Quantitative Measurement of Serum Hepatitis B Surface Antigen Using an Immunoradiometric Assay in Chronic Hepatitis B

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Abstract

Purpose Measurement of serum hepatitis B virus surface antigen (HBsAg) levels is important for the management of chronic hepatitis B patients in terms of monitoring response to antiviral therapy. This study aimed to evaluate the diagnostic performance of a new diagnostic kit, which quantitatively measures serum HBsAg level using an immunoradiometric assay (IRMA)-based method. Measurements were compared with those obtained using a chemiluminescent microparticle immunoassay (CMIA)-based method.

Methods The blood samples of 96 patients with chronic hepatitis B were used in this study. Copy numbers of serum hepatitis B virus (HBV) DNA were determined in 23 of these samples. The correlation between and the concor-

dance of IRMA and CMIA results were determined using Pearson's correlation coefficients. *P* values of 0.05 were considered to be statistically significant throughout.

Results Laboratory diagnoses based on IRMA were wholly in agreement with those based on CMIA. Furthermore, serum HBsAg levels by IRMA were found to be highly correlated with those determined by CMIA (correlation coefficient $R^2 = 0.838$, $p < 0.001$). Serum HBsAg level and serum HBV DNA copies were found to be linearly related by both methods ($R^2 = 0.067$, $p = 0.316$ by IRMA, and $R^2 = 0.101$, $p = 0.215$ by CMIA).

Conclusion The diagnostic performance of the investigated IRMA method of determining HBsAg levels was found to be comparable with that of a CMIA-based method in chronic hepatitis B patients.

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Introduction

Hepatitis B virus (HBV) infection is a major global health problem. It is the leading cause of both liver cirrhosis and hepatocellular carcinoma, and is responsible for more than 500,000 deaths per year [1, 2]. Chronic HBV carriers are the main source of HBV infection in the population, and thus, the detection of HBV infection in pregnant woman and blood donors is required to prevent spread of the infection. Despite a national vaccination campaign, 5.1% of males and 4.1% of females older than 10 years of age are hepatitis B surface antigen (HBsAg)-positive in South Korea [3, 4].

HBsAg is a major envelope protein of HBV, and can serve as an epitope and provide the host with immunity [5, 6]. Recently, antiviral agents such as lamivudine and interferon- α (IFN- α) have been used as standard therapies for the treatment of chronic hepatitis B, and new drugs have been or are being developed to treat refractory mutant viral infections [7, 8].

Quantitative measures of HBsAg level in serum are important for monitoring response to anti-viral treatment during the management of patients with a chronic hepatitis B virus infection [9]. Several assay methods are currently in use, such as radioimmunoassays (RIAs), electrochemiluminescence immunoassays (ECLAs), and chemiluminescent microparticle immunoassays (CMAs), to measure HBsAg levels in serum, and in particular, a new diagnostic kit for the quantitative measurement of the serum HBsAg using an immunoradiometric assay (IRMA) method was recently developed.

This study was undertaken to evaluate the diagnostic performance of this new quantitative HBsAg kit. To do so, we compared serum HBsAg levels determined by IRMA and CMIA in patients with chronic hepatitis B infection.

Materials and Methods

Patients and Samples

The serum samples of 96 patients with chronic hepatitis B infection that visited Seoul National University Boramae Medical Center were used in this study; HBV DNA copy number was determined in 23 of these samples. The samples were requested by the Department of Nuclear Medicine for evaluation of HBsAg by a qualitative test, and we reused the samples.

Control Materials

An HBsAg standard obtained from the World Health Organization (WHO), of known concentration [National Institute for Biological Standards and Control (NIBSC) material code 80/459], was used to produce standard IRMA and CMIA curves. In addition, the following control materials—HBsAg, subtype *adw2*, and genotype A (NIBSC code 00/588—were used to evaluate the ability of the two techniques to detect mutant HBsAg.

IRMA

The IRMA kit (RIAKEY, Shin Jin Medics, South Korea) was used according to the manufacturer's instructions. Briefly, a sample was incubated with primary antibody-coated beads for 1 hour. The beads were removed by washing four times with

washing solution, and then treated with a second antibody conjugated with I-125 for 30 min. Afterward, radioactivity was measured using a gamma counter in (CPM) (Packard, Ill., USA). Serum HBsAg levels were determined by reading CPM values off using a standard curve. A sample was considered positive when the HBsAg serum level exceeded 0.1 IU/ml (detection range: 0.05–250 IU/ml). When the HBsAg level was found to exceed the detection range, the test was repeated after dilution.

CMIA

A commercial CMIA diagnostic kit (ARCHITECT HBsAg, Abbott Laboratories, Abbott Park, Ill., USA) was used for comparison purposes. Its detection range was from 0.05 to 250 IU/ml. The results of this system were measured using the calibration curve, which was obtained by using six standard solutions. As mentioned above for the IRMA test, when the tested HBsAg level exceeded the detection range, the sample was diluted and the test was re-performed.

The Determination of Hepatitis B Virus DNA Copy Numbers

HBV DNA copy numbers were determined using the COBAS Amplicor HBV Monitor Test (Roche Diagnostics, Branchburg, N.J., USA) or a COBAS Taqman 48 Analyzer (Roche Diagnostics, Branchburg, N.J., USA). The lower detection limits of these tests were 300 and 70 copies/ml, respectively.

Statistical Analysis

Fisher's exact test was used to compare categorical variables, and concordance rates between IRMA and CMIA for detecting serum HBsAg were calculated. Pearson's correlation coefficients (R) were used to examine the nature of the correlation between IRMA and CMIA results. MedCalc 11.1.1.0 for Windows was used throughout, and p values <0.05 were considered significant.

Results

Comparison between IRMA and CMIR for the Control Material

Both the IRMA and CMIR methods showed good correlation with known concentrations of the NIBSC control materials; for code 80/459 material ($R^2 = 1.000$, $p < 0.001$ for IRMA and $R^2 = 0.991$, $p < 0.001$ for CMIR) (Fig. 1a) and code 00/588 material ($R^2 = 1.000$, $p < 0.001$ for IRMA and $R^2 = 0.997$, $p < 0.001$ for CMIA) (Fig. 1b).

Fig. 1 Comparison of the results obtained using the IRMA (immunoradiometric assay) and CMIA (chemiluminescent microparticle assay) kits for WHO standard materials. National Institute for Biological Standards and Control (NIBSC) code 80/459 (**a**) and code 00/588 (**b**). For the material coded 80/459, HBsAg levels obtained using the two methods were concurred with the concentrations of standard solutions. (between the concentrations of standard solutions and IRMA results; $y=0.965x + 0.143$, $R^2 = 1$, $p<0.001$; and between the concentrations of standard solutions and CMIA results by; $y=1.012x + 3.031$, $R^2 = 0.991$, $p<0.001$). Similarly, for the material coded 00/588, the results of the two methods concurred well (the correlation between standard solutions and IRMA results was described by: $y=1.113x - 0.063$, $R^2 = 1$, $p<0.001$; and between standard solutions and CMIA results by: $y=1.357x - 0.033$, $R^2 = 0.997$, $p<0.001$)

Comparison between IRMA and CMIR using Patient Serum Samples

Of the 96 samples analyzed, 31 (32.3%) were positive for HBsAg. All samples positive or negative by IRMA were also positive or negative by CMIA. Accordingly, the concordance rate was 100% ($p<0.0001$) (Table 1). Levels of serum HBsAg measured by IRMA and CMIA were highly correlated ($R^2 = 0.838$, $p<0.001$ in total samples, $R^2 = 0.760$ in positive samples) (Fig. 2). In cases with a high

HBsAg level, IRMA results tended to be higher than CMIA results.

Correlation between HBsAg Level and HBV DNA Copy Number

Twenty-three samples were examined for HBV DNA copy number, and 17 of the 23 had a HBV DNA copy number of >20 IU/ml. These 17 samples showed a trend toward a positive correlation between serum HBsAg level and HBV DNA copy number by IRMA and CMIA ($R^2 = 0.067$, $p=0.316$ by IRMA, and $R^2 = 0.101$, $p=0.215$ by CMIA method). However, these correlations were not significantly different (Fig. 3a, b).

Discussion

HBV infection is endemic in South Korea and an important cause of liver cirrhosis and hepatocellular carcinoma. It was reported that the prevalence of HBV infection in South Korea during the 1990s was about 5.7% [10]. HBsAg measurement is mandatory not only for the diagnosis of HBV infection but also for monitoring the response to treatment [11, 12]. These measurements can be performed using IRMA, EIA, and CMIA, though CMIA is currently used for quantitative purposes [13].

Accordingly, an accurate diagnostic kit is required to measure serum HBsAg and manage chronic hepatitis B virus infections. Furthermore, HBsAg monitoring can be useful in hepatitis B virus e antigen (HBeAg)-positive patients undergoing conventional interferon α treatment [14]. In addition, in patients being treated with pegylated interferon alfa-2a and/or lamivudine, the loss of HBsAg is an important finding for predicting sustained virological response [15, 16], as seroconversion of HBsAg suggests a more favorable prognosis and reduced risks of developing cirrhosis and hepatocellular carcinoma [17, 18].

CMIA has been widely used to quantify serum HBsAg levels. In one study, it was reported that Architect HBsAg QT is capable of detecting HBsAg at 0.2 ng/ml [19]. CMIA is performed using an automatic calibrator to produce a calibration curve and interpret sample results rapidly. The IRMA method utilizes an automatic gamma counter that fulfills a similar function to an automatic calibrator. The IRMA kit used in the present study is expected to provide similar diagnostic accuracies as the CMIA method at a lower cost. In most cases with a high HBsAg level ($>5,000$ IU/ml), IRMA results tended to be higher than CMIA results. The detection range without dilution of both methods is from 0.05 to 250 IU/ml. To measure higher levels of HBsAg (more than 250 IU/ml) the sample should be diluted. This finding might be attributable to manual

Table 1 Comparison of HBsAg reactivities using the IRMA and CMIA methods ($n=96$)

		CMIA result		Total	Significance level
		Reactive	Non-reactive		
IRMA result	Reactive	31	0	31	$p<0.0001$
	Non-reactive	0	65	65	
	Total	31	65	96	

dilution. In the detection range (0.05–250 IU/ml), there was no such finding in the patient's sample or standard material measurement.

A previous study reported that CMIA is more sensitive than radioimmunoassay-based methods [20]. It was considered that mutation in the “a” determinant of HBV S antigen coding DNA could influence the ability of a monoclonal-based assay to detect HBsAg [21]. The IRMA diagnostic kit used in this study is based on a polyclonal/monoclonal antibody, which includes the subtypes *ad* and *ay*. In South Korea, *adr* is the predominant HBV serotype, followed by *adw* [22]. The new IRMA kit showed a good correlation with WHO control materials, which were based on the *ad* and *adw* subtypes, respectively. In a previous study, it was reported that levels of HBsAg by IRMA show high concordance rates with CMIA-determined levels [13], and in the present study, IRMA and CMIA diagnostic results concurred completely. However, further studies are required to confirm the diagnostic accuracy of this new IRMA-based kit in various HBV mutation and clinical settings.

In the present study, the correlation between HBsAg amount and HBV DNA copy number was not found to be

significant. However, the relationship between HBsAg level and HBV DNA copy number in chronic hepatitis patients depends on various clinical parameters, clinical situation, and immune clearance levels. In a previous study, a significant negative correlation was found between HBV DNA copy numbers and HBsAg levels in non-cirrhotic chronic hepatitis B patients [23]. In another study, it was

Fig. 2 Correlation between IRMA results and CMIA results of total patients' serum HBsAg titers. IRMA and CMIA results were linearly related, but IRMA tended to produce slightly higher results than CMIA ($y=1.201x + 554.071$, $R^2 = 0.838$, $p<0.001$) **Fig. 3** Correlations between serum HBV DNA copy numbers and HBsAg levels as determined by IRMA and CMIA. Correlations between serum HBV DNA copy numbers and HBsAg levels were similar for IRMA (a) and CMIA (b) (IRMA: $y=853.313x + 5985.207$, $R^2 = 0.067$, $p=0.316$; CMIA: $y=803.650x + 2213.549$, $R^2 = 0.101$, $p=0.215$)

found that HBsAg levels change during the different clinical phases of chronic hepatitis B [24]. Further studies are required to evaluate serum HBsAg level changes in terms of clinical status.

Antiviral agents primarily target HBV replication. HBsAg is produced by the circular DNA of HBV, and therefore, its production is dependent on the replication of viral DNA. Therefore, prognosis after HBsAg seroconversion is generally good [25]. However, in the presence of conditions such as liver cirrhosis and hepatocellular carcinoma the prognostic meaning of negative HBsAg seroconversion is unclear [26]. Serum HBsAg is considered as an important surrogate marker of treatment response, especially in Asian chronic HBV patients [27]. Furthermore, the prevalences of HBV genotypes show regional differences, and as a result, WHO recommended that regulatory authorities devise HBsAg panels using subtypes and genotypes prevalent in their regions [28]. We believe that the new diagnostic IRMA kit specifically designed for use in South Korea will achieve a position of clinical importance in terms of treatment and the development of targeted antiviral drugs.

In conclusion, the new IRMA diagnostic kit for quantifying serum HBsAg levels showed comparable diagnostic performance to a CMIA diagnostic kit. Furthermore, the HBsAg levels obtained using the two kits were found to be highly correlated.

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