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Comparative evaluation of automated chemiluminescence tests and RIBA assay used in HCV diagnosis.

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Abstract

Introduction: Hepatitis C, caused by hepatitis C virus (HCV) can be a mild illness lasting a few weeks or can cause lifelong liver cirrhosis and cancer. Today although the sensitivity of diagnostic tests is increasing; it has often been associated with decreased specificity so the rate of false-positive test results is increasing. The aim of this study was to compare the false-positive rates of anti-HCV results.
Methods: During the period of 18.07.2011 to 18.12.2013; blood samples of patients admitted to Konya Numune Hospital were screened for anti-HCV using chemiluminescence immunoassay (CIA). After 2012; the new version of same anti-HCV test was used. Borderline and reactive results were retested and tests which were reactive in repeated CIA were confirmed by a recombinant immunoblot-assay (RIBA). Subjects with a positive RIBA test were considered to have been as true positive anti-HCV.
Results: A total of 54178 sera were tested for anti-HCV during the period of 18.07.2011 to 18.12.2013 and 649 sera were positive with chemiluminescence method. 374 of reactive cases were confirmed by RIBA. The RIBA results showed 171 (45.7 %) negative, 163 (43.5 %) positive, and 40 (10.7 %) indeterminate results. By using the new version of the test; the rate of false positive and indeterminate anti-HCV test results decreased from 75.1% to 35.5 %.
Conclusions: In this study it was observed that lower false positive rates of newly developed test. Lowering the false positive rate of ELISA tests will provide more confidence to use these tests in the diagnosis of HCV. There is a need for further studies on this issue.

Keywords: Hepatitis C virus (HCV), Anti-HCV, HCV-RIBA.

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Introduction

Hepatitis C virus (HCV) is an enveloped positive-stranded RNA virus of the Flaviviridae family. HCV is endemic in most parts of the world [1]. HCV infection is often asymptomatic; however, the majority of HCV-infected individuals develop a persistent chronic infection and nearly in 20% of these subject; cirrhosis occurs [1-3]. Patients with HCV-related cirrhosis have an increased risk of developing hepatocellular carcinoma [4]. HCV is transmitted through exposure to infected blood, blood products and body fluid [1-3]. Also, invasive procedures can be a risk for transmission [5]. People with a history of intravenous drug use, and those with HIV infection, hemophilia, leprosy is an important risk factor for HCV infection [6].

Diagnosis of HCV infection is a crucial public health problem in the general population, especially for the high-risk patients such as those undergoing hemodialysis, blood donors, etc. [4]. Also, in the elderly population, there is a risk as before HCV wasn't screening in blood donors. Definitive diagnosis of HCV infection is difficult, and treatment is highly expensive and has severe side effects. For diagnosis of HCV infection; detection of HCV antigen-specific antibodies, viral RNA or HCV core antigens can be used [4-7]. Antibody detection tests like chemiluminescent immunoassay (CIA) and enzyme-linked immüno-sorbant assay (ELISA) tests are the most frequently serological tests which are used for diagnosis of HCV infection [4,6-8]. These serological tests are rapid, easy and cheap but false positive results frequently have been observed especially for antibody tests [9]. CDC has recommended that a person can be considered to have serologic evidence of HCV infection only after an anti-HCV screening-test-positive result has been verified by a more specific serologic test, e.g., the recombinant immunoblot assay (RIBA) or a nucleic acid test (NAT) [10].

The studies have been performed for decreasing false positive ratios for the serological test. The aim of this study was to

determine the false-positive rates of anti-HCV results in a state hospital and to compare the false-positive rates of the previous version and new version of a chemiluminescence immunoassay for the anti-HCV test.

Methods

Patients admitted to Konya Numune Hospital's clinics with various clinical symptoms included in this study from July 2011 to December 2013. A total of 54178 blood samples were taken from these patients, and anti-HCV tests were performed by using chemiluminescence immunoassay (CIA) (Cobas®e 601, Roche Diagnostics, Mannheim, Germany).

For CIA; samples with cutoff-index <0.90 are nonreactive, between ≥ 0.9 and <1.0 are considered as borderline and ≥ 1 was accepted as reactive. When anti-HCV was positive or borderline by CIA, samples were retested and tests which were reactive in repeated CIA were confirmed by RIBA "Recombine HCV IgG" (Mikrogen Diagnostic, Germany). Only one serum sample of patients who found positive previously tested with RIBA test, others were excluded from the study. 374 of 649 reactive cases were confirmed by using RIBA. For CIA patient with positive RIBA test was considered to have true anti-HCV that shows exposure to the virus and so active or past infection.

The new version of the test developed by this company was used after July 2012 in our hospital. Previous version of CIA test was used for testing up to July 2012. False positivity ratios of previous and new versions of the chemiluminescence immunoassay were compared according to RIBA tests.

Statistical analysis was made by the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA, version 17.0) for Windows software program. The relationship between the false-positive rates of the previous version and new version of a chemiluminescence immunoassay for anti-HCV test was compared with Chi-Square Tests.

Results

A total of 54178 sera were tested for anti-HCV, and a total of 649 (%1.2) sera were positive with chemiluminescence method during July 2011 to December 2013. 261 of 21833 tests which were studied with the previous version were positive, and 388 of 32345 tests which were studied with new version were positive.

374 of 649 reactive cases were confirmed by using RIBA. Of 374 tests positive with chemiluminescence method, 171 (45.7 %) were negative, 163 (43.5 %) were positive, and 40 (10.7 %) were indeterminate by RIBA. The test results of previous and new versions of chemiluminescence method were compared according to RİBA in Table 1.

Table 1: Comparison of the test results of previous and new versions of chemiluminescence method according to RİBA.

		RIBA			Total
		Negative*	Positive**	Indeterminate***	
Previous version	Count	121	49	27	197
	% within version	61.40%	24.90%	13.70%	100.00%
New version	Count	50	114	13	177
	% within version	28.20%	64.40%	7.30%	100.00%
Total	Count	171	163	40	374
	% within version	45.70%	43.60%	10.70%	100.00%

*There is no band; **There are two or more HCV bands; ***There is positivity in one band

Differences were observed between previous version and new version of a chemiluminescence immunoassay for anti-HCV test and these differences were statistically significant ($p<0.001$). It is observed that the rates of false positive and indeterminate anti-HCV test results decreased by using the new version of the test.

Discussion

The hepatitis C virus is most commonly transmitted through exposure to infected blood and body fluid [2]. Today especially in some countries which having chronic infection rates as high as 5% and above; unsafe injections have an important role in transmission [11]. Hepatitis C virus can cause chronic hepatitis and hepatocellular carcinoma [12]. Hepatitis C is generally considered to be a curable disease but treatment is not well tolerated in some patients [13]. There is no vaccine for hepatitis C so raising awareness for preventing transmission is very important [11].

For the diagnosis of acute HCV infection, both clinical signs and laboratory findings are necessary, because infection is often asymptomatic [6,14]. The HCV genome consists of seven functional regions: the core, the envelope, including the E1 and E2 regions, and the nonstructural region, including NS2, NS3, NS4, and NS5 [4]. Today, for the detection of antibodies against HCV (anti-HCV); third-generation tests have been widely used due to their increased sensitivity and specificity

[8-10]. Third-generation tests like CIA for testing anti-HCV contains reconfigured core and NS3 antigens and an additional antigen (NS5) [8-10]. So it shortens the time for detection of antibody to an average of 7-8 weeks after infection. But because of the addition of more antigens into the third generation tests, both the sensitivity and specificity had increased and another problem; the increase in the rate of false positivity had occurred. To perform confirmatory tests on samples with low sample/cut-off (S/CO) ratios to avoid false positive results has become mandatory [8,15]. The studies are underway for earlier serological identification of HCV and to improve the sensitivity and specificity.

After exposure to the virus, HCV RNA, the first marker is usually detectable by 1-2 weeks and anti-HCV is usually detectable by 8-10 weeks [16]. The presence of anti-HCV antibodies may usually indicate HCV infections, but it may show a false positive [17]. RIBA is a reliable supplemental test for discriminating between true-and false-positive results of anti-HCV tests which is the most common method of serological diagnosis of antibody against HCV [7,16,18,19]. HCV RNA is another test that can be useful to confirm of anti HCV. RIBA should be tested to report anti-HCV as negative when HCV RNA is negative anti HCV positive [10,18]. Pereira et al., [20] reported that had no detectable HCV-RNA in sera of individuals who has anti-HCV positive and indeterminate RIBA results. Cross-reactive circulating antigens and antibodies may cause false positive results such as pregnancy in women, nephrotic syndrome autoimmune diseases etc. [21,22]. False positive results of antibody screening tests for Hepatitis C virus may lead to the unnecessary use of expensive tests like HCV RNA or RIBA for confirmation [18,23]. So we used only RIBA test to confirm anti HCV antibody.

Keşli et al., [9] reported that the false seropositivity rates by using the Cobas601 was 52.2% (71/136) with confirmed by RIBA. Moretti et al. [24] reported that the 313 positive patients with CIA was confirmed by RIBA and 222 (71.0%), 46 (14.7%), and 45 patients (14.3%) were positive, negative, and indeterminate, respectively. In this study, it is seen that a false positive rate decreases from 61% to 28% with new version CIA.

The overall prevalence of HCV is estimated at nearly 3% [2,25]. To confirm active HCV infection, medical evaluation and treatment; nucleic acid testing is required. And if RIBA is negative, no further evaluation of the person is needed. Sometimes HCV RNA result can be negative in active HCV infection or intermittent positivity can be detected. So the positivity of HCV RNA shows the infection but the negativity of HCV RNA does not show absence of the infection. In China: serum samples from blood centres in China were tested for HCV antibodies and later RIBA was performed. HCV RNA couldn't be detected in 5.72% of serum samples which were RIBA-positive [26].

As it is known; HBV infection is more common all around the world. There is a vaccine for HBV. So in the future the seroprevalance of HBV infection may decrease but it is shown in Iran that the prevalence of HCV is rising, especially among younger men, and in the future perhaps hepatitis C would be the most common cause of chronic viral liver disease so planning the prevention of HCV infections becomes more of a social than a medical problem in future [27].

It is important to provide more reliable results for physicians and their patients. For persons being tested for HCV infection for the first time, if there is no clinical sign the diagnosis is difficult and further testing is more reliable in diagnosis of HCV infectious. So reactive tests especially with low S/CO ratios should be confirmed with confirmatory tests to avoid false positive results.

In this study it was observed that lower false positive rates of newly developed test. The false positive rate was significantly decreased by using the new test statistically. Lowering the false positive rate of CLA and like tests will provide more confidence to use these tests in the diagnosis of HCV. But there is still need to using of the confirmation tests when tested a positive test with CIA and like tests. There is a need for further studies on this issue.

References

1. Craxì A, Laffi G, Zignego AL. Hepatitis C virus (HCV) infection: a systemic disease. Mol Aspects Med 2008; 29: 85-95.
2. Khan A, Tareen AM, Ikram A, Rahman H, Wadood A, Qasim M, Khan K. Prevalence of HCV among the young male blood donors of Quetta region of Balochistan, Pakistan. Virol J 2013; 10: 83.
3. Chiquete E, Sánchez LV, Maldonado M, Quezada D, Panduro A. Prediction of the hepatitis C viremia using immunoassay data and clinical expertise. Ann Hepatol 2005; 4: 107-114.
4. Ismail N, Fish GE, Smith MB. Laboratory evaluation of a fully automated chemiluminescence immunoassay for rapid detection of HBsAg, antibodies to HBsAg, and antibodies to hepatitis C virus. J Clin Microbiol 2004; 42: 610-617.
5. Piro L, Solinas S, Luciani M, Casale A, Bighiani T, Santonocito D, Girelli G. Prospective study of the meaning of indeterminate results of the recombinant immunoblot assay for hepatitis C virus in blood donors. Blood Transfus 2008; 6: 107-111.
6. Caruntu FA, Benea L. Acute hepatitis C virus infection: Diagnosis, pathogenesis, treatment. J Gastrointestin Liver Dis 2006; 15: 249-256.
7. Kaya S, Cicioglu Aridogan B, Sesli Cetin E, Adiloglu AK, Demirci M. Comparison of polymerase chain reaction and serological methods in the diagnosis of hepatitis C virus infection. Suleyman Demirel Uni Tip Fak Derg 2007; 14: 10-14.
8. Zer Y, Karaoğlan I, Çiçek H, Karağoz ID, Sağlam M. Evaluation of the patients with low levels of anti-HCV positivity. Mikrobiyol Bul 2009; 43: 133-139.
9. Kesli R, Ozdemir M, Kurtoglu MG, Baykan M, Baysal B. Evaluation and comparison of three different anti-hepatitis

C virus antibody tests based on chemiluminescence and enzyme-linked immunosorbent assay methods used in the diagnosis of hepatitis C infections in Turkey. J Int Med Res 2009; 37: 1420-1429.

10. Alter MJ, Kuhnert WL, Finelli L, Centers for Disease Control and Prevention. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. MMWR Recomm Rep 2003; 52: 1-13.
11. World Health Organization. Hepatitis C: Fact sheet No. 164. Geneva: Switzerland.
12. Naderi M, Gholipour N, Zolfaghari MR, Moradi Binabaj M, Yegane Moghadam A, Motalleb G. Hepatitis C virus and vaccine development. Int J Mol Cell Med 2014; 3: 207-215.
13. Webster DP, Klenerman P, Dusheiko GM. Hepatitis C. Lancet 2015; 2.
14. Allison RD, Conry-Cantilena C, Koziol D, Schechterly C, Ness P, Gibble J, Kleiner DE, Ghany MG, Alter HJ. A 25-year study of the clinical and histologic outcomes of hepatitis C virus infection and its modes of transmission in a cohort of initially asymptomatic blood donors. J Infect Dis 2012; 206: 654-661.
15. Pereira FM, Sant'Anna Zarife MA, Bertollo LA. Comparison of two automated hemiluminescence tests for the detection of antibodies against the hepatitis C virus. Rev Pan-Amaz Saude 2010; 1: 17-21.
16. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. Clin Chem 2000; 46: 2027-2049.
17. Centers for Disease Control and Prevention (CDC). Testing for HCV infection: an update of guidance for clinicians and laboratorians. MMWR Morb Mortal Wkly Rep 2013; 62: 362-365.
18. Sayan M, Meriç M, Mutlu B, Celebi S, Willke A. Low positive anti-HCV microparticle enzyme immunoassay results: do they predict hepatitis C virus infection?. Mikrobiyol Bul 2006; 40: 81-84.
19. Makuria AT, Raghuraman S, Burbelo PD, Cantilena CC, Allison RD, Gibble J, Rehermann B, Alter HJ. The clinical relevance of persistent recombinant immunoblot assay-indeterminate reactions: insights into the natural history of hepatitis C virus infection and implications for donor counseling. Transfusion 2012; 52: 1940-1948.
20. Pereira FM, Zarife MA, Reis EA, G Reis M. Indeterminate RIBA results were associated with the absence of hepatitis C virus RNA (HCV-RNA) in blood donors. Rev Soc Bras Med Trop 2014; 47: 12-17.
21. Sharma UK, Stramer SL, Wright DJ, Glynn SA, Hermansen S, Schreiber GB, Kleinman SH, Busch MP; Retrovirus Epidemiology Donor Study. Impact of changes in viral marker screening assays. Transfusion 2003; 43: 202-214.
22. Rahman M, Khan SA, Lodhi Y. Unconfirmed reactive screening tests and their impact on donor management. Pak J Med Sci 2008; 24: 517-519.
23. Takeuchi T, Katsume A, Tanaka T, Abe A, Inoue K, Tsukiyama-Kohara K, Kawaguchi R, Tanaka S, Kohara M. Real-time detection system for quantification of hepatitis C virus genome. Gastroenterology 1999; 116: 636-642.
24. Moretti M, Pieretti B, Masucci A, Sisti D, Rocchi M, Delprete E. Role of signal-to-cutoff ratios in hepatitis C virus antibody detection. Clin Vaccine Immunol 2012; 19: 1329-1331.
25. Anand BS, Velez M. Assessment of correlation between serum titers of hepatitis C virus and severity of liver disease. World J Gastroenterol 2004; 10: 2409-2411.
26. Zhang K, Wang L, Sun Y, Zhang R, Lin G, Xie J, Li J. Improving the safety of blood transfusion by using a combination of two screening assays for hepatitis C virus. Transfus Med 2014; 24: 297-304.
27. Merat S, Rezvan H, Nouraie M, Jafari E, Abolghasemi H, Radmard AR, Zaer-rezaii H, Amini-Kafiabad S, Maghsudlu M, Pourshams A, Malekzadeh R, Esmaili S. Seroprevalence of hepatitis C virus: the first population-based study from Iran. Int J Infect Dis 2010; 14: e113-116.

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