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New approaches to in vitro diagnosis of hepatitis C infection a reason for post transfusion hepatitis: Diagnostic value of determination of hepatitis C virus core antigen

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ABSTRACT

In between the dates of February 2008–March 2009, by applying to Istanbul University CTF Microbiology and Clinical Microbiology Basic Sciences Branch and Duzen laboratories, 123 cases, where HCV RNA and anti-HCV positivity are identified with molecular (real-time PCR) and serologic (ELISA) methods as a positive control group, and 48 cases where HCV RNA and anti-HCV negativity are identified as a negative control group are established. The values of sensitivity, specificity, positive and negative approximation of recently developed HCV Core Ag (Abbott Diagnostics, Germany) kit are determined successively as 94.3%, 97.9%, 99.1%, 87%, 95.3% and 88%. Although the new HCV Ag assay is clearly not sensitive enough to replace HCV NAT it may serve as a valuable tool in the HCV diagnostic algorithm as it is able to pick up a great majority of anti-HCV and HCV RNA positive samples, thus allowing a timely and less expensive serological diagnosis of an active HCV infection. This may be an advantage for labs that do not have access to PCR easily.

1. Introduction

Hepatitis C virus (HCV) is a RNA virus of Flaviviridae family. It was first defined as non-A and non-B hepatitis in 1975, and the genome belonging to the dominant virus was cloned in 1989. HCV is one of the main causes of acute and chronic hepatitis across the world. Even though the main transmission way for the virus is blood transfusion, HCV may also contaminate through tissue transplantation, drug usage via injection, sexual intercourse with infected individuals or interfamily relations, perinatal and percutaneous transmission, use of contaminated tools, etc. [1–4]. For specific HCV in vitro diagnosis, HCV antibody test and RT-PCR, as well as HCV RNA tests are frequently used. Sensitivity and specificity issues have been observed for years in the commercial kits that determine anti-HCV response. Such issues particularly reveal themselves as false negativity due to late response of antibody or binding to non-specific antigenic epitope or false positivity developed due to some other mechanisms. Neither addition of new antigenic determinants nor modifications specific to experiment process could have bring a solution for issues in antibody-based HCV diagnosis. Therefore, 4th generation ELISA kits have been introduced into the market for early detection of lately developed responses of HCV antibody. Because of the limited characteristics of diagnostic antibody tests, RIBA is offered as the validation kit; however, since this test is not used very often, diagnosis is mostly performed using the RT-PCR (reverse transcription-polymerase chain reaction) method and HCV RNA method. [5–7] Ongoing researches in the field of creating the practical, cost-effective tests with optimal sensitivity and

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specificity to screen donor bloods for HCV infections or to diagnose clinical HCV cases are currently under review. This researches are important in some clinical pictures that are frequently have problems about indicating whether antibody positivity resulted from a past or a current infection and hemodialysis patients that has mute antibody response due to immunesuppression. For these reasons detecting viral antigen may help supporting the in vitro diagnosis in such situations [8,9]. In this study we aimed to examine the diagnostic performance of newly developed HCV core antigen test in diagnosis of Hepatitis C infection for the first time in Turkey.

2. Material and methods

In our planned, methodology-based, cross-sectional study conducted between February 2008 and March 2009, 123 cases clinically diagnosed with viral hepatitis using clinical findings, biochemical data (ALT, bilirubin, etc.) and radiodiagnostic data were included; serologic and molecular tests were requested from these 123 subjects, who applied to Istanbul University, Cerrahpasa Faculty of Medicine, Department of Microbiology and General Clinic Microbiology and Düzen Laboratory, and who were detected positive with anti-HCV (Murex, Abbott, South Africa) test and were also found to be positive using Realtime PCR and HCV RNA as reference test method. HCV RNA and anti-HCV negativity 48 healthy blood donors detected to be HCV RNA-and anti-HCV-negative, who were seronegative with regard to donor viral factors, were included in the study as negative control group. Recently developed antibodies, in which specific monoclonal anti-HCV are placed in solid phase Hepatitis C virus core antigen zone, HCV Ag (Abbott Diagnostics, Germany) kit for detecting antigen, were examined in terms of sensitivity, specificity, positive and negative predictive values, accuracy and kappa values in diagnosis of HCV infection. All statistical evaluations were carried out using SPSS 15.0 (Statistical Packages for Social Sciences; SPSS Inc., Chicago).

3. Results

Of the 123 subjects with actual Hepatitis C (HCV RNA-positive) included in the study, 116 (94%) cases were detected to be positive in HCV antigen test and 7 were found to be negative. On the other hand, of the 48 negative control subjects, who tested negative for anti-HCV and HCV RNA, 1 subject (2%) tested positive and 47 (98%) subjects tested negative in HCV antigen test. Based on the existing data, sensitivity, specificity, positive and negative predictive values, accuracy and kappa values as the diagnostic performance parameters of recently developed HCV antigen test (Abbott Diagnostics, Germany) were detected as 94.3%, 97.9%, 99.1%, 87%, 95.3% and 88%, respectively (Table 1).

In our study, when the positivity of HCV Ag test was examined according to various viral load distribution patterns between 0 and 100,000 IU/ml of viral load quantity determined with real time as HCV RNA, HCV antigen test was determined to be negative for 6 cases with viral load lower than 2000 IU/ml, HCV Ag test was found to be negative for one subject with 33,200 IU/ml viral load and no negativity was determined for the other patterns (Table 2).

Table 1
Diagnostic performance parameters for HCV antigen test.

	Reference test	–(HCV-RNA)
HCV-Ag test	Actual positive	Actual negative
Positive	116	1
Negative	7	47
Total	123	48

4. Discussion

Hepatitis C seen commonly worldwide is a serious health issue in terms of morbidity and mortality across the world and in our country. Health professionals, patients undergoing transplantation and hemodialysis are the main risk groups defined. Several reasons, such as exhibiting chronic persistent characteristics, adverse proceeding in patient's prognosis for certain genotypes (e.g. type 1 b), frequently occurring exacerbation and relapse during treatment, occurrence of hepatocellular carcinoma resulting with higher rate in comparison to the other viral factors of hepatitis, under development of preventive inoculation, indicate the severity of the HCV infections in a universal and national extent. Due to such reasons, the importance of acting in accordance with the preventive measures without being exposed to HCV infections and development of recent test methods devoted to correct and duly diagnosis to prevent transmission of the infection for especially the public health, as well as usage of such methods for diagnosing clinical cases and donor screenings related to production of blood and blood products, are emphasized. Research for easily applied and high speed tests providing high sensitivity and specificity based on serologic tests for diagnosing HCV infections is still continued, as the diagnostic sensitivity and specificity issues of antibody search-based test kits used in laboratory tests for HCV are not resolved and no standardization can be achieved; therefore, studies focusing on this field draw special attention [10–13].

Since the antibody response for patients exposed to HCV infection is obtained late (extending up to 6 months), the diagnostic performance of test kits vary, while the tests do not give a clue with respect to whether antibody response determined shows actual viremia, which makes routine diagnostic validation and molecular tests during treatment follow-ups still necessary. Researchers put serious efforts to develop HCV antigen kits in recent years that will reveal performance at least close to the results of molecular methods with regard to sensitivity and specificity and that will minimize the serologic problems encountered during the first stage of diagnosis of HCV infections, and try to present the diagnostic performances with a series of clinical case studies. This is the first study performed with commercial kit for detecting antigen in our country. The performance of the kit based on obtaining core antibodies through placing monoclonal antibodies with solid

Table 2
Viral load distribution and HCV core Ag results in HCV RNA – positive subject.

HCV-RNA (IU/ml)	HCV-RNA	HCV core Ag	
	Number of positive subjects	Positive subjects	Negative subjects
0–1000	4	0	4
1001–2000	2	0	2
2001–10000	2	2	0
10001–100000	13	12	1[a]
>100000	102	102	0
Total	123	116	7

[a] 33,200 IU/ml is determined as viral load.

phase, which was developed for the core zone of HCV genome, was assessed with 123 actual HCV and 48 actual negative subjects. The sensitivity, specificity, Real-time PCR (RT-PCR) and kappa (compliance) values were determined as 94.3%, 97.9% and 88%, respectively. Among the studies performed with the same kit in other countries with 5403 specimens from volunteer blood donors, hospitalized patients and individuals with medical conditions unrelated to HCV infection showed that the core antigen tested positive for 196 (99.5%) out of 197 actual HCV infected subjects with positive HCV-RNA. 97% positivity is determined using another immunoradiometric antigen test and positivity is designated as 98% with another RT-PCR method experienced [14]. Again in 2007, Dawson et al. from USA found HCV core antigen test positive for 175 persons, where they compared the same antigen kit with qualitative HCV-RNA in the study including 185 subjects, and declared the sensitivity of test to be 94.6% [15]. 100% sensitivity is determined with HCV core antigen test from 385 clinical examples in a study performed by Ross et al. from Germany, in 2009 [16]. In the study with 282 cases performed by Park et al in 2010, automatic HCV core Ag testing shows a high correlation with quantitative RT-PCR and is indicated that this antigen may be an alternative to the method's determined by PCR assay [17]. While the study carried out by Dawson et al. [15] brought similar conclusions with our study, other three studies resulted with higher sensitivity rates than the one detected in our study.

However, the common conclusion of our study and other four studies is that HCV antigen test has a sensitivity close to RT-PCR result in the diagnosis of HCV infections and diagnostic performance level of the test proved to be sufficient. In the study performed, viral load was lower than 2000 IU/ml in 6 HCV RNA positive and HCV Ag negative subjects. This value can be accepted as the lower limit for analytical sensitivity for HCV Ag test for our study. We consider that HCV Ag Negative results obtained from a HCV RNA-positive (33,200 IU/ml) subject may be due to genotype differences. The genotype determination was not performed because of the insufficiency of patients' sera or the death of patients. Similar results were reported in the studies performed by Morota et al. in Japan on diagnostic relations of HCV antigen test with genotypes (4a/4c) [14]. At the same time, a study that performed by Krajden et al from Canada in 2004, indicates that second-line core antigen test has 27,000 IU/ml antigen detecting limit (Limit of Detection-LOD) and has a limited value for the diagnosis of active HCV infection as a confirmatory test in viremic specimens, which means quantitative PCR RNA testing is still maintain its importance [8]. Another study performed by Schnuriger et al., in 2006 indicates that the sensitivity of HCV core antigen test is significantly lower than PCR testing and a sensitive PCR method is required in patients with acute HCV infection [18]. Also They found that for the diagnosis of hepatitis C infection specially in high-risk group, the long period of HCV seronegativity should be reduced. However Fabrizi et al emphasized in their study in 2005 the usefulness of total HCV core Ag in performing HCV RNA measurement among hemodialysis patients without needing any special equipment or training [19]. In addition to these studies Bouzgarrou et al., in 2005, Medhi et al., in 2008 and Miedouge et al., in 2010 reached similar conclusions in their studies [20–22].

As a result, the diagnostic performance parameters of recently developed HCV antigen test kits satisfy the scientific criteria in screening and diagnostic tests. Although our study does not include hemodialysis patients and with the foresight of the literatures that we accessed, emphasize on the importance of using HCV core antigen test in acute HCV infection in these group of patients, due to our feature, the test kit can serve as a valuable tool in the HCV diagnostic algorithm as it is capable of collecting the majority of anti-HCV and HCV RNA-positive samples, thus allowing a timely and costefficient serological diagnosis of an active HCV infection. This may be an advantage for labs that do not have easy access to PCR.

References

[1] Huarte MP, Casi MA. Virology, diagnostic tests, epidemiology and transmission mechanisms of hepatitis C virus infection. An Sist Sanit Navar 2004;27:41–9.
[2] Davison SM, Mieli-Vergani G, Sira J, Kelly DA. Perinatal hepatitis C virus infection: diagnosis and management. Arch Dis Child 2006;91:781–5.
[3] Lima LP, Pedro RJ, Rocha MD. Mother-to-infant transmission of hepatitits C virus (HCV) in Brazil. J Trop Pediatr 2004;50:236–8.
[4] Schackman BR, Oneda K, Goldie SJ. The cost effectiveness of elective cesarian delivery to prevent hepatitis C transmission in HIV-coinfected women. AIDS 2004;18:1827–34.
[5] Laperche S, Le Marrec N, Girault A, Bouchardeau F, Servant-Delmas A, Maniez-Montreuil M. Simultaneous detection of hepatitis C virus (HCV) core antigen and anti-HCV antibodies improves the early detection of HCV infection. J Clin Microbiol 2005;43:3877–83.
[6] Echevarría J, Avellón A, Jonas G, Hausmann M, Vockel A, Kapprell HP. Sensitivity of a modified version of the ARCHITECT® Anti-HCV test in detecting samples with immunoblot-confirmed, low-level antibody to hepatitis C virus. J Clin Virol 2006;35:368–72.

[7] Kim S, Kim JH, Yoon S, Park YH, Kim HS. Clinical performance evaluation of four automated chemiluminescence immunoassays for hepatitis C virus antibody detection. J Clin Microbiol 2008;46:3919–23.
[8] Krajden M, Shivji R, Gunadasa K, Mak A, McNabb G, Friesenhahn M. Et al Evaluation of the core antigen assay as a second line supplemental test for diagnosis of active hepatitis C virus infection. J Clin Microbiol 2004;42:4054–9.
[9] Reddy AK, Dakshinamurty KV, Lakshmi V. Utility of HCV core antigen ELISA in the screening for hepatitis C virus infection in patients on hemodialysis. Indian J Med Microbiol 2006;24(1):55–7.
[10] Leary TP, Gutierrez RA, Muerhoff AS, Birkenmeyer LG, Desai SM, Dawson GJ. A chemiluminescent, magnetic particle-based immunoassay for the detection of hepatitis C virus core antigen in human serum or plasma. J Med Virol 2006;78:1436–40.
[11] Comanor L, Elkin C, Leung K, Krajden M, Kronquist K, Nicolas K. Successful genotyping of previously failed and low viral load specimens sung an HCV RNA qualitative assay based on transcription mediated amplification in conjunction with the line probe assay. J Clin Virol 2003;28:14–26.
[12] Barut HS, Gunal O. Global and national epidemiology of hepatitis C. Klimik Derg 2009;22:8–43 [in Turkish].
[13] Lauer GM, Walker BD. Hepatitis C virüs infection. N Engl J Med 2001;345:41–52.
[14] Morota K, Fujinami R, Kinukawa H, Machida T, Ohno K, Saegusa H. A new sensitive and automated chemiluminescent microparticle immunoassay for quantitative determination of hepatitis C virus core antigen. J Virol Methods Apr 2009;157:8–14.
[15] Dawson GJ. HCV core antigen detection in seropositive samples. J Med Virol 2007;79:52–3.
[16] Ross RS, Viazov S, Salloum S, Hilgard P, Gerken G, Roggendorf M. Analytical performance characteristics and clinical utility of a novel assay for quantification of total hepatitis C virus core antigen. J Clin Microbiol 2010;48:1161–8.
[17] Park Y, Lee JH, Kim BS, Kim do Y, Han KH, Kim HS. New automated hepatitis C virus (HCV) core antigen assay as an alternative to real-time PCR for HCV RNA quantification. J Clin Microbiol 2010;48:2253–6.
[18] Schnuriger A, Dominguez S, Valantin MA, Tubiana R, Duvivier C, Ghosn J. Early detection of hepatitis C virus infection by use of a new combined antigen-antibody detection assay: potential use for high-risk individuals. J Clin Microbiol 2006;44:1561–3.
[19] Fabrizi F, Lunghi G, Aucella F, Mangano S, Barbisoni F, Bisegna S. Novel assay using total hepatitis C virus (HCV) core antigen quantification for diagnosis of HCV infection in dialysis patients. J Clin Microbiol 2005;43:414–20.
[20] Bouzgarrou N, Fodha I, Othman SB, Achour A, Grattard F, Trabelsi A. Evaluation of a total core antigen assay for the diagnosis of hepatitis C virus infection in hemodialysis patients. J Med Virol 2005;77:502–8.
[21] Medhi S, Potukuchi SK, Polipalli SK, Swargiary SS, Deka P, Chaudhary A. Diagnostic utility of hepatitis C virus core antigen in hemodialysis patients. Clin Biochem 2008;41:447–52.
[22] Miedouge M, Saune K, Kamar N, Rieu M, Rostaing L, Izopet J. Analytical evaluation of HCV core antigen and interest for HCV screening in haemodialysis patients. J Clin Virol 2010;48:18–21.