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Comparison of the Elecsys HBsAg II Assay and the Architect Assay for Quantification of hepatitis B surface antigen in patients with chronic hepatitis B

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SUMMARY

Quantitative hepatitis B surface antigen (HBsAg) is a valuable tool in hepatitis B virus (HBV) disease diagnosis and management for evaluating the effectiveness of antiviral therapy. The aim of the current research was to compare the performances of the Elecsys HBsAg II and Abbott Architect HBsAg assays in chronic hepatitis B (CHB) patients. Between May 2014 and December 2014, 72 CHBs were tested using Abbott Architect HBsAg QT and Roche Elecsys HBsAg II assay. After transformation to log (10) IU/mL, the results of the two assays were compared using the interclass correlation test, the Pearson correlation test and Bland–Altman analyses. We also analyzed all the parameters in on-treatment patients and naive patients with Pearson correlation test. There was a significant overall correlation between the Elecsys and Architect assays. We also analyzed all the parameters in naive and on-treatment patients. There was a significantly good correlation between the two assays in untreated patients and on-treatment patients. In this study, there was a significant correlation between the results of the Elecsys HBsAg II and Abbott Architect HBsAg assays in the overall and naive/on-treatment CHB patients. Additionally, we found that mean HBsAg levels resulting from the Architect assay were higher than those obtained by Elecsys assay.

Keywords: Hepatitis B surface antigen, chronic hepatitis B, quantification.

INTRODUCTION

Hepatitis B virus (HBV) infection is a frequently encountered disease in the world which accounts for more than 350 million HBV carriers [1]. The HBV carrier prevalence is about 10-20% in some geographic areas (Southeast Asia, China, sub-Saharan Africa) [2]. Patients with chronic hepatitis B infection have a high risk of life threatening conditions as cirrhosis and/or hepatocellular carcinoma. The estimated worldwide mortality rate is between 0.5-1.2 million deaths per year. Hepatitis B surface antigen (HBsAg), which reflects the transcriptional activity of cccDNA (covalently closed circular DNA) and integrated HBV DNA sequence, is released through a complex mechanism [3]. Hepatitis B surface Antigen (HBsAg) is the essential step in diagnosing HBV infection [4]. HBsAg level is highest during the immune-tolerant phase due to high levels of HBV replication, and this level progressively declines from the immune tolerance to the inactive (low replicative immune control) phase [5]. Formerly,

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HBsAg was used as a qualitative diagnostic test for hepatitis B infection. Recently, quantitative serum HBsAg assays have begun to be utilized [6, 7]. Recent studies have demonstrated that quantification of HBsAg (qHBsAg) may be a useful tool for clinicians in management of chronic hepatitis B [8, 9].

In terms of tailoring the treatment duration in patients affected by chronic hepatitis B (CHB), an individualized approach can be anticipated via quantitative HBsAg [10, 11]. Quantification of HBsAg can be measured by a number of methods ranging from radioimmuno-assays (RIA) to fully automated chemiluminescence immunoassays (CLIA). Both forms of HBsAg and mutants can be detected by more sensitive CLIA [12].

Recently, multiple diagnostic assays are utilized for quantification of HBsAg. The most commonly used assay is the Architect assay, yet HBsAg quantification may also be measured using the Elecsys. Therefore, the aim of the present study was to compare the performances of the Elecsys HBsAg II and Abbott Architect HBsAg assays in patients with CHB.

PATIENTS AND METHODS

The study was carried out between May 2014 and December 2014; a total of 72 consecutive patients (44 males and 28 females) affected by chronic hepatitis B attending the Infectious Diseases and Clinical Microbiology outpatient clinic of our hospital were included. CHB patients coinfected with hepatitis C virus (HCV), human immunodeficiency virus (HIV) and hepatitis delta virus (HDV) infection, patients with chronic renal failure and patients with autoimmune liver disease were excluded from the research. All patients were grouped as naïve and anti-viral treatment status.

Complete clinical evaluations of all patients' clinical data were taken by means of the hospital information system. All study subjects' sera were tested for routine hepatitis B immunoserological markers (HBsAg, HBeAg, anti-HBe, anti-HBc total/IgM) by commercial methods (Architect i2000SR, Abbott Diagnostics, IL, USA). All sera were tested for quantitative HBsAg by both commercially automated available quantitative assay (Architect HBsAg quantitation QT assay, Abbott Diagnostics, IL, USA) and (Elecsys HBsAg II assay, GmbH, Mannheim, Germany). A quantitative PCR method (Applied Biosystems, ABI 7500 Real Time PCR System) was utilized to determine HBV-DNA levels.

HBsAg quantitation

Architect HBsAg QT

The HBsAg levels were measured with Architect HBsAg QT assay on the fully automated Architect instrument as per the manufacturer's protocol and results were expressed as IU/ml. This is a two-stage CLIA, with flexible assay protocols referred to as chemiflex. In the first stage, sample and anti-HBs coated paramagnetic micro particles are combined. Micro particles of anti-HBs are bound with the HbsAg present in the sample. After washing, the acridinium labeled anti-HBs conjugate is then added. The subsequent chemiluminescent reaction is measured in relative light units (RLUs). There is a relationship between the amounts of HBsAg in the sample and the RLUs identified by the optical system. A previously generated calibration curve is employed for determining the concentration of HBsAg in the specimen. This allows the calculation of HBsAg in undiluted serum/plasma samples from 0.05 to 250 IU/ml and sixty (60) samples with HBsAg levels >250 IU/ml were retested at a dilution of 1:500 and 1:1000. Two samples with HBsAg levels <0.05 IU/ml at a dilution 1:100 were retested undiluted. The calibration of the Abbott Architect HBsAg assay gives the results in IU/ml [11].

Elecsys HBsAg II

Quantitation of HBsAg was measured using the Elecsys HBsAg II quantitative assay. Detection of HBsAg by Elecsys utilizes a sandwich principle and this assay has two stages: first, a complex is formed with 2 monoclonal HBsAg-specific antibodies, one of which is biotinylated, and the other labeled with a ruthenium complex. This complex joins to the solid phase through interaction of biotin and streptavidin after attachment of streptavidin-coated microparticles [13]. The mixture is subsequently aspirated into a measuring cell, where application of a voltage induces chemiluminescent emission, which is measured by a photomultiplier. All serum samples were tested at a dilution 1:400. The Elecsys HBsAg II quantitative assay is calibrated to give results in terms of international units per milliliter (IU/mL) [13].

Statistical analysis

Quantitative variables were expressed as mean values with standard deviation. The correlation between HBsAg levels by both the methods was done by Pearson's correlation coefficient test, interclass correlation test and Bland-Altman analyses. Statistical analysis was done using Statistical Package for the Social Sciences software (SPSS) for Windows (Chicago, Illinois, USA) version 17.0. All *P* values less than 0.05 were considered significant.

RESULTS

Of the 72 (29 naïve, 43 on-treatment) cases, 44 (61,1%) were male and 28 (38,9%) were female. The mean age of the patients was 44,9±15,64. The mean HBsAg titer was 5893±7127 IU/ml in Architect method and this value was 3265±1633 IU/ml in Elecsys method. The mean value of HBV DNA is level is 375819247±1941669832 IU/ml. There was a significant association between fibrosis score and HBsAg level in naïve CHB patients and HBsAg level was significantly lower in the group with high [3-6] fibrosis scores (p=0,029). There was a significant correlation between HBsAg levels measured by Architect assay and HBV DNA levels in naïve CHB patients (p<0,014 r=0,453). However, there was no correlation between HBsAg levels measured by Elecsys and HBV DNA levels (p=0,142; r=0,279). We also analyzed all the parameters in on-treatment patients, except HBV DNA level. The HBV DNA data of on-treatment patients were almost of low titer or undetectable. Additionally, the mean HBsAg levels resulted by the Architect assay were higher than those obtained by the Elecsys assay.

The overall correlation between Elecsys and Architect assays

We have detected a significant overall correlation between the Elecsys and Architect assays. There was a high correlation between two methods with Interclass Correlation analysis (r=0.838; p<0.001) (Table 1, Figure 1), Pearson correlation analysis and Bland-Altman correlation analysis (Figure 2).

Table 1 - Correlation between HBsAg measurements using the Architect HBsAg QT assay and the Elecsys HBsAg by interclass correlation test (r=0.838; p<0.001).

	Intraclass correlation	*95% Confidence Interval*		*p*
		Lower	*Upper*	
Single measures	0.717	0.584	0.813	0.0001
Average measures	0.838	0.737	0.897	

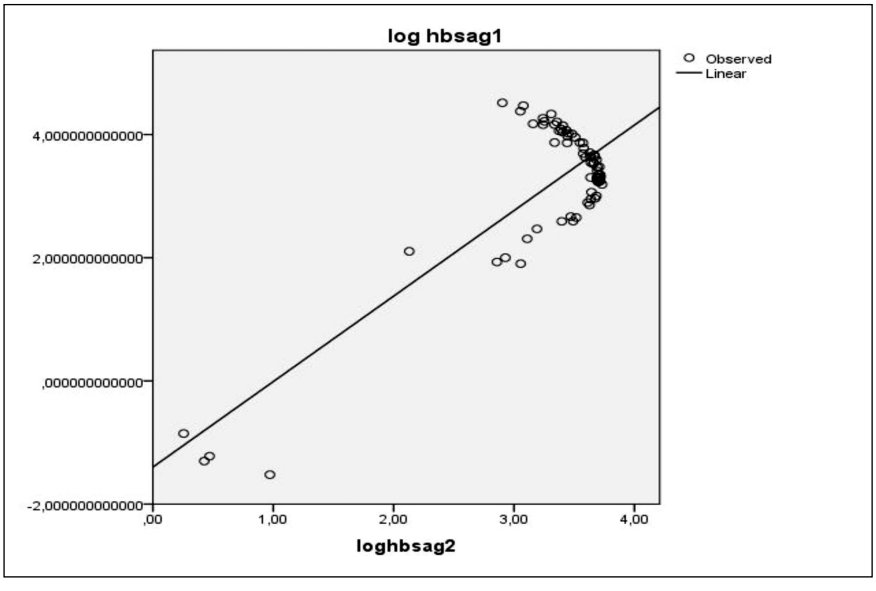

Figure 1 - Correlation between HBsAg measurements using the Architect HBsAg QT assay and the Elecsys HBsAg II.

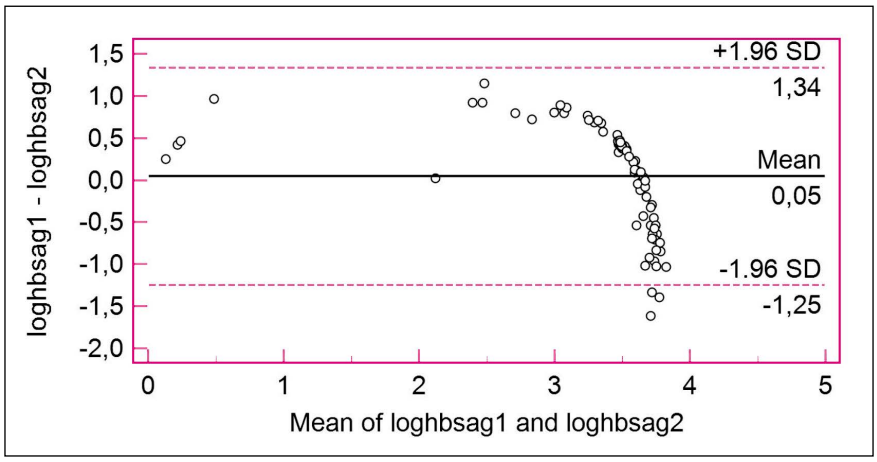

Figure 2 - Bland-Altman plot of HBsAg measurements using the Architect HBsAg QT assay and the Elecsys HBsAg II. Dashed lines represent 95% confidence limits.

Table 2 - Comparison of HBsAg 1 and HBsAg 2 on limits of agreement (Architect HBsAg QT: logHBsAg 1; Elecsys HBsAg: logHBsAg2).

Method	*n*	*d*	*Standard Deviation*	*Limits of agreement*	
				d ± 1.96 Standard Deviation*	
LogHBsAg1 – LogHBsAg2	72	0.045	0.659	-1.246	1.337

*It is accepted to compute 95% limits of agreement for each comparison (average difference ± 1.96 standard deviation of the difference), which tell us how far apart measurements by 2 HBsAg methods were more likely to be for the CHB patients. Evaluation of LogHBsAg1 can be found 1,246 units lower or 1,337 units higher than LogHBsAg 2.

Additionally, we assessed the agreement between two measurement methods by using Bland and Altman plots (Figure 2) and by calculating limits of agreement (Table 2). It is accepted to calculate 95% limits of agreement for each comparison (average difference ± 1.96 standard deviation of the difference), which tell us how far apart measurements by two HBsAg methods were more likely to be for the CHB patients.

Evaluation of LogHBsAg1 (Architect HBsAg QT) can be found 1,246 units lower or 1,337 units higher than LogHBsAg 2 (Elecsys HBsAg) (Figure 2) and (Table 2).

Moreover, we utilized the coefficient of variation to assess the repeatability and to decide which method is superior or more useful. The coefficient of variation expresses the standard deviation as a percentage of the mean.

We have calculated the coefficient of variation for LogHBsAg 1 (Architect HBsAg QT) and LogHBsAg 2 (Elecsys HBsAg) as 22.2 and 30.9, respectively. As a result, it can be stated that LogHBsAg 1 (Architect HBsAg QT assay) has a better repeatability and is superior to LogHBsAg 2 (Elecsys assay).

Correlation between Elecsys and Architect assays among the naive and on-treatment patients

We also analyzed all the parameters in on-treatment patients and naïve patients. A significantly good correlation was found between the Architect and Elecsys HBsAg assays in untreated patients (n=29; p=0.002; r: 0.547) and on-treatment patients (n=43; p=0.001; r: 0.923) with Pearson correlation analysis.

DISCUSSION

The CHB infection is a serious public health problem because of its worldwide distribution and potential complications. Long-term suppression of viral replication is important to reduce the complications of CHB infection. Monitoring of CHB patients receiving antiviral treatment is crucial since current treatment options have restricted favorable outcome in achieving durable endpoints and antiviral resistance may arise during long-term therapy [10, 14, 15]. The most frequently used method for treatment follow-up in CHB patients is serial serum HBV DNA mea-

surement, which is expensive and technically challenging.

On the other hand, the technique for measuring quantitative HBsAg is relatively easy and inexpensive. Recently, qHBsAg has been assessed as an alternative parameter for monitoring treatment response (9). Recent studies have demonstrated that decrease in HBsAg levels during treatment can foresee the likelihood of HBsAg clearance and sustained virological response to antiviral treatment [9, 15]. For this reason, the importance of HBsAg quantification is increasing day by day as it can be utilized in the individualization of therapy. Thus, there is likely to be an increasing need for reliable assays for HBsAg quantification [11].

Recent studies have demonstrated that HBsAg quantification could be useful complement to HBV DNA levels in tracking the clinical evaluation and treatment of patients with chronic HBV infection (3). Moreover, guidelines such as EASL and NICE indicate the importance of co-operative use of qHBsAg and HBV-DNA for diagnosis and monitoring of CHB treatment. Consistent with other studies, our study showed that levels of HBsAg test measured by the Roche Elecsys HBsAg II had a significant correlation with those attained using the Architect method [3,16]. In one of those studies, Sonnoveld et al. demonstrated that there was a high correlation and close agreement between quantitative HBsAg levels conducted with Elecsys and Architect assays [7]. It was stated in previous studies that the advantage of the Elecsys assay was its automatic on-board dilution, allowing a range of HBsAg measurement of 20 to 52,000 IU/ml. The accessibility of this assay has already been observed in a multicenter study among 611 CHB patients, in which HBsAg levels were able to be quantified in 72% of the samples on the first attempt [17].

On the other hand, HBsAg levels may be measured within a relatively limited range (from 0.05 to 250 IU/ml) by the Architect assay. Patient serum samples with values that exceed 250 IU/ml may be measured after being re-calculated as an "IU/mL" unit when diluted at a ratio of 1:500 due to automatic or manual dilution protocol. Although it seems to be a disadvantage, Architect assay has a higher sensitivity and specificity when compared to Elecsys assay. According to our study, the results obtained from Architect method were found to be better than Elecsys method. In spite of the strong overall correlation between these two methods, a few differences were observed in our study as Architect assay was better in terms of correlation with serum HBV DNA levels in treatment naïve group.

Additionally, Architect assay was found to be a superior and more useful measurement method according to the statistical evaluation.

There are also many studies investigating the correlation between qHBsAg and HBV DNA levels. Most of those studies demonstrated a significant relationship between these markers. Ganji et al. investigated the correlation between HBsAg quantitative assay results and HBV DNA levels in chronic HBV patients. Although a significant relationship was found in HBeAg positive patients, no meaningful relation was found in "e" negative patients (18). In another study, Alghamdi et al. also demonstrated a significant relationship between these markers in HBeAg negative patients and stated that they could be considered as a predictor if they are used together [19]. Additionally, Günal et al. also demonstrated a significant correlation between HBV DNA levels and qHBsAg levels and generated similar results with our study [20].

To sum up, the Architect method is fully able to quantify the levels of serum HBsAg CHB, with very high correlation and accuracy compared to the Elecsys assay.

For this reason, both methods may be utilized in clinical practice in various stages or monitoring treatment. Also, HBsAg quantification can specify additional information about the derivation of the natural history of chronic hepatitis B infection. The ready availability of standardized assays for quantitative HBsAg has opened a novel window in the field of gastro-hepatology. Although HBsAg cannot substitute for HBV DNA, in comparing HBsAg level with HBV DNA, because of inexpensiveness of HBsAg quantification, its utilization in daily practice is expected to become widespread in daily practice in the near future. Further studies are required to clarify the role of HBsAg in clinical fields.

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REFERENCES

[1] Maddrey W.C. Hepatitis B: an important public health issue. *J. Med. Virol.* 61, 3, 362-366, 2000.
[2] Alter M.J., Hadler S.C., Margolis H.S., et al. The changing epidemiology of hepatitis B in the United States: need for alternative vaccination strategies. *JAMA* 263, 9, 1218-1222, 1990.
[3] Liao C.C., Hsu C.W., Gu P.W., Yeh C.T., Lin S.M., Chiu C.T. Comparison of the elecsys HBsAg II assay and the architect assay for quantification of hepatitis B surface antigen in chronic hepatitis B patients. *Biomed. J.* 38(3), 250-256, 2015.
[4] Dienstag J.L. Hepatitis B virus infection. *N. Engl. J. Med.* 359, 14, 1486-1500, 2008.
[5] Nguyen T., Thompson A.J., Bowden S., et al. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. *J. Hepatol.* 52, 508-513, 2010.
[6] Wursthorn K., Jaroszewicz J., Zacher B.J., et al. Correlation between the Elecsys HBsAg II assay and the Architect assay for the quantification of hepatitis B surface antigen (HBsAg) in the serum. *J. Clin. Virol.* 50, 292-296, 2011.
[7] Sonneveld M.J., Rijckborst V., Boucher C.A., et al. A comparison of two assays for quantification of Hepatitis B surface Antigen in patients with chronic hepatitis B. *J. Clin. Virol.* 51, 3, 175-178, 2011.
[8] Tseng T.C., Kao J.H. Clinical utility of quantitative HBsAg in natural history and nucleos(t)ide analogue treatment of chronic hepatitis B: new trick of old dog. *J. Gastroenterol.* 48, 1, 13-21, 2013.
[9] Liaw Y.F. Clinical utility of hepatitis B surface antigen quantitation in patients with chronic hepatitis B: a review. *Hepatol.* 53, 2121-2129, 2011.
[10] Liver EAFTSOT. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J. Hepatol.* 57, 167-185, 2012.
[11] Gupta E., Pandey P., Kumar A., Sharma M., Sarin S. Correlation between two chemiluminescence based assays for quantification of hepatitis B surface antigen in patients with chronic hepatitis B infection. *Indian J. Med. Microbiol.* 33, 1, 96, 2015.
[12] Verheyen J., Neumann-Fraune M., Berg T., Kaiser R., Obermeier M. The detection of HBsAg mutants expressed in vitro using two different quantitative HBsAg assays. *J. Clin. Virol.* 54, 3, 279-281, 2012.
[13] Lee H.J., Kim S.Y., Lee S.M., et al. Elecsys hepatitis B surface antigen quantitative assay: performance evaluation and correlation with hepatitis B virus DNA during 96 weeks of follow-up in chronic hepatitis B patients. *Ann. Lab. Med.* 32, 420-425, 2012.
[14] Lok A.S., McMahon B.J. AASLD practice guideline update. *Hepatology* 50(3), 661-662, 2009.
[15] Jaroszewicz J., Serrano B.C., Wursthorn K., et al. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection: a European perspective. *J. Hepatol.* 52, 4, 514-522, 2010.
[16] Mühlbacher A., Weber B., Bürgisser P., et al. Multicenter study of a new fully automated HBsAg screening assay with enhanced sensitivity for the detection of HBV mutants. *Med. Microbiol. Immunol.* 197, 55-64, 2008.
[17] Zacher B., Moriconi F., Bowden S., et al. Multicenter evaluation of the Elecsys hepatitis B surface antigen quantitative assay. *Clin. Vaccine Immunol.* 18, 11, 1943-1950, 2011.
[18] Ganji A., Esmaeilzadeh A., Ghafarzadegan K., Helalat H., Rafatpanah H., Mokhtarifar A. Correlation between HBsAg quantitative assay results and HBV DNA levels in chronic HBV. *Hepat. Mon.* 11, 342-345, 2011.
[19] Alghamdi A., Aref N., El-Hazmi M., et al. Correlation between hepatitis B surface antigen titers and HBV DNA levels. *Saudi J. Gastroenterol.* 19, 252-257, 2013.
[20] Günal Ö., Barut Ş., Etikan İ., Duygu F., Tuncel U., Sünbül M. Relation between serum quantitative HBsAg, ALT and HBV DNA levels in HBeAg negative chronic HBV infection. *Turk. J. Gastroenterol.* 25, 142, 2014.