., 2003).

A large number of immunologically-based tests have been developed over the last 20 years for the detection of HCV core antigens in plasma or serum as potential alternatives to HCV RNA tests. In first-generation HCV core antigen tests, there is no preliminary procedure intended to differentiate between antigen and antibody. Therefore, HCV core antigens can only be detected in the period up to the emergence of anti-HCV antibodies using first-generation tests (Takahashi et al., 1992; Aoyagi et al., 1999; Tanaka et al., 1995; Kashiwakuma et al., 1996; Icardi et al. 2003). With second-generation tests, preliminary procedures aimed at distinguishing HCV core antibodies and anti-HCV antibody complexes are performed beforehand. HCV core antigens can thus be detected during seroconversion. Preliminary procedures are performed manually in these tests carried out on microplates, and the lowest detectable limit is about 1.5 pg/ml (Tobler et al., 2005; Tanaka et al., 2006; Fabrizi et al., 2005).

A fully automated system has recently been developed by ABBOTT Diagnostics (Abbott Park, IL, USA) for the detection of HCV core antigen in serum or plasma including preliminary procedures for differentiation of antigen-antibody complexes. This test also provides quantitative results. The lowest detectable limit in the test is 3 fmol/l (0.06 pg/ml). This value is approximately 25 times lower than that for previous tests (Mederake et al., 2009).

The purpose of this study was to compare the results of the architect HCV core antigen test, which has newly been introduced in Turkey with the HCV RNA results obtained from the Abbott RealTime™ HCV RNA test (Abbott Molecular Inc., Des Plaines, IL, USA) and to determine the correlation between the two.

MATERIALS AND METHODS

In this study, HCV RNA test and HCV core antigen test were consecutively and simultaneously administered on three hundred and twenty-five sera samples between June 2010 and July 2012. All the samples were anti-HCV reactive.

Anti-HCV levels in the serum samples reaching our laboratory were determined using the chemiluminescent microparticle immunoassay (CMIA) technique with Architect Anti-HCV kits (Abbott Diagnostics, Wiesbaden, Germany) on an Architect I2000SR (Abbott Diagnostics, Abbott Park, IL, USA) device. Sample/cutoff values (S/CO) ≥ 1 were regarded as reactive.

HCV core antigen levels in serum were investigated using the Architect HCV Ag test (Abbott Diagnostics, Wiesbaden, Germany) with CMIA technology following the manufacturer's recommendations. Since the HCV core antigen test and Anti-HCV antibody test were investigated on the same device, it was subjected to daily maintenance using a 0.5% sodium chloride solution in order to prevent cross-contamination. The lowest level detectable by the test, 3 fmol/l (0.06 pg/ml), was adopted as the cut-off value.

HCV RNA isolation was performed using the Sample Preparation System (Promega Corporation Madison, WI, USA) on an Abbott *m2000sp* platform (Abbott Molecular Inc., Des Plaines, IL, USA). HCV RNA levels were determined using the Abbott *m2000rt* Instrument System (Abbott Molecular Inc., Des Plaines, IL, USA) and Abbott RealTime™ HCV test (Abbott Molecular Inc., Des Plaines, IL, USA). This test, based on the quantitative determination of the HCV viral load in plasma using the real time polymerase chain reaction method (RT-PCR) after reverse transcription, was performed with a 0.2 ml plasma sample, in line with the manufacturer's instructions. The test's lowest detection limit, 30 IU/ml, and values above that were regarded as positive.

Statistical analysis was performed using SPSS 13.0 (Series no: 9069728). Descriptive data were expressed as number and percentage. The Spearman rank test and linear regression analysis were used to determine the correlation between HCV RNA and HCV core antigen levels.

RESULTS

One hundred and sixteen of the 325 samples were detected positive by the two tests. HCV RNA levels were greater than 30 IU/ml in 119 of the 325 samples (35.69%). HCV core antigen levels were greater than 3 fmol/l in 120 samples (36.92%) (Table 1). When the HCV RNA test was regarded as a reference test, HCV core antigen test sensitivity, specificity, PPV and NPV rates were calculated as described by Akobeng AK (2007), and found as 97.48, 98.06, 96.67 and 98.54%, respectively. The Spearman's correlation coefficient (*r*) was determined as 0.899 ($p < 0.0001$) at comparison of HCV RNA and HCV core antibodies (Figure 1). HCV core antigen was deter-

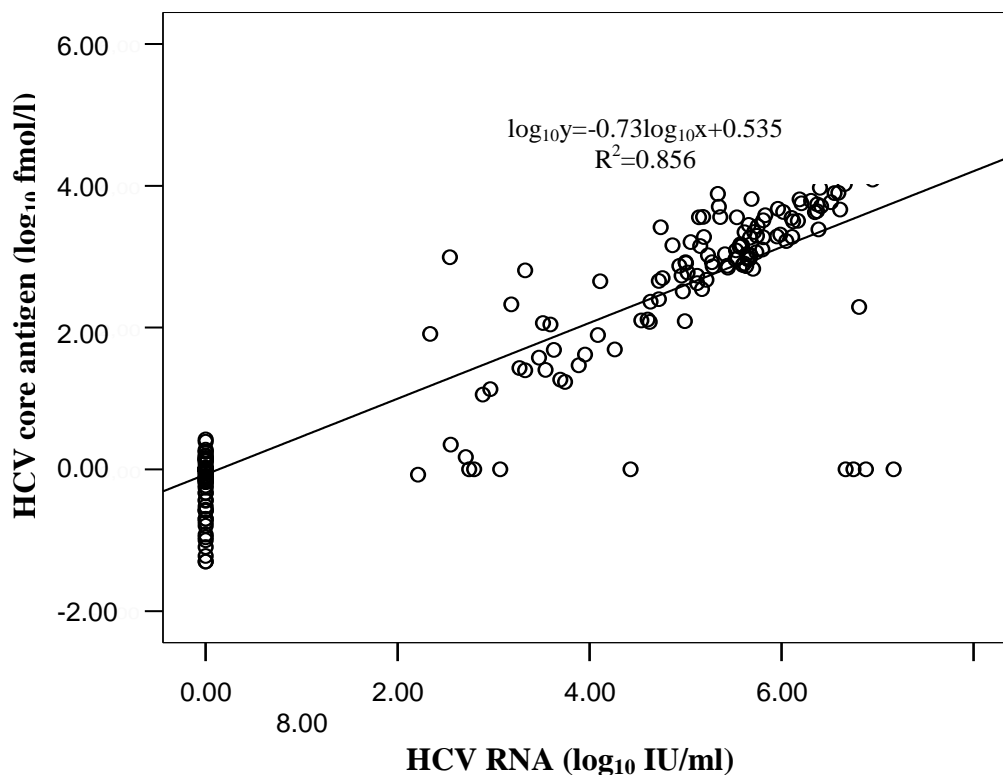


Figure 1. Comparison of HCV RNA and HCV core antigen levels (n=325). (The graph was prepared on the basis of the logarithm of the results obtained in both tests).

Table 2. Results from inconsistent HCV RNA and HCV core antigen tests.

Specimen number	HCV RNA level (IU/ml)	HCV core antigen level (fmol/l)
1	< 30	3.04
2	< 30	6.40
3	< 30	8.86
4	ND*	3.78
5	357	2.23
6	163	0.84
7	513	1.49

*ND: Not detected

mined as negative in four HCV RNA positive samples, and positive in three HCV RNA negative tests. Detailed results from the seven samples in which the HCV RNA and HCV core antigen tests were inconsistent are shown in Table 2.

DISCUSSION

This study compared HCV RNA test and Architect HCV core antigen test results in specimens with anti-HCV antibodies identified as reactive. The ability of Architect HCV core antigen test's to provide quantitative results is

being fully automated, and having a low detection limit of 3 fmol/l represent superior features compared to previous HCV core antigen tests. The device's total working time, that is, maintenance once daily has been performed and is approximately 40 min; some five times shorter than that of previously manufactured tests (around 3.5 h) (Mederake et al., 2009; Ergunay K et al., 2011; Morota et al., 2009; Ross et al., 2010; Kesli et al., 2011; Park et al., 2010; Medici MC et al., 2011).

Positivity rates of HCV RNA, and HCV core antigen tests were found to be quite similar, 36.61 and 36.92%, respectively. When the HCV RNA test was taken as refe-

rence, the HCV core antigen test exhibited rather high sensitivity, specificity, PPV and NPV (97.48, 98.06, 96.67 and 98.54%, respectively). These values are slightly higher than those obtained in previously manufactured tests. Fabrizi et al. (2005) used the second-generation Ortho Trak-C test (Ortho-Clinical Diagnostics, Raritan, N.J., USA) and determined sensitivity, specificity, PPV and NPV of 92.7, 97.4, 94.7 and 96.5%, respectively, in hemodialysis patients.

HCV RNA and HCV core antigen test results were inconsistent in seven specimens in our study. The HCV RNA test was lower than 30 IU/ml or negative in four of these specimens in those HCV core antigen levels were positive with amount lower than 10 fmol/l. The manufacturers recommend that if HCV core antigens are measured at 3 to 10 fmol/l, the test should be repeated two times, and that if positivity above 3 fmol/l is seen in at least one of these tests, the test should be interpreted as positive. Samples with a level of 3-10 fmol/l were not retested in our study for economic reasons. This may be regarded as a limitation of the study. The possibility of there being insufficient RNA for quantification in specimens identified as HCV core antigen positive and with HCV RNA \leq 30 IU/ml must also be borne in mind.

HCV RNA was positive in three samples although the HCV core antigen test results of them were below 3 fmol/l. HCV RNA levels in these three samples were 10^3 IU/ml or less. We therefore think that care is needed when interpreting HCV core antigen results if HCV RNA levels are $\leq 10^3$ IU/ml.

When the correlation between HCV RNA and HCV core antigen levels was analyzed, the correlation coefficient between the two tests was quite high ($r=0.899$). Kesli et al. (2011) determined HCV RNA levels using the Qiagen HCV RNA test (Qiagen, Hilden, Germany) and identified a correlation with the Architect HCV core antigen test as 0.864. Ergünay et al. (2011) determined HCV RNA levels with COBAS Ampliprep/COBAS Taqman HCV Real-time PCR (Roche Diagnostics, Germany) and calculated a correlation coefficient as 0.915. Mederacke et al. (2009) determined HCV RNA levels using Cobas Taqman or Amplicor HCV Monitor (Roche Diagnostics, Germany) and reported a correlation coefficient as 0.75. Medici et al. (2011) determined a correlation coefficient ranging from 0.713 to 0.870 for the HCV core antigen test and different HCV RNA kits. The values determined in our study and the results from other studies show that HCV RNA tests and the HCV core antigen test give quite compatible results.

In conclusion, the Architect HCV core antigen test and HCV RNA test produced highly compatible findings in our study. The HCV RNA test is still regarded as standard in the detection of active infection in individuals in whom anti-HCV antibodies are detected, in the confirmation of anti-HCV antibody tests and in the commencement and monitoring of treatment (Alter et al., 2003). However, we conclude that the HCV core antigen test can be used as

an alternative to HCV RNA tests, particularly when the HCV RNA test is unavailable.

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