Research Article

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Performance of Rapid Test in Detection of Anti-HCV in Frozen Sera

Dondurulmuş Serumlarda Anti-HCV Saptanmasında Hızlı Testin Performansı

Zafer MENGELOĞLU, Özlem BUCAK, Esra KOÇOĞLU, Tekin TAŞ, Şeyda KARABÖRK

Abant İzzet Baysal University Faculty of Medicine, Department of Medical Microbiology, Bolu, Turkey

ABSTRACT

Objective: Hepatitis C is a common infectious disease throughout the world. Rapid test of hepatitis C, detecting anti-HCV, is a useful test that is easy to perform as well as it may have lack of sensitivity and specificity. In the present study, it was aimed to investigate whether the rapid test of HCV infection can still reveal accurate results in anti-HCV-positive sera frozen for a few years.

Materials and Methods: Sera of a total of 100 patients that were determined to be positive for anti-HCV using chemiluminescence technology system (Architect i2000sr, IL, USA) were stored at -200 °C for a maximum period of 3.5 years. The sera were thawed and tested again for anti-HCV using the Nanosign rapid test kit (Bioland, Korea) for HCV infection.

Results: The positivity rate of the test was 37%. In addition, the rapid test revealed a very low positivity rate as 5.2% in sera with a level of anti-HCV below 10.0 S/CO. In contrast, the positivity rate was 71.4% in samples with high anti-HCV levels. A significant positive correlation was found between positivity levels and anti-HCV levels ($r=0.708$, $p<0.001$). No correlation was found between positivity levels and time passed after freezing the samples ($r=-0.91$, $p=0.367$). Besides this, no significant difference was observed amongst the groups in terms of the time intervals of freezing ($p>0.05$).

Conclusion: The findings of this study suggest that the rapid test of HCV infection is not reliable in frozen sera with low anti-HCV levels due to the instability of the molecules in the samples, and the time passed after freezing of the sample doesn't change the results of the tests. *(Viral Hepatitis Journal 2014; 20(1): 8-10)*

Key words: Rapid test, anti-HCV, frozen sera, HCV

ÖZET

Amaç: Hepatit C, dünya genelinde yaygın enfeksiyöz bir hastalıktır. Anti-HCV'yi saptayan Hepatit C hızlı testinin duyarlılık ve özgüllüğü düşük olabilmekle birlikte kolay uygulanabilen kullanışlı bir testtir. Bu çalışmada, HCV enfeksiyonu hızlı testinin birkaç yıldır dondurulmuş olarak bekletilen anti-HCV pozitif serum örneklerinde hâlâ doğru sonuçlar verip vermediğinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Kemilüminisans teknolojisini kullanan sistemle (Architect i2000 sr, IL, ABD) anti-HCV pozitif saptanan toplam 100 hastaya ait serumlar -20 °C'de en fazla 3,5 yıllık bir süre saklandı. Serumlar çözdürüldü ve Nanosign hızlı test kiti (Bioland, Güney Kore) kullanılarak anti-HCV için tekrar test edildi.

Bulgular: Testin pozitiflik oranı %37 olarak bulundu. Ek olarak, hızlı test, anti-HCV düzeyi 10 S/CO'nun altında olan serumlarda %5,2 gibi çok düşük bir pozitiflik oranı verdi. Bunun tersine, yüksek anti-HCV düzeyi bulunan örneklerde bu oran %71,4 idi. Pozitiflik dereceleri ve anti-HCV düzeyleri arasında anlamlı pozitif korelasyon bulundu ($r=0,708$, $p<0,001$). Pozitiflik dereceleri ile dondurulma üzerinden geçen süre arasında herhangi bir korelasyon bulunmadı ($r=-0,91$, $p=0,367$). Bunun yanı sıra, dondurulma zaman aralıkları bakımından gruplar arasında anlamlı bir farklılık gözlenmedi ($p>0,05$).

Sonuç: Bu çalışmanın bulguları, örneklerdeki moleküllerin kararsızlığına bağlı olarak HCV hızlı testinin düşük anti-HCV bulunduran dondurulmuş serumlarda güvenilir olmadığını ve örneklerin dondurulmasından sonra geçen sürenin test sonuçlarını değiştirmediğini göstermektedir. *(Viral Hepatit Dergisi 2014; 20(1): 8-10)*

Anahtar Kelimeler: Hızlı test, anti-HCV, dondurulmuş serumlar, HCV

Introduction

Hepatitis C is a widespread infectious disease throughout the world, and may cause asymptomatic disease as well as chronic infections with high morbidity and mortality rates. Clinical and laboratory diagnosis of this infection is urgent. Serological testing for hepatitis C antibody (anti-HCV) is the primary way to identify patients with hepatitis C virus (HCV) infection. Testing has been recommended particularly for risky groups, pregnant women, infants born to anti-HCV-positive mothers, sex partners and household contacts of HCV-infected persons, persons born in countries with high anti-HCV prevalence, intravenous drug users, hemodialysis patients, blood donors and persons infected with human immunodeficiency virus (1,2).

HCV particles persist in blood, lymphocytes, and liver despite the immune response to most of the HCV proteins. Detection of anti-HCV has become the most practical means of diagnosing both present and past infection by allowing high-throughput screening in clinical laboratories and blood transfusion centers. False results still exist as one of the cons of anti-HCV test (1,3,4,5).

Address for Correspondence: *Zafer Mengeloğlu MD, Abant İzzet Baysal University Faculty of Medicine, Department of Medical Microbiology, Bolu, Turkey*
Phone: +90 374 253 46 56/32 64 E-mail: mengeloglu@gmail.com **Received:** *29.07.2013* **Accepted:** *15.11.2013*
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Rapid test of hepatitis C, known as cassette test, is a useful test that is easy to perform, however it may have lack of sensitivity and specificity. Rapid test detects anti-HCV in serum. In many small-sized laboratories, the rapid test has still been used in the diagnosis of HCV infection. It is used in blood transfusion centers in cases of emergencies for severely injured patients as well (2,6).

Sera of the patients have been stored in freezers for long times for many reasons such as repeating tests, hanging on for further or additional tests, or for some judicial occasions. The time passed in the freezer for the serum samples may be important in some tests in terms of the probable decrease in sensitivity as there is a question whether the anti-HCV molecules keep their stability despite the storage conditions. It is crucial for some cases to know whether the test is reliable in sera frozen for many years (2,6).

In the present study, it was aimed to investigate whether the rapid test of HCV infection can still reveal accurate results in anti-HCV-positive sera frozen for some years.

Material and Methods

Rapid Tests

Serum samples of a total of 100 patients were used for the study. All the sera that were detected positive for anti-HCV using chemiluminescence technology system (Architect i2000sr, IL, USA) were stored at -20 °C. The freezing time ranged from one month to 3.5 years. The sera were thawed for the first time, and tested again for anti-HCV using only the Nanosign rapid test kit (Bioland, Korea) for HCV infection. The chemiluminescence system was not used again simultaneously with the rapid test. The rapid test kit used in the study has a band coated with HCV antigenes as core, NS3, NS4, and NS5. The tests were performed following the recommendations of the manufacturer. Despite it is not noted by the manufacturer, the tests were scored ranging from negative to "+++" by two observers in order to clarify and analyse results better. The sera were groupped in terms of the freezing periods according to years (as <1 year, 1-2 years, 3 years, and >3 years). Chemiluminescence test results determining the anti-HCV level as "Signal to cut-off (S/CO)" was used as the quality control standard of the study, and so, positivity rate was calculated dividing the number of positive samples detected by rapid test by the number of real positive samples detected by the chemiluminescence system.

Statistical Analysis

Statistical analysis was performed using SPSS 15.0 software (IBM SPSS Inc., Chicago, IL, USA). Whether the continuous variables were normally distributed was assessed using the Shapiro-Wilk test. The Levene test was used to evaluate the homogeneity of variances. Spearman's rank correlation test was used for correlation and relation between indicated parameters. Values were presented as mean ± standard deviations. Group means were compared by one-way analysis of variance (ANOVA). The Kruskal-Wallis test was used to compare median values. A p value <0.05 was considered to indicate statistical significance.

Results

A total of 33 (33%) sera were detected as positive and 67 (67%) as negative for anti-HCV by the rapid test. Only three of 58 (5.2%) sera that had anti-HCV level between 1-10.0 S/CO with chemiluminescence system were positive using the rapid test (Table 1).

According to these findings, rapid test revealed a very low positivity rate of 5.2% in sera with anti-HCV levels below 10.0 S/CO. In contrast, 30 of the rest 42 sera with anti-HCV levels above 10.0 S/CO were positive, meaning of a positivity rate of 71.4% in high anti-HCV level samples.

A highly significant positive correlation was found between positivity levels and anti-HCV levels using Spearman test ($r=0.708$, $p<0.001$, correlation is significant at the 0.01 level by 2-tailed analysis). No correlation was found between positivity levels and time passed after years ($r=-0.91$, $p=0.367$, Spearman test). Besides, no significant difference was observed amongst the groups in terms of the time intervals of freezing ($p>0.05$) (Table 2). These findings suggest that the time passed after the first freeze of the sera didn't cause any impact on the results of rapid tests.

Discussion

Testing for HCV infection is an urgent requirement for the patients in risky group due to the high morbidity- and mortality-rates of the disease. Screening is a basic tool used to identify the disease even in the period before the symptoms occur. Rapid assays are amongst the popular methods in diagnosis of HCV infection particularly in developing countries. Rapid tests have some advantages such as being easy to perform, not requirement of expensive equipment or experienced staff, and providing immediate results. These tests can provide better flexibility and accessibility in various clinical settings (7). However, the main drawbacks of rapid assays are relatively lower clinical sensitivity and the operator's subjectivity in the interpretation of the test results (8). Importance of specificity, accuracy, and reproducibility of the test results is incontrovertible. It has been an important issue whether the time passed after the freezing of the clinical samples has any impact on the accuracy of the test results in diagnosis of hepatitis C.

In the present study, it was shown that the time passed after the freezing didn't change the test results using the rapid assay. The time used in this study ranged from one month to 3.5 years. The sera with higher anti-HCV levels revealed positive

Table 1. Distribution of sera according to levels of anti-HCV and positivity of rapid tests

Test result by rapid assay	n	Mean±SD*	Anti-HCV level (S/CO)	
			1.0-10.0	>10.0
Negative	67	4.77±4.31	55	12
+	27	13.19±2.13	3	24
++	4	15.63±1.40	0	4
+++	2	13.76±0.51	0	2
Total	100	7.66±5.56	58	42

* Standard deviation.

Table 2. Distribution of sera according to positivity levels of rapid test and time passed after freezing of sera

Test result	n	Storage time			
		<1 year	1-2 years	2-3 years	>3 years
Negative	67	16	15	20	16
+	27	8	7	6	6
++	4	1	2	1	0
+++	2	1	0	0	1
Total	100	26	24	27	23

results while the ones with lower levels revealed negative without any differences in terms of the time passed. The only determinant which effects the results was the initial anti-HCV level detected using chemiluminescence method. According to these findings, we can consider that rapid test cannot diagnose the HCV infection in the sera with low-level of anti-HCV with a low positivity rate of 5.2%, and we cannot rule out the infection if the rapid test kit reveals negative. Our findings suggest that the results don't differ with the time passed after freezing of the sample. Our false negative test results can be due to either the instability of the molecules in the serum samples due to the storage conditions or the inactive carriers who may have low anti-HCV levels, because rapid tests are reported to have quite higher sensitivity rates than our study showed (6).

In the present study, the positivity levels of the rapid test were found to be positively correlated with anti-HCV levels of the sera. The positivity levels even didn't differ due to the time of freezing. These findings suggest that positive results of the rapid test is reliable and a high positive result is likely to mean a high anti-HCV level. The sensitivity of the rapid HCV test in field studies is reported to range from 88.3% to 99.3% (9). However, the specificity rates reveal higher in most studies (6).

HCV rapid assay test cannot be solely relied on as an accurate diagnostic tool for screening infection of HCV particularly in high-risk group patients (10). Rapid HCV tests are not widely recommended for use even in blood transfusion centers due to their lower sensitivities (11). Besides this, the study by Scheiblauer et al (6). who evaluated a total of 44 rapid test assay concludes that these tests could be useful in small-sized laboratories and for epidemiological studies.

Bienek and Charlton (12) compared the effects of storage conditions of sera on the test results of HBV, HCV and HIV infections. They concluded that the diagnostic performance of the tests vary among products and storage conditions. In our study, the storage conditions of the sera were all the same in terms of temperature and humidity.

Comert et al. (13) reported no significant loss in viral load of HCV-RNA despite the freeze-thaw cycles, however, HCV-RNA molecules can differ from anti-HCV in terms of the molecular structure, and hence, the stability can be different. So we accepted chemiluminescence method as the quality control standard because chemiluminescence system detects anti-HCV just like the rapid test we used in the present study. We considered that using immuneblot or PCR tests as the reference methods might cause false analysis in calculating the positivity rates due to the different molecules that each of them detect.

HCV exhibits different genotype distributions and the antiviral antibody concentration in humoral fluids can vary with respect to geographic location (7). Therefore, the performance of rapid test kits are suggested to be evaluated using clinical specimens from the country that the study is being performed in (7). However, due to a report suggesting that the test performances of rapid assays is not related to the genotype, it is difficult to conclude with a reason for this (6). In the present study, we didn't perform any molecular tests for the distribution of HCV genotypes of the sera we used, so we are not able to discuss the reason of the low positivity rate concerning the genetical differences.

Previously, in another study, we found that freeze-thaw cycles decreased the positivity rates of the rapid test, moreover, the decrease was more as the number of the cycles increased (Unpublished data). So, in the present study, we used the sera that haven't been thawed until the study not to effect the test results.

In the present study, the positivity rate was only expressed about the frozen-thawed samples, so it doesn't give information about the reliability of the test results conducted with fresh samples.

Conclusions

The findings of the present study suggest that the rapid test of HCV infection is not reliable in frozen sera with low levels of anti-HCV and the time passed after freezing of the sample doesn't change the results of the tests. Besides, a negative rapid test result doesn't rule out the infection.

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Conflict of interest: None declared.

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