Research Article [Araştırma Makalesi]

The Utility of ANTI-HCV S/CO Ratio, HCV-RNA and ALT Test in Predicting Viremia in ANTI-HCV Positive Patients

[ANTİ-HCV Pozitif Hastalarda Viremi Belirlenmesinde ANTİ-HCV S/CO, HCV-RNA ve ALT Testlerinin Kullanımı]

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ABSTRACT

Hepatitis C Virus (HCV) infection is frequently diagnosed by detection of antibody against the HCV (Anti-HCV). The seropositivity of anti-HCV could reflect chronic infectious status and/or previous infection. Detection of HCV-RNA by PCR is still laborious, too expensive, requires specific expertise and facilities, and usually used to confirm positive serology. This study was performed in anti-HCV positive patients to determine the relationship between anti-HCV Sample rate / Cutoff rate (S/CO) ratios, HCV-RNA and Alanine aminotransferase (ALT) levels. In addition, the utility of anti-HCV S/CO ratio values in predicting HCV viremia in anti-HCV positive patients were evaluated. Serum samples of 124 patients were tested for anti-HCV by a MEIA technique, for HCV-RNA by a quantitative PCR and for ALT by IFCC UV test. S/CO values for anti-HCV test and ALT levels were correlated with the quantitative values of HCV-RNA (respectively, r=0.824, p<0.0001 and r=0.795, p<0.0001). HCV-RNA values were found to be negative in samples with S/CO ≤ 25.9. HCV-RNA positivity was found in 100 % of samples with a S/CO > 25.9. All HCV-RNA negative cases had normal serum ALT levels (24.67 ± 8.56 U/L) and relatively low S/CO values (3.81 ± 4.32) for anti-HCV tests. HCV-RNA positive other cases had greater S/CO values (119.53 ± 37.99) and elevated serum ALT levels (99.53 ± 49.96 U/L). The sensitivity and specificity of anti-HCV in the diagnosis of HCV viremia was 100 % and specificity was 100 % respectively when S/CO ratio was set 25.9 as a cutoff. Among patients with high S/CO ratios, the likelihood of HCV-RNA positivity was directly related to S/CO ratio. S/CO values may be used to predict HCV viremia in Anti-HCV positive individuals and therefore, quantitative HCV-RNA testing could not be routinely required for all patients.

Key Words: Anti- HCV, HCV-RNA, ALT , Hepatitis C virus infection.

ÖZET

Hepatit C Virüs (HCV) enfeksiyonu sıklıkla HCV antikorunun saptanması ile tanılanır. Anti-HCV'nin pozitifliği kronik enfeksiyonu ve/ veya önceden geçirilen enfeksiyonu gösterebilir. PCR ile HCV-RNA nın tespit edilmesi hala zahmetli ve çok pahalıdır; belli bir bilgi ve beceri gerektirir, genellikle de pozitif serolojiyi doğrulamak için kullanılır. Bu çalışma, anti-HCV'si pozitif olan hastalarda, anti-HCV Sample rate /Cutoff rate (S/CO) oranı, HCV-RNA ve Alanin aminotransferaz (ALT) düzeyleri arasındaki ilişkileri saptamak için yapıldı. Ayrıca, anti-HCV pozitif olan hastalarda vireminin belirlenmesinde, anti-HCV S/CO oranı düzeylerinin kullanımı değerlendirildi. 124 hastanın serum örneklerinde MEIA tekniği ile anti-HCV, kantitatif PCR ile HCV-RNA ve IFCC'nin UV testi ile ALT tayin edildi. Anti-HCV testi S/CO değerleri ve ALT düzeyleri, HCV-RNA'nın kantitatif miktarları ile direk olarak ilişkili idi (sırasıyla, r=0.824, p<0.0001 ve r=0.795, p<0.0001). HCV-RNA değerleri, S/CO ≤ 25.9 olan örneklerde negatif bulundu. S/CO>25.9 olan örneklerin % 100'ünde HCV-RNA pozitif bulundu. HCV-RNA negatif olan tüm hastalar normal serum ALT düzeyleri (24.67±8.56 U/L) ve düşük anti-HCV S/CO değerlerine (3.81 ± 4.32) sahiptiler. HCV-RNA pozitif olan diğer hastalar daha yüksek S/CO değerleri ve (119.53 ± 37.99) ve yükselmiş serum ALT düzeylerine (99.53±49.96 U/L) sahipti. HCV viremisinin tanılanmasında, S/CO için eşik değeri 25.9 alındığında anti-HCV'nin duyarlılığı ve seçiciliği % 100 idi. S/CO oranları yüksek olan hastalarda, HCV-RNA pozitiflik olasılığı doğrudan S/CO oranı ile ilişkili idi. S/CO değerleri, anti-HCV pozitif kişilerde HCV viremisini belirtmek için kullanılabilir ve böylece tüm hastalar için rutin olarak HCV-RNA testinin yapılması gerekli olmayabilir.

Anahtar Kelimeler: Anti-HCV, HCV-RNA, ALT, Hepatit C virüs enfeksiyonu.

INTRODUCTION

Hepatitis C Virus (HCV) is a single - stranded RNA virus that belongs to the family Flaviviridae. HCV was initially recognized as non-A, non-B hepatitis virus in 1974 until cloning of the etiological agent in 1989. It has been estimated that approximately 3 % (170 million people) of the world's population is infected with HCV (1- 5).

Transmission is mainly associated with infected blood products, intravenous drug abuse, accidental needle sticks or perinatal infection although other less common routes such as vertical or sexual transmission are reported (1,3,5). Hepatitis C is the leading cause for end-stage liver complications, including hepatocellular carcinoma and the need for liver transplantation; the frequency of these is expected to increase two-to threefold by 2030 (6).

Accurate diagnosis of active HCV infection is important not only because of the associated morbidity and mortality but also because of the possibility of spontaneous or pharmacology-induced sustained virologic cure (2). Laboratory assays that are available for the diagnosis and management of HCV infection include 1- serologic tests to detect HCV antibodies, 2- molecular tests to detect and quantitate HCV-RNA, and 3- genotyping techniques. Assays to detect and quantify HCV core antigen, have also been developed (1,2,4,5,7).

The diagnosis of hepatitis C virus (HCV) infection is based on the detection of anti-HCV antibodies in serum, generally by means of enzyme-linked immunosorbent assays (ELISA). Anti-HCV can indicate one of three possible conditions: Current active infection with HCV, past infection with HCV, or a false positive reaction. Detection of HCV-RNA by polymerase chain reaction (PCR) is considered the gold standard to confirm the diagnosis of HCV infection and for assessing viremia in patients during and following antiviral therapy (4,5,8 - 9).

In this study, we aimed to determine the relationship between quantitative Anti-HCV, quantitative HCV-RNA and Alanine aminotransferase (ALT) levels in Anti-HCV positive patients. We also evaluated the performance of different MEIA S/CO values in the identification of viremic from non-viremic Anti-HCV positive patients.

MATERIALS AND METHODS

From November 2005 to February 2006, 124 anti-HCV positive patients were included in this study. Patients (48 female and 76 male) were evaluated at the Hepatology Unit of T.Yüksek İhtisas Hospital. All patients were negative for hepatitis B surface antigen and anti-HIV. Serological and biochemical tests were carried out on serum samples the same day. For the anti-HCV test, each sample was centrifuged at an RCF of at least 10 000 × g for 10 minutes. The PCR test was carried out on serum samples; specimens were divided in aliquots and kept frozen -70 °C until tested.

Serum samples were assayed for anti-HCV using HCV version 3.0 AxSYM (Lot No: 35378HN00, Abbott Diagnostics). The AxSYM HCV 3.0 is a micro-particle en-zyme immunoassay (MEIA). The results are expressed as the ratio between the signal detected on the sample and the cutoff value (S/CO). This is a semi-quantitative assay in which S/CO value is directly proportional to the quantity of antibodies directed to HCV. In the anti-HCV test, an S/CO equal or greater than 1.00 is considered reactive. The index calibrator and the assay controls were tested. The specificity of assay was 99.84 % and sensitivity was 100 %.

The quantitative determination of HCV-RNA in serum was performed by the COBAS AMPLICOR HCV test (Roche Diagnostics). HCV-RNA was detected using a reverse transcriptase PCR using primers from the 5'-untranslated region (5'-UTR). The test amplifies a target sequence of 244 nucleotides defined by primers KY78 and KY80 and localized in the 5'- UTR region and has a nominal sensitivity of 50 IU/ml. The results were expressed in IU/ml. The assay was performed in the presence of known amounts of internal quantitation standard (QS). The Cobas Amplicor HCV Monitor Test, v2.0, is being calibrated to the World Health Organization International Standard for HCV. Serum ALT activities were measured by IFCC UV Test using Modular PP autoanalyzer (Roche Diagnostics).

Anti-HCV positive patients were divided in two groups according to the presence or absence of serum HCV-RNA by PCR. S/CO and ALT values were analyzed in each group. We also classified samples in three groups according to the S/CO values. Sensitivity and specificity of different S/CO values in detecting viremic patients were calculated. ROC curve was performed for S/CO values. Statistical analysis was performed using SPSS program. Results were expressed as mean ± standart deviation. Unpaired T test was used for comparisons. A p value less than 0.05 was considered statistically significant. Correlations were analyzed by Spearman's correlation coefficient.

RESULTS

124 anti-HCV positive patients confirmed by a third- generation test, were included in this study.

Sample rate /Cutoff rate (S/CO) values for anti-HCV tests and ALT levels were correlated with the quantitative values of HCV-RNA (respectively, r= 0.824, p<0.0001 and r=0.795, p<0.0001). HCV-RNA values were found to be negative in samples with S/CO ≤ 25.9. HCV-RNA positivity was found in 100 % of samples with S/CO>25.9. HCV-RNA values were between 10^2 and 10^7 IU/ml in samples with a S/CO>25.9. All HCV-RNA negative cases had normal serum ALT levels (24.67±8.56 U/L) and relatively low S/CO values (3.82±4.32) for anti-HCV tests. HCV-RNA positive other cases had greater average S/CO values (119.53±37.99) and elevated serum ALT levels (99.53±49.96 U/L). Average ALT levels versus Anti-HCV S/CO values are shown in figure 1. S/CO values and ALT levels were significantly different between HCV-RNA positive and negative patients (p<0.0001 for both) (Figure 2, 3).

We further analyzed the best cut-off value of the S/CO in differentiating viremic from non viremic patients. The S/CO value of 25.9 showed sensitivity (True positive ratio) of 100 % and 1-specificity (False positive ratio) 0 % in discriminating both categories of HCV infected patients.

DISCUSSION

Hepatitis C has emerged as an important public health problem that has affected 170 million people worldwide and is currently the most common indication for orthotopic liver transplantation. The disease, characterized by asymptomatic onset, is often discovered incidentally through blood tests obtained during physical examination or before blood donation. An estimated 20 % of persons with chronic HCV infection develop cirrhosis over the course of 25 year, and some will progress to end-stage liver disease and hepatocellular carcinoma (1,5,8,10).
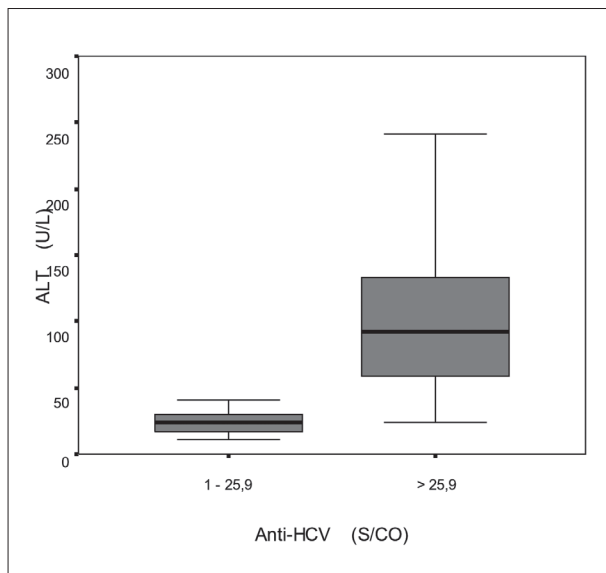

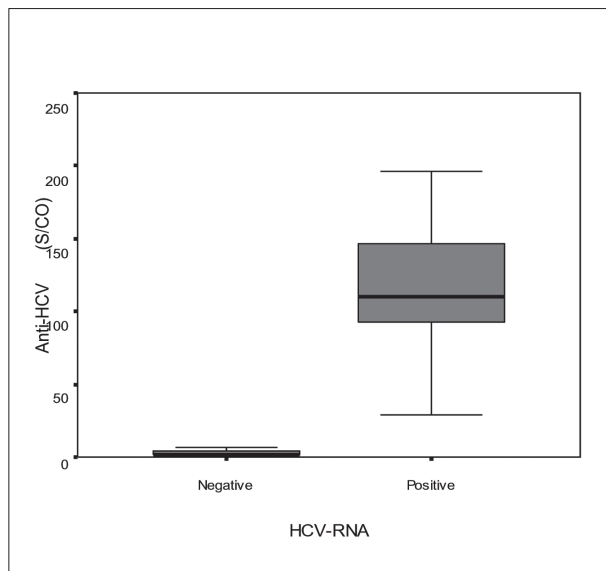**Figure 1.** ALT activities versus Anti-HCV (S/CO) values

Figure 2. Anti-HCV (S/CO) values versus HCV-RNA status
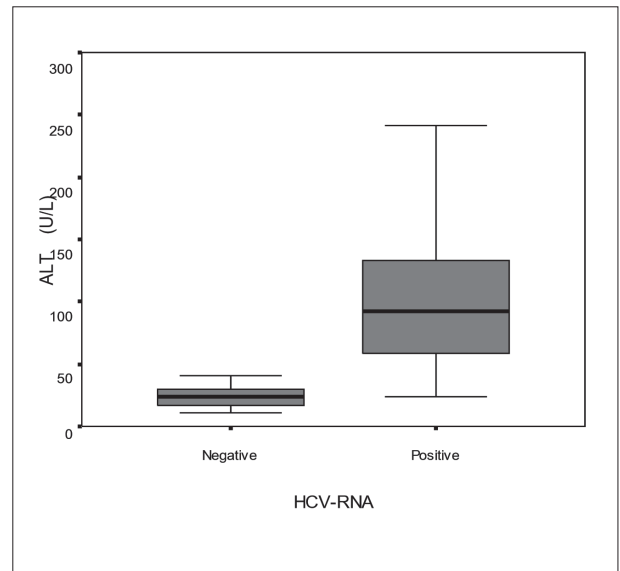

Figure 3. ALT activities versus HCV-RNA status

There have been remarkable advances in diagnostic testing for HCV over the past decade. This has included progressive improvement in both the sensitivity and specificity of tests for antibodies to HCV. These tests now provide rapid and inexpensive means of identifying individuals who have been infected with hepatitis C. Qualitative and quantitative tests for HCV-RNA have become the gold standard of successful antiviral therapy (9-13).

Testing for hepatitis C virus (HCV) infection typically begins with measurement of antibodies to HCV proteins using enzyme immunoassays (EIA). Currently, laboratories use either second- or third-generation EIA tests that detect antibodies to one or more of several recombinant or synthetic proteins produced by genes from different areas of the HCV genome. Anti-HCV EIA results are interpreted by comparison of absorbance readings with a defined cutoff value. Although EIA tests provide a quantitative absorbance result, they are usually reported simply as positive or negative (4,9,12- 13).

Because of the possibility of false-positive results, especially in low prevalence settings such as testing of blood donors, positive anti-HCV results are usually confirmed by additional tests. Recently, the Centers for Disease Control (CDC) revised guidelines for laboratories, recommending confirmatory testing for samples with low S/CO ratios. When EIAs are used, they suggested a S/CO ratio < 3.8 to identify low-positive samples (6). The current clinical practice after identifying a positive anti-HCV EIA result is to measure HCV-RNA to assess whether viremia is present (6,9 - 10,12 - 13).

Several studies have shown that individuals with low positive anti-HCV results were typically negative when tested by HCV-RNA (6,12,14). Our results confirm those of previous studies, indicating that low positive anti-HCV EIA results frequently represent false positive reactivity In this study, when the MEIA positivity was expressed as

a S/CO ratio, taken as a semi-quantitative index, a good correlation with HCV-RNA was found. In our study, HCV-RNA values were found negative in 61 patients with S/CO ≤ 25.9. All of the strong positive samples (S/C0>25.9) had positive HCV-RNA values.

 A possible S/CO value to separate viremic and non-viremic populations could be of help in the first line diagnosis of hepatitis C. The S/CO values such as 26, 32, 34 and 40 were determined in the third generation anti-HCV assay to distinguish viremic from non-viremic patients in several published studies (4,6,10,14-16).

In this study, the sensitivity of anti-HCV in the diagnosis of HCV viremia and the specificity was 100 % when the cutoff for S/CO was set at 25.9. In conclusion, by establishing 25.9 as cutoff value of the S/CO in the third generation anti-HCV assay, it is possible to distinguish between viremic and non-viremic patients. Based on our results, subjects with S/CO values < 25.9 were more likely to be cases of past infection or of nonspecific reaction. Most of the subjects with S/CO > 25.9 could represent current or persistent infection.

ALT values are considered an important tool for screening liver disease and they are good reflectors of disease activity in chronic viral hepatitis (11,17). Our data show that the relationship between serum ALT values and anti-HCV antibody was exclusively related to the association between raised aminotransferase values and HCV viremia. HCV-RNA positive patients showed higher hepatic enzyme levels than patients with no detectable HCV-RNA. All HCV-RNA negative cases had normal serum ALT levels. ALT levels and S/CO values were significantly different between HCV-RNA positive and negative patients. The finding of an association between HCV viremia and increased ALT levels likely reflects a link between virus replication and liver damage.

Consequently, infection is more likely in those cases in which there is a strong antibody reaction, while in cases with lower antibody reactivity it is more likely that the infection is on its way out or past. In conclusion, it is important for laboratories to report the S/CO value whenever a positive anti-HCV result is found. Our data demonstrate that viremic HCV patients had higher S/CO values in the MEIA test in comparison with non viremic patients. Based on our results, the cutoff value of the S/CO was 25.9 in differentiating viremic from non-viremic patients. Thus, a threshold set at 25.9 S/CO may be suggested for performing a supplemental assay on MEIA anti-HCV.

HCV-RNA testing is expensive and time consuming, requires a sophisticated molecular laboratory, and may not be always readily available in underdeveloped parts of the world, where the greatest numbers of HCV infected patients are found. Although false positive EIA results are a problem in low prevalence settings, the accuracy of the third –generation test is very good in high–prevalence populations, and therefore, HCV-RNA testing may not be necessary in high-risk patients with positive anti-HCV results.

In conclusion, anti-HCV S/CO ratio is important even in high-risk individuals; laboratories should report the S/CO ratio along with anti-HCV EIA results. Anti-HCV S/CO ratio values may be used to predict HCV viremia in anti-HCV positive individuals. ALT activity measurements, along with anti-HCV S/CO ratio improve the quality of screening for potentially infectious patients. However, we think, additional studies are helpful to predict practically viremia by using Anti-HCV S/CO values.

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