Full Length Research Paper

Evaluation of diagnostic performance of new antigen-based enzyme immune assay for diagnosis of Hepatitis C virus (HCV) infections

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Laboratory diagnosis of Hepatitis C virus (HCV) is sometimes problematic. A novel immune enzyme assay (HCV-Core Antigen, Abbott Laboratories/Germany) has been recently licensed for detection of HCV antigen in human serum. In this study, we aimed to evaluate diagnostic performance of this new test in comparison with the HCV-RNA quantification and anti-HCV antibody (anti-HCV-Ab) analyses. A total of 152 serum samples of which 112 anti-HCV-Ab positive (range 1.08 to 386.54 s/co) and 40 anti-HCV-Ab negative (<1 s/co) were analyzed with HCV-RNA and HCV-Ag tests. According to HCV-RNA detection, sensitivity and specificity of HCV-Ag test was measured as 96.9 and 100%, respectively and of anti-HCV-Ab were measured as 100 and 60%, orderly. An excellent positive predictive value for HCV-Ag test was detected as 100%, whereas 28.5% for anti-HCV-Ab test. Pearson correlation analysis showed that there was a statistically significant and strong relationship (p < 0.001, R: 0.773) between HCV-Ag and HCV-RNA quantification analysis. The correlation between the two tests showed an exponential trend (R^2: 0.949). These results suggest that HCV-Ag test may be a reliable assay for HCV antigen detection, which is also well-correlated with serum viral load. However, large studies, including different HCV genotypes and with extreme viral quantity, are required to assess analytic potency of this novel kit.

Key words: Hepatitis C, diagnosis, ELISA, HCV core antigen, HCV-RNA.

INTRODUCTION

Hepatitis C virus (HCV) infection is a global public health problem considering over 170 million people infected throughout the world ("WHO", 1997). This infection may occur as acute hepatitis of which 75% are asymptomatic, and approximately 70% can evolve into chronic infection that is the second most common cause of cirrhosis and hepatocellular carcinoma all over the world (WHO, 1997;

Marcellin, 1999).

Transmission of HCV generally occurs with blood transfusion, sexual contact, intra-familial contagion, and intravenous drug use. Considering the HCV is the leading cause of transfusion-associated hepatitis, effectively screening of the donors' blood samples plays an important role in preventing the transmission (Harris et al., 2001; Nolte et al., 1995).

Anti-HCV-Ab EIA method has been used for HCV screening not only for blood banks but also for routine medical application in many countries for years. Widespread usage of this method is most likely due to its

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Table 1. Sensitivity, specificity, positive predictive value and negative predictive value of tests.

Variable	HCV-RNA			
	Sensitivity (%)	Specificity (%)	PPV*(%)	NPV** (%)
HCV-Ag	96.9	100	100	99.1
Anti-HCV-Ab	100	60	28.5	100

*positive predictive value, ** negative predictive value.

relatively low cost and easy to use features. Regarding the some certain conditions such as sero-conversion phase of the disease, there has been a need to confirm the result of anti-HCV-Ab analyze with immunoblotting or nucleic acid amplification tests. However, these assays require intensive labor, high cost and large facilities with high cost (Park et al., 2010). Therefore, requirement of an effective, antigen-based and easily-applicable kit has been elucidated.

In the present study, we investigated the diagnostic power of a newly-presented commercial ELISA kit detecting HCV core antigen in comparison with the currently used assays.

MATERIALS AND METHODS

Samples

The serum samples included in the present investigation were randomly selected from patients with suspected HCV infection who were referred to Malatya State Hospital between January to July 2010. The serum samples were stored at -40°C until the analysis were performed.

Serological assays

Anti-HCV-Ab and HCV-Ag were studied with Abbott architect system using a two-step chemiluminescent micro particle immunoassay technology (Abbott Laboratories/Germany) qualitatively and quantitatively, respectively. The results are evaluated according to the manufacturer instructions as following, for anti-HCV-Ab test; <1 s/co nonreactive, ≥1 s/co reactive; and for HCV core antigen test; <0.06 pg/ml non-reactive, 0.06-0.2 pg/ml gray zone, and >0.2 pg/ml reactive.

Molecular analysis

HCV RNA was isolated from serum samples via Qiagen automated extraction kit. The quantitative viral load investigation was performed with real-time polymerase chain reaction by Rotor Gene Q 6000 analyzer (Qiagen, Germany).

Statistical analysis

Statistical analysis was performed using SPSS 12.0 for Windows (SPSS Inc, Il/US). The sensitivity and specificity of ELISA tests were examined comparing with PCR assay. Correlation between HCV core antigen test and HCV-RNA RT-PCR was evaluated by Pearson correlation analysis.

RESULTS

A total of 112 anti-HCV positive and 40 anti-HCV negative samples from 152 patients were enrolled in the study. Of the 112 patients whose sera were positive for anti-HCV-Ab assay, 51 were female, 59 were male and the mean age was 49.08 years (SD: 19.36). The mean age of the 40 anti-HCV negative patients consist of 16 female and 24 male, and mean age was 41.25 years (SD: 16.77). Among 112 anti-HCV positive samples 31 were positive for HCV-Ag test. The average serum antigen amount was measured as 55.87pg/ml (SD: 75.21). None of the patients whose anti-HCV-Ab assay was negative revealed positive result for HCV-Ag test.

In the molecular analysis, HCV-RNA was detected in 32 of 112 anti-HCV positive samples. Any detectable HCV-RNA couldn't be determined among anti-HCV negative samples. Of the 32 HCV-RNA positive samples, 31 were positive with HCV-Ag test. HCV-Ag test resulted as gray zone (0.08 pg/ml) in one patient whose anti-HCV-Ab and HCV-RNA were positive.

According to HCV-RNA detection, sensitivity and specificity of anti-HCV-Ab were measured as 100 and 60%, and of HCV-Ag tests were measured as 96.9 and 100%, respectively. For HCV-Ag assay, positive and negative predictive values were detected as 100 and 99.1%, orderly. Sensitivity, specificity, positive and negative predictive values of HVC-Ag and anti-HCV-Ab tests in comparison with HCV-RNA analysis is shown on Table 1.

A strong and significant correlation ratio was detected between HCV-Ag and HCV-RNA tests using Pearson correlation analysis. (P < 0.001, R = 0.773). The correlation of these two tests showed exponential trend (R^2: 0.949). The correlation analysis results are shown on Figure 1.

DISCUSSION

The prevalence of HCV infections appears to decrease in the last decade owing to the newly introduced diagnostic tools. However it's still an important public health threat especially in the developing countries ("WHO", 1997; Perz et al., 2006). Diseases occurring as a result of HCV infections have a significant impact on both the life of the effected individual as well as the reimbursement system
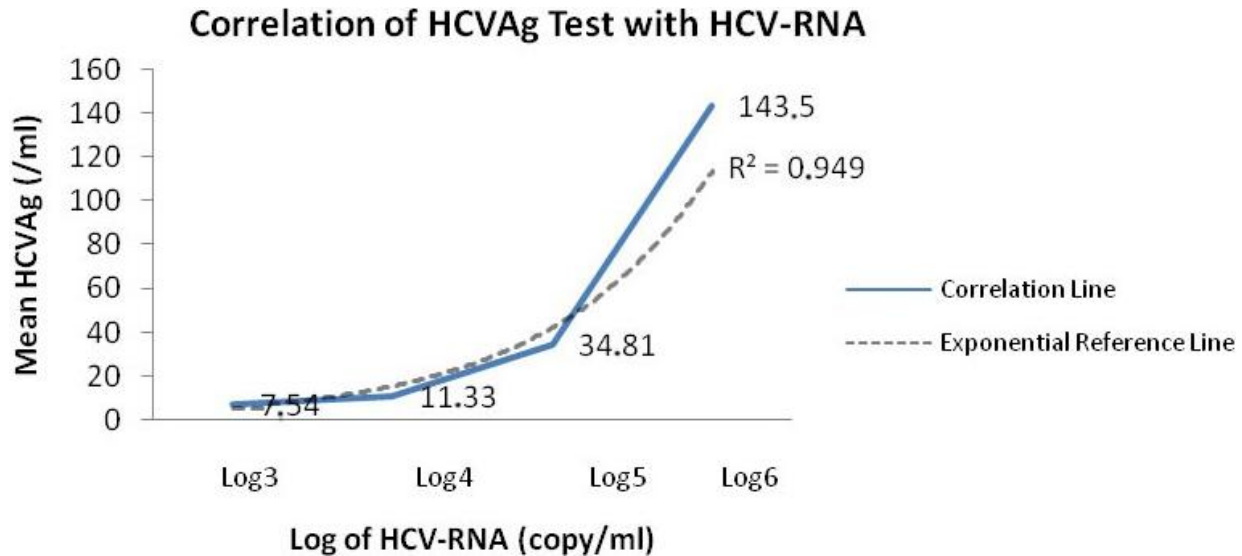
Correlation of HCVAg Test with HCV-RNA

Figure 1. Correlation of HCV-Ag test with HCV-RNA.

of different countries ("WHO", 1997). Because treatment of HCV induced disorders are noticeably costly. On the other hand, once liver disease is established, hepatitis C can require intensive healthcare resources and generate very high costs, particularly in patients with end-stage liver disease (Kim, 2002).

The common route of infection occurs via intravenous drug injections in developed countries whereas unsafe injections and usage of contaminated equipment in invasive healthcare-procedures account for the transmission of the virus in under developed countries (Wasley and Alter, 2000). Screening of HCV is of great importance owing to the fact that 75% of the cases are asymptomatic. Currently antibody tests have been used in the screening of HCV infections. No antibody response may be elicited in some cases up to 6 months. Moreover hemodialysis patients, HIV and immunocompromised subjects may not reveal antibody response. (Schneeberger et al., 1998; Hoofnagle, 2002; Hadlich et al., 2007; El-Emshaty et al., 2011). Insidious nature of HCV infections and insufficiency of existing screening tests necessitate a new diagnostic tool to detect HCV antigen in blood for early intervention.

Although not cost effective some countries have been using molecular analysis of HCV for the purpose of screening. (Chiavetta et al., 2003). Since labor intensive and high technology depended it is not rational to apply these tests in high HCV prevalent countries.

In the present study, we have studied 152 samples, of which 23 were taken from patients with chronic HCV infection. Of 112 anti-HCV antibody positive samples, 80 were negative with HCV-RNA assay. Although the negative HCV-RNA results can be due to the non-viremic stage of the disease, it can be depended on the recovery from the infection as well. In our study HCV-Ag detected

31 of 32 HCV-RNA positive samples. The only sample that HCV-Ag test failed to detect was 0.08 pg/ml interpreted as grayzone.

The compatibility between molecular analysis of virus and HCV core ag tests were examined by HCV-RNA positive commercial samples including genotypes 1a, 1b, 2a, 2b, 3a, 3k, 4a, 5a and 6a. The investigators have detected a good correlation ($r = 0.74$) between these two assays in the quantitation of viral load (Morota et al., 2009). Furthermore, Park et al. (2010) confirmed a correlation of 94% between newly presented antigen test and molecular test using the method of real-time quantitative reverse transcription-PCR. Similarly, in our study the percent agreement between these assays was 94%. On account of this equivalency, HCV-Ag test would be used for both monitoring the progress of the disease and assessment of antiviral therapy.

Based on the study of Park et al. (2010), HCV-Ag test can be used instead of HCV-RNA test due to the necessity of qualified personnel, time-consuming and expensive properties of molecular analysis.

In the meantime anti-HCV cannot be considered as a redundant test. Even though antibody test cannot be able to distinguish acute and chronic infection, its reactivity is an evidence of virus admission in chronic infection, while HCV-RNA and HCV-Ag are negative (Ross et al., 2010). In the present study, 23 chronically HCV infected patients have an antibody response reactive in range of 1.26 to 16.81 s/co with RNA and Ag test negative results. Hence the antigen and antibody assays could be used concurrently in order to avoid both false positive and false negative results for HCV infections (Laperche et al., 2005; Alzahrani, 2008).

The limitation of our investigation is the limited number of positive samples and the lack of specimens including

window period of HCV infection (Anti HCV negative, HCV-Ag positive pattern), whether the patients bearing this pattern could give an idea of the sensitivity of new HCV-Ag assay in antibody false negative population. Nevertheless, the correlation between Antigen test and HCV-RNA was convincing according to the number of samples studied.

The second limitation can be assessed as; HCV-Ag test's ability in detecting virus independently from genotype differences. Although in our study we couldn't examine the various genotypes, it is well known that HCV genotypes 1b comprise over than 75% of Turkish HCV (Altuglu et al., 2008).

In conclusion, in our study we compared the newly introduced HCV antigen test with both commonly used anti-HCV antibody test and HCV-RNA test which is the reference method for HCV infections. According to our data HCV antigen test were highly positive in HCV-RNA positive samples. However, anti-HCV antibody should be used concurrently not to miss the chronic disease in non-viremic phase in which antigen test and RNA negative.

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