Original Article

Diagnostic validity of the chemiluminescent method compared to polymerase chain reaction for hepatitis B virus detection in the routine clinical diagnostic laboratory

Mohammad-Hassan Khadem-Ansari[1], Mir-Davood Omrani[2], Yousef Rasmi[1,3], Arsalan Ghavam[4]

[1]Departments of Biochemistry, [2]Genetics, Faculty of Medicine, [3]Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran, [4]Biology, Faculty of Sciences, Hacettepe University, Ankara, Turkey

Abstract

Background: Hepatitis B virus (HBV) is the most common significant chronic viral infection world-wide. Hepatitis B surface antigen (HBsAg) has been the principal target for laboratory testing to identify active infection by HBV. We aimed to find out diagnostic validity of the Liaison chemiluminescent method compared to the polymerase chain reaction (PCR) method for HBV detection in the routine clinical diagnostic laboratory.

Materials and Methods: From 350 patients suspicious of having infection with HBV, serum samples were separated and used for testing HBsAg by two methods of Liaison chemiluminescent immunoassay, with HBsAg confirmatory test and PCR method.

Results: According to the PCR results as assumed as gold standard method with 100% sensitivity and specificity, detection rate sensitivity of chemiluminescent with confirmatory test was 96% and its specificity was 100%, and for chemiluminescent without confirmatory test sensitivity and specificity were 100% and 70%, respectively. Also for chemiluminescent with confirmatory test, positive predictive value (PPV) was 100% and its negative predictive value (NPV) was 97%, compared to chemiluminescent without confirmatory test with PPV and NPV equal to 71% and 100%, respectively.

Conclusions: It is possible to conclude that in the majority of the HBV cases, the diagnostic value of chemiluminescent method compared to the PCR method is acceptable, except in low indexes positive cases that need further investigation with the PCR method.

Key Words: Chemiluminescent, diagnostic value, hepatitis B virus, polymerase chain reaction

Address for correspondence:
Dr. Yousef Rasmi, Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran. E-mail: rasmiy@umsu.ac.ir
Received: 12.12.2012, **Accepted:** 01.12.2013

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.133178

INTRODUCTION

Hepatitis B virus (HBV) continues to be a major global public health problem. Two billion people have been infected worldwide; 400 million infected with chronic form of HBV infection, and over 520,000 die each year. Over 70% of the chronic hepatitis B patients in the world are Asians.[3-5] The rate of chronic hepatitis B infection in Iran differs based on the regions and studied groups.[1,2] A prevalence rate of 1.7% in the Fars province

How to cite this article: Khadem-Ansari M, Omrani M, Rasmi Y, Ghavam A. Diagnostic validity of the chemiluminescent method compared to polymerase chain reaction for hepatitis B virus detection in the routine clinical diagnostic laboratory. Adv Biomed Res 2014;3:116.

and 5% in Sistan-Balouchestan province was reported by Alavian *et al*.[6] The number of HBV patients in Iran is declining from 1.3% to 0.8% due to the national vaccination program implementation since 1993.[7]

Without intervention, approximately 15% to 40% of chronically infected individuals will eventually develop cirrhosis, end-stage liver disease or hepatocellular carcinoma, or require liver transplantation.[8] HBV is mainly transmitted by parenteral route and percutaneous route, which occurs through the sharing of needles by intravenous drug users or reuse of contaminated sharp instruments for tattoos, acupuncture, or ear piercing and sexual contact.[9,10]

HBV surface antigen (HBsAg) is the established serological marker used routinely for the diagnosis of acute or chronic HBV infection, the screening of blood or organ donors, and the surveillance of persons at risk of acquiring or transmitting HBV. Finding possibilities for improving the quality of present HBsAg assays in the market and to prevent wrong diagnosis because of false-negative HBsAg results is the priorities of many health system facilities.[11]

Newly developed HBsAg assays show a performance increase in terms of specificity and sensitivity, allowing the detection of HBsAg. Nowadays Liaison HBsAg assay uses chemiluminescent immunoassay (CLIA) technology routinely for determination of HBsAg in human serum or plasma samples.[12-14] But even having validation certificate from Europe for these companies does not guarantee highest specificity and sensitivity for their products and it is necessary for every country to check their quality and find their limitations. Since this kit is in use in many routine laboratories, and there are few questions about the specificity of the technique in detecting low indexes positive cases, therefore it was needed to compare the efficacy of this method with other techniques such as polymerase chain reaction (PCR).

The aims of this study were to determine whether the detection rate of HbsAg levels in routine clinical samples by chemiluminescent test systems is comparable and accurate within acceptable limits.

To our knowledge, this report may provide the first systematic and comprehensive comparison of currently marketed CLIA technology (Liaison HBsAg) with PCR assays.

MATERIALS AND METHODS

Five milliliter blood sample was obtained from 350 patients suspicious of having HBV and had referred to the educational hospitals of Urmia University of Medical Sciences, Urmia, Iran, from April until November 2008. Serums were separated after 15 minutes at room temperature by bench centrifuge and stored at –40°C upon to be tested. Patient serums were divided into two groups. The first aliquot used for testing HBsAg by CLIA and HBsAg confirmatory test. The second aliquot was used for determination of the presence of the HBV by real-time PCR method.

DNA of all the samples were extracted using Qiagen extraction kit (Hamburg, Germany).

Preparation of the samples for PCR was performed as previously described with some modification.[15]

Chemiluminescent immunoassay

The Liaison HBsAg method (Dia-Sorin, Saluggia, Italy) for determination of HBsAg is a direct sandwich CLIA. The minimum volume required is 300 µl (150 µl specimen plus 150 µl dead volume). During the first incubation, HBsAg present in calibrators, samples, or controls binds to the solid phase (magnetic particles) coated with mouse monoclonal antibodies. After first washing step and during the second incubation, a sheep polyclonal isoluminol-antibody conjugate reacts with HBsAg already bound to the solid phase. After the second incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added, and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-polyclonal antibody conjugate, is measured by a photomultiplier in relative light units and is indicative of the HBsAg concentration present in calibrators, samples, or controls. The analyzer automatically calculates HBsAg levels, expressed as index values. Manufacturers determine an "index value" that balances the necessary high sensitivity for detecting the antigen with the need to avoid false-positive results. Samples with HBsAg levels of 1.1 graded as reactive and below 0.9 index values should be graded as negative. Samples with HBsAg levels ranging between an index values of 0.9 and 1.1 graded equivocal (borderline). All tests were carried out with positive and negative HBsAg controls.

Confirmatory test

According to the Dia-sorin recommendations sample results graded as equivocal or above 1.1 was re-evaluated using HBsAg confirmatory test. It has been our practice to follow all repeatedly reactive or borderline results with a neutralization confirmatory assay.

A specimen is not confirmed positive when the index value of the non-neutralized aliquot (mixed with

specimen diluent) is less than 0.9 irrespective of the outcome of percent neutralization (=negative specimen).

A specimen is not confirmed positive when the index value of the non-neutralized aliquot (mixed with specimen diluent) is greater than or equal to 0.9 and percent neutralization is less than 50% (=presence of an interfering substance).

A specimen is confirmed positive when the index value of the non-neutralized aliquot (mixed with specimen diluent) is greater than or equal to 0.9 and percent neutralization is greater than or equal to 50%.

PCR method

Using Robogene hepatitis B virus kit (Roboscreen, GmbH, Leipzig-Germany), the presence of HBV in the samples were determined. In PCR experiment each 50-100 IU/ml of serum corresponds to 250-500 copies of HBV per ml. Reaction carried out in the Rotor-GENE 3000 Research (Corbett real time PCR machine; Hamburg, Germany) according to the manufacture instructions.

Negative and positive results were confirmed by running of 5 µl of PCR products of HBV- DNA on 2% agarose gel.

The calculation of accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), and Pearson correlation values were carried out according to Irwig and collogues methods.[16]

Study activities were undertaken only after review and approval by the Reaserch Ethics Committee at UMSU. All procedure involving patients were in accordance with the Helsinki declaration 1975 as revised 1983.

Statistical tests

The statistic analysis were calculated using the free online software from http://www.quantitativeskills.com/sisa/statistics/diagnos.htm.

RESULTS

From 350 studied cases, 200 cases were negative with both PCR and Liaison chemiluminescent method with confirmatory test. Sixty from 200 above mentioned cases, that expected according to the defined threshold for Liaison be positive (>1.1), did not show positive signal by confirmatory test. In addition, six cases that were positive by both PCR and Liaison chemiluminescent methods were negative by CLIA with confirmatory test. The rest of the samples (144 cases) with result index above 30 were positive by either PCR or chemiluminescent with or without confirmatory tests [Table 1].

True positive (TP), true negative (TN), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Pearson correlation values for chemiluminescent with or without confirmatory tests compared to the PCR were calculated and summarized in Table 2.

According to the PCR results as assumed as a gold standard method with sensitivity and specificity of 100%, detection rate sensitivity of chemiluminescent with confirmatory test was 96% and its specificity was 100%, but for chemiluminescent without confirmatory test, sensitivity and specificity was 100% and 70%, respectively. Also for chemiluminescent with confirmatory test, PPV was 100% and its NPV were 97%, compared to chemiluminescent without confirmatory test with PPV and NPV equal to 71% and 100%, respectively.

DISCUSSION

The usual criteria for analysis of HBsAg are detection of HBsAg and result confirmation by antibody neutralization. HBsAg is an HBV serologic marker that plays a major role in the diagnosis of HBV infection.[17] American Association for the Study of

Table 1: HBsAg status in 350 studied cases by PCR and Liaison Chemiluminescent methods with or without confirmatory tests. Chemiluminescent index value was set according to the manufacture recommendations

Number of cases	PCR	Index value	Without confirmatory test	With confirmatory test
140	Neg	<0.9	Neg	Neg
60	Neg	1.1-5.8	Pos	Neg
6	Pos	11.8-22	Pos	Neg
4	Pos	30-60	Pos	Pos
13	Pos	64-180	Pos	Pos
127	Pos	>240	Pos	Pos

PCR: Polymerase chain reaction, Neg: Negative, Pos: Positive

Table 2: True positive, true negative, sensitivity, specificity, PPV, NPV, and pearson correlation values of the chemiluminescent with and without confirmatory test compared to the PCR method

Type of test	True positive *N* (%)	True negative *N* (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Chemiluminescent with confirmatory	144 (41)	200 (57)	96	100	100	97
Chemiluminescent without confirmatory	150 (43)	140 (40)	100	70	71	100
PCR	150 (100)	200 (100)	100	100	100	100

PPV: Positive predictive value, NPV: Negative predictive value, PCR: Polymerase chain reaction

Liver Diseases suggest that patients chronically infected with HBV should be monitored (clinically, biochemically and virologically) on a regular basis (6-12 monthly).[18] These guidelines are basically in agreement with the recommendations of the European Viral Hepatitis Educational Initiative.[19] A new-generation chemiluminescent HBsAg assay performed on the Liasion Chemiluminescent measures HBsAg with a method that involves two steps: Initial screening and confirmation neutralization.[20,21]

Based on our knowledge, the first comprehensive comparison between Liasion Chemiluminescent test system competency and PCR method for detecting HBV antigen or DNA in serum was carried out. We observed that a relatively high percentage of individuals with negative HBsAg were always HBV- DNA negative too (<0.9 index) and vise versa individuals with high index HBsAg (>22 index) were always HBV- DNA positive. This result is true in about 98.3% (344 cases) of our studied cases. But in six cases (1.7%), with weakly positive result (11.8-22 index) that did not pass the confirmation neutralization step. The initial positivity result and the subsequent failure at the confirmation step for some of our cases led us to question the clinical significance of these results and led to the further investigation using other methods like PCR method.

When these six cases were tested with the PCR method, a positive signal was found. This findings shows either, higher sensitivity of PCR method compared to the Liasion Chemiluminescent method for detecting remaining HBV- DNA or presence of mutation in HBV- DNA that can cause HBsAg conformational changes.

As Ly *et al*. (2006) reported, there are several results of HBsAg-negative virus carriers (HBV- DNA positive) with immunosilent infections.[14] They concluded that natural variation and mutations can induce HBsAg conformational changes. Since many HBsAg immunoassays use monoclonal antibodies with epitopes directed against the major hydrophilic region, in particular against the "a" determinant, amino acid substitution in this region may account for false-negative results in immunoassays.[14,22]

O'Brien[23] also found that weakly positive results were more likely to fail neutralization than were strongly positive results, and suggested that this confirmatory test be used only in samples with weakly positive results. Similar recommendations have been made by the Centers for Disease Control for weakly positive anti-HCV results.[24] Weakly positive results are also more likely to be falsely positive with other infectious disease assays, including anti-HIVand anti-HBc.[25-27]

In addition, Chen and Kaplan study[20] indicates that laboratories need to be aware of the performance of their HBsAg assays. At least, laboratories should know the analytical performance of their assays near the cut off concentration, and use neutralization assays in samples with weakly positive HBsAg results.

It has been recognized for many years that there is a delay between HBV infection and appearance of HBsAg; during this "window" period, the individual may still be infectious.[26]

To overcome these chemiluminescent method limitations we suggest using the PCR method as a gold standard method for detecting and confirming the presence of HBV DNA in low indexes cases.

Also to avoid the reporting of false-negative or false-positive HBsAg results, we suggest that any sample, from any manufacturer, with a low-positive index that does not pass the assay's confirmation test not be designated "negative or positive" until HBV DNA are measured with the PCR method for providing a clearer picture of the patient's status.

Additional researchs are needed to address several questions relating to the prevalence and significance of mutants that are not detected by HBsAg assays, and to the need for increased sensitivity of HBsAg assays to reduce the window period and the occurrence of occult HBV infections.

Based on the finding of this study, it was possible to conclude that in the majority of the HBV cases, the diagnostic chemiluminescent method value compared to the PCR method is acceptable except in low indexes positive cases that need further investigation with the PCR method.

REFERENCES

1. Jahani MR, Motevalian SA, Mahmoodi M. Hepatitis B carriers in large vehicle drivers of Iran. Vaccine 2003;21:1948-51.
2. Mohebbi SR, Amini-Bavil-Olyaee S, Zali N, Noorinayer B, Derakhshan F, Chiani M, *et al.* Molecular epidemiology of hepatitis B virus in Iran. Clin Microbiol Infect 2008 14:858-66.
3. Belongia EA, Costa J, Gareen IF, Grem JL, Inadomi JM, Kern ER, *et al*. NIH consensus development statement on management of hepatitis B. NIH Consens State Sci Statements 2008;25:1-29.
4. Andre F. Hepatitis B epidemiology in Asia, the Middle East and Africa. Vaccine 2000;18 Suppl 1:S20-2.
5. Bhattacharya S, O'Donnell K, Dudley T, Kennefick A, Osman H, Boxall E, *et al.* Ante-natal screening and post-natal follow-up of hepatitis B in the West Midlands of England. QJM 2008;101:307-12.
6. Alavian SM, Bagheri-Lankarani K, Mahdavi-Mazdeh M, Nourozi S. Hepatitis B and C in dialysis units in Iran: changing the epidemiology. Hemodial Int 2008;12:378-82.
7. Alavian SM, Fallahian F, Lankarani KB. The changing epidemiology of viral hepatitis B in Iran. J Gastrointestin Liver Dis 2007;16:403-6.

8. Lai CL, Ratziu V, Yuen MF, Poynard T. Viral hepatitis B. Lancet 2003;362:2089-94.
9. Lin HJ, Lai CL, Lau JY, Chung HT, Lauder IJ, Fong MW. Evidence for intrafamilial transmission of hepatitis B virus from sequence analysis of mutant HBV DNAs in two Chinese families. Lancet 1990;336:208-12.
10. Mast EE, Weinbaum CM, Fiore AE, Alter MJ, Bell BP, Finelli L, *et al*. Janssen RS, Ward JW: A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: Recommendations of the Advisory Committee on Immunization Practices Part II: immunization of adults. MMWR Recomm Rep 2006; 55(RR-16):1-33; quiz CE31-4.
11. Krajden M, McNabb G, Petric M. The laboratory diagnosis of hepatitis B virus. *Can* J Infect Dis Med Microbiol 2005;16:65-72.
12. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2001;34:1225-41.
13. Huzly D, Schenk T, Jilg W, Neumann-Haefelin D. Comparison of nine commercially available assays for quantification of antibody response to hepatitis B virus surface antigen. J Clin Microbiol 2008;46:1298-306.
14. Ly TD, Servant-Delmas A, Bagot S, Gonzalo S, Ferey MP, Ebel A, *et al*. Sensitivities of four new commercial hepatitis B virus surface antigen assays in detection of HBsAg mutant forms. J Clin Microbiol 2006;44:2321-26.
15. KhademAnsari MH, Omrani M, Movahedi V. Comparative evaluation of immunochromatographic rapid diagnostic tests (Strip and Device) and PCR methods for detection of human hepatitis B surface antigens. Hepatitis Mon 2007;7:373-7.
16. Irwig L, Bossuyt P, Glasziou P, Gatsonis C, Lijmer J. Designing studies to ensure that estimates of test accuracy are transferable. BMJ 2002;324:669-71.
17. Kramvis A, Kew M, Francois G. Hepatitis B virus genotypes. Vaccine 2005;23:2409-23.
18. Lok AS, McMahon BJ. Chronic hepatitis B: update of recommendations. Hepatology 2004;39:857-61.
19. Thomas HC. Best practice in the treatment of chronic hepatitis B: A summary of the European Viral Hepatitis Educational Initiative. J Hepatol 2007;47:588-97.
20. Chen D, Kaplan LA. Performance of a new-generation chemiluminescent assay for hepatitis B surface antigen. Clinical chemistry 2006;52:1592-8.
21. Kessler HH. Comparison of currently available assays for detection of hepatitis B virus DNA in the routine diagnostic laboratory. Expert Rev Mol Diagn 2005;5:531-6.
22. Koyanagi T, Nakamuta M, Sakai H, Sugimoto R, Enjoji M, Koto K, I*et al*. Analysis of HBs antigen negative variant of hepatitis B virus: Unique substitutions, Glu129 to Asp and Gly145 to Ala in the surface antigen gene. Med Sci Monit 2000;6:1165-9.
23. O'Brien JE. Hepatitis B surface antigen: Decreased need for confirmation of reactive results. Clin Chem 2000;46:582.
24. Alter MJ, Kuhnert WL, Finelli L. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. MMWR Recomm Rep 2003, 52(RR-3):1-13, 15; quiz CE11-14.
25. Schwartz JS, Dans PE, Kinosian BP. Human immunodeficiency virus test evaluation, performance, and use. Proposals to make good tests better. JAMA 1988;259:2574-9.
26. Dufour DR. Hepatitis B surface antigen assays: Are they good enough for their current uses? Clin Chem 2006;52:1457-9.
27. Carlson JR, Bryant ML, Hinrichs SH, Yamamoto JK, Levy NB, Yee J, *et al*. AIDS serology testing in low- and high-risk groups. JAMA 1985;253:3405-8.

Source of Support: Nil, **Conflict of Interest:** None declared.