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The Evaluation of Quantitative Hbsag Assay and HBV-DNA Assay in Chronic Hepatitis B Infection

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Abstract

Chronic hepatitis B infection increases the risk of developing liver failure, liver cancer or cirrhosis, leading to death in case of bad management of the infection. Polymerase chain reaction assays for measuring HBV DNA has been used for many years for diagnosis and monitoring purposes in patients with chronic hepatitis B, but they are too expensive. Quantitative Hepatitis B Surface Antigen (HBsAg) had been suggested as a similar biomarker with HBV DNA. Recent studies and emerging data have shown that HBsAg levels change dynamically during the natural course of a chronic HBV infection. The aims of this study are to show the correlation between quantitative HBsAg and HBV-DNA levels and to analyze if quantitative HBsAg assay can possibly substitute HBV-DNA assay to optimize the management of chronic hepatitis B patients in our daily clinical practice. A total of 200 samples (52 females and 148 males) from different patients with chronic hepatitis B infection, without starting the treatment, are involved in this study. These specimens were tested for quantitative HBsAg, HbeAg, anti-Hbe, anti-Hbc and anti-Hbc-IgM levels with Cobas 6000 Roche instrument and HBV DNA levels with Cobas Taq Man instrument, at "Intermedica" Medical Clinic, in Tirana, Albania. Furthermore, the results were statistically processed with SPSS program (version 15.0; SPSS, Inc., Chicago, IL). The evaluation of the results showed a different correlation between quantitative HBsAg assay and HBV DNA assay at different phases of chronic hepatitis B infection; at Immune-Tolerant phase (IT), the correlation was good [r=0.676 (p<0.01)]. The quantitative HBsAg and HBV DNA relationship was stable in patients that resulted positive for HBeAg. This means a stable relationship between viral replication and HBsAg levels (ccDNA levels) before the immune-clearance phase. There was no correlation between these assays during the other phases of infection. In conclusion, there is a weak correlation between quantitative HBsAg assay and HBV DNA assay and quantitative HBsAg assay cannot substitute the HBV DNA assay, it cannot be used as the only biomarker for chronic hepatitis B infection.

Keywords: Quantitative HbsAg; HBV DNA; Chronic hepatitis B; Electrochemiluminescence

Introduction

Chronic Hepatitis B Virus (HBV) infection is a major global health problem which infects approximately 350 million people worldwide. The prolonged infection increases the risk of developing liver failure, cirrhosis or Hepatocellular Carcinoma (HCC) [1-3] leading to death.

Since 1960, the surface Hepatitis B Virus Antigen (HBsAg) has been used as a hallmark of chronic HBV infection [4]. Recent studies with quantitative assays have shown that serum HBsAg level changes significantly during different phases of chronic HBV infection by inversely correlating with HBV immune control: The higher the control the lower the level of HbsAg [5]. These results are in accordance with the hypothesis that the level of HBsAg reflects the complex interaction between the virus and the immune system and provides additional information on the viral load, measured through HBV DNA level [6]. HBsAg production changes quantitatively and qualitatively over time of infection, and is dynamically regulated during different phases of infection [7].

Several studies have shown a clear correlation between serum levels of the surface hepatitis B antigen and circular DNA. HBsAg is the main protein of the viral envelope, and serological measurement of it has led to the diagnosis of hepatitis B infection. If the infection occurs, HBsAg is the first detectable immunological serum marker, which is usually present from weeks to months before the onset of clinical symptoms. In case of recovery of acute HBV infection, HBsAg is no longer detectable in serum 6 months after its last recent onset [8,9].

If HBsAg persists for more than 6 months after acute hepatitis, the presence of chronic hepatitis B infection should be assumed. A Chronic hepatitis B virus patient with high levels of aminotransferases, high viral load of HBV DNA and histological abnormalities should be considered for therapy [10].

Various therapies are used to treat patients with HBV infection. The current standard for controlling these therapies is

the determination of HBV DNA. They should lower the viral load as much as possible, theoretically below the lower limit of detection of the PCR Real-Time method (10-15 IU/mL), to ensure the virological suppression and prevention of complications. However, the ideal end of therapy is the steady loss of HBsAg with or without anti-HBs seroconversion.

However, recent studies suggest monitoring the interferon alpha pegylated therapy by HBsAg and HBV DNA determination [11-13]. Furthermore, the determination of HBsAg is likely to predict stable virological response and loss of HBsAg. Further studies are needed to confirm these findings and to determine the importance of HbsAg determination in monitoring chronic hepatitis B infection.

Chronic Hepatitis B Virus (HBV) infection is classified into four phases (or types of immune response): immune-tolerant phase, immune-clearance phase, chronic active phase, and chronic inactive phase, which correlate with patients' immune response to HBV.

Also, the initial immune response and the subsequent initial stage of chronic infection usually depend on the age at which the patient has acquired HBV, the results of HBV therapy and the genotype of HBV.

Phases of chronic HBV infection are not always considered as static and patients who first develop the immunotolerant phase usually progress to the immunoactive phase and then to the chronic inactive carrier condition [14].

Methodology

A total of 200 samples (52 females and 148 males) from different patients with chronic hepatitis B infection, without starting the treatment, are involved in this study. These patients were diagnosed with Chronic Hepatitis B at the Gastro hepatology Clinic of Mother Teresa University Hospital Center in Tirana, based on their disease history, ultrasound, biochemical and immunological examinations. Serum samples were tested, from March 2012 to April 2013, for quantitative HBsAg HbeAg, anti-Hbe, anti-Hbc and anti-Hbc-IgM levels with Cobas 6000 Roche instrument and HBV DNA levels with Cobas Taq Man instrument, at "Intermedica" Medical Clinic, in Tirana, Albania determination.

Based on international protocols, patients were classified into four groups:

Immune tolerant phase patients, who resulted positive for HBeAg with normal ALT values.

Immune-clearance phase patients, who resulted positive and negative for HBeAg with high values of ALT and HBV DNA.

Chronic active patients who resulted negative for HBeAg with high values of quantitative HBsAg (4-5 log IU/ml) and moderate growth ALT values.

Chronic inactive carrier who resulted negative for HBeAg with normal values of ALT and HBV DNA.

Blood sampling and centrifugations were performed in two different ways: gel tube and centrifugations for 10 min with 30,000 revolutions/min for quantitative HbsAg; EDTA tube and centrifugations for 30 min with 1500 revolutions/min for HBV DNA. The examinations for quantitative HbsAg were performed within 2 hours of receiving the patient's blood. This determination was performed with sandwich principle of electrochemiluminescence technique, applied on Cobas 6000 instrument and the results are expressed in IU/mL. Samples were stored at-20 degrees Celsius to BMM at the time of the HBV DNA determination, which was performed with PCR-RT technique applied on Cobas TAQ-MAN instrument and the results, are expressed in IU/mL.

Furthermore, the results were statistically processed with SPSS program (version 15.0; SPSS, Inc., Chicago, IL).

Results and Discussion

The evaluation of results showed high levels of HBV DNA [log 8 (IU/ml)] and quantitative HBsAg [approximately (log 5(IU/ml)] at immune tolerant phase. Also, the first group (immune tolerant phase patients) showed high levels of HBV DNA and quantitative HBsAg, comparing it with the other three groups. The correlation between quantitative HBsAg assay and HBV DNA assay **(Figure 1)** was good at immune-tolerant phase [r=0.676 (p<0.01)].

There was no correlation between HBV DNA levels and HBeAg, ALT or AST levels. The quantitative HBsAg and HBV DNA relationship was stable in patients that resulted positive for HBeAg. This means a stable relationship between viral replication and HBsAg levels (ccDNA levels) before the immune-clearance phase.

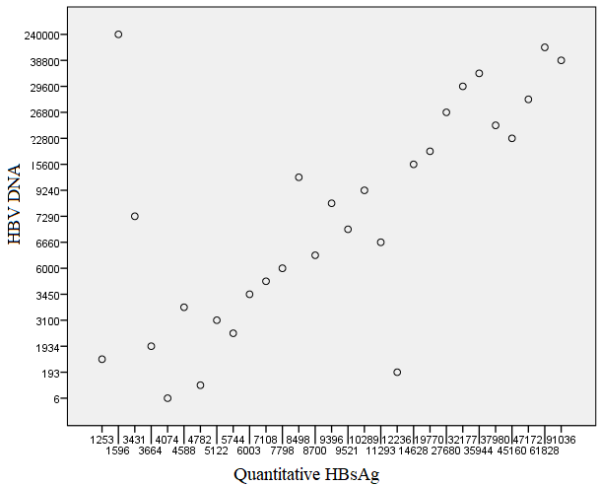

Figure 1: Analysis of correlation between quantitative HBsAg assay and HBV DNA assay at Immune tolerant phase, with few values that deviate the linear line. This means that quantitative HBsAg assay and HBV DNA assay have a good concordance between them, as biomarkers for chronic hepatitis B infection at Immune tolerant phase.

The evaluation of results showed low levels of HBV DNA and higher levels of quantitative HBsAg at chronic active phase. There was no correlation between quantitative HBsAg assay and HBV DNA assay **(Figure 2)**, r=0.247.

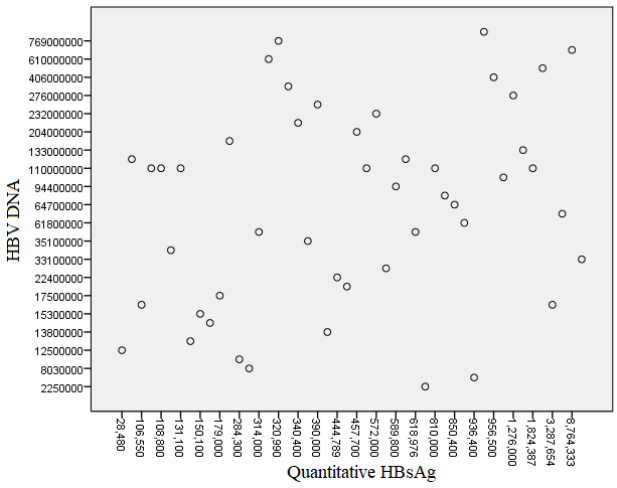

Figure 2: Analysis of correlation between quantitative HBsAg assay and HBV DNA assay at chronic active phase, with too many values that deviate the linear line. This means that quantitative HBsAg assay and HBV DNA assay have not a good concordance between them, as biomarkers for chronic hepatitis B infection at chronic active phase.

An immune control of viral replication at immune-clearance phase, with increasing levels of HBsAg/HBV DNA report, is caused by decreasing levels of HBV DNA and increasing levels of quantitative HBsAg. The evaluation of results showed no correlation between quantitative HBsAg assay and HBV DNA assay, r=0.120 **(Figures 3 and 4)**.

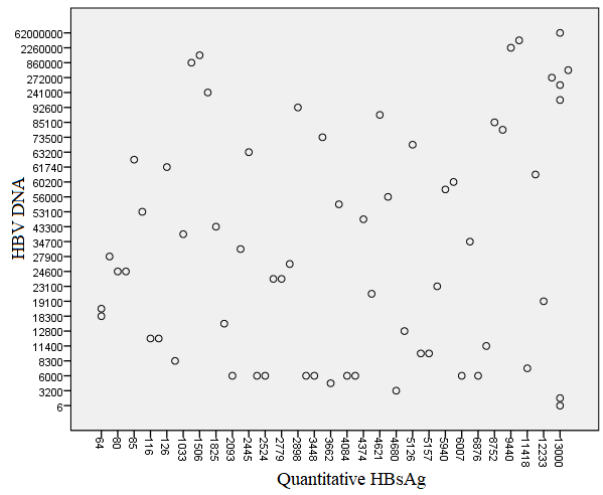

Figure 3: Analysis of correlation between quantitative HBsAg assay and HBV DNA assay at immune-clearance phase, with too many values that deviate the linear line. This means that quantitative HBsAg assay and HBV DNA assay have not a good concordance between them, as biomarkers for chronic hepatitis B infection at immune-clearance phase.

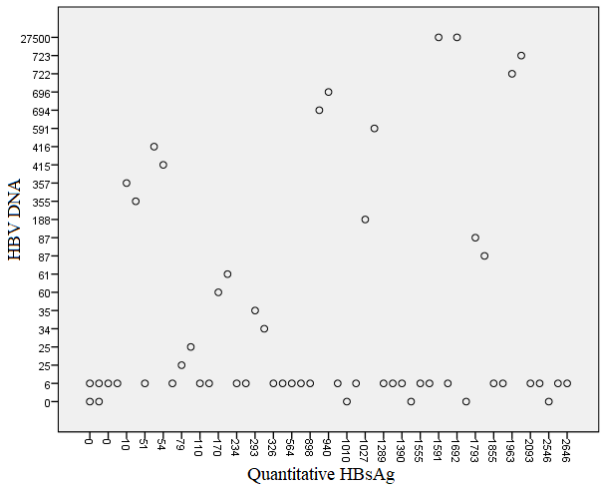

Figure 4: Analysis of correlation between quantitative HBsAg assay and HBV DNA assay at chronic inactive phase, with too many values that deviate the linear line. This means that quantitative HBsAg assay and HBV DNA assay have not a good concordance between them, as biomarkers for chronic hepatitis B infection at chronic inactive phase.

The evaluation of results showed low levels of HBsAg and normal levels of transaminases (ALT, AST) at chronic inactive phase (negative values of HBeAg), and no correlation between quantitative HBsAg assay and HBV DNA assay, r=0.22 (Figure 4). These reflect the immune clearance tendency.

Furthermore, the evaluation of results showed that active phase patients were younger than inactive phase patients. Also, the number of infected males (145) resulted higher than infected females (51). 33% of patients resulted positive for HBeAg, and 67% of patients resulted negative for HBeAg. Also, patients who resulted positive for HBsAg had higher levels of HBV DNA than patients who resulted negative for HBeAg.

The results of this study are in concordance with some studies conducted in Iran. This is thought to be related with the same genotype through these populations [15]. On the other hand, the results of this study are not in concordance with some other studies conducted in Korea [16-21].

Conclusion

The evaluation of the results showed a different correlation between quantitative HBsAg assay and HBV DNA assay at different phases of chronic hepatitis B infection; at Immune-Tolerant phase (IT), the correlation was good [r=0.676 (p<0.01)]. There was no correlation between these assays during the other phases of infection.

In conclusion, there is a weak correlation between quantitative HBsAg assay and HBV DNA assay. So, quantitative HBsAg assay cannot substitute the HBV DNA assay (except the immune-tolerant phase) and it cannot be used as the only biomarker for chronic hepatitis B infection.

References

1. Lee WM (1997) Hepatitis B virus infection. N Engl J Med 337:1733-1745.
2. Wright TL, Lau JYN (1993) Clinical aspects of hepatitis B virus infection. Lancet 342:1340-1344.
3. Hoofnagle JH, di Bisceglie AM (1997) The treatment of chronic viral hepatitis. N Engl J Med 336:347-356.
4. Hoofnagle JH (1981) Type B hepatitis: Virology, serology and clinical course. Semin Liver Dis 1:7-14.
5. Ji Lee H, Young Kim S, Min Lee S, Heo J, Hoi Kim H, et al. (2012) 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. 32: 420-425.
6. Scaglioni PP, Melegari M, Wands JR (1996) Recent advances in the molecular biology of hepatitis B virus. Baillieres Clin Gastroenterol 10:207-225.
7. Lin LY, Wong VW, Zhou HJ, Chan HY, Gui HL, et al. (2010) Relationship between serum hepatitis B virus DNA and surface antigen with covalently closed circular DNA in HBeAg-negative patients. J Med Virol 82:1494-1500.
8. Coursaget P, Yvonnet B, Bourdil C (1987) HBsAg positive reactivity in man not due to hepatitis B virus. Lancet 2:1354-1358.
9. Shiels MT, Taswell HF, Czaja AJ (1987) frequency and significance of concurrent hepatitis B surface antigen and antibody in acute and chronic hepatitis B. Gastroenterol 93:675-680.
10. European Association for the Study of the Liver (2019) EASL Clinical Practice Guidelines: Management of chronic hepatitis B. J Hepatol 50:227-242.
11. Marcellin P, Bonino F, Lau GK (2009) Sustained response of hepatitis B e antigen-negative patients 3 years after treatment with peginterferon alpha-2a. Gastroenterol 136:2169-2179.
12. Perillo R (2009) Hepatitis B surface antigen quantification as a current-day paradox: Obtaining the gold in the face of dimishing returns. Hepatol 49:4.
13. Brunetto MR, Moriconi F, Bonino F (2009) Hepatitis B Virus Surface Antigen Levels: A Guide to Sustained Response to peginterferon alpha-2a in HBeAg-Negative Chronich Hepatitis B. Hepatol 49:1141-1150.
14. Lok AS, McMahon BJ (2007) Chronic hepatitis B. Hepatol 45:507-539.
15. Ganji A, Esmaeilzadeh A, Mokhtarifar A (2011) Correlation between HBsAg quantitative assay results and HBV DNA levels in chronic HBV Hepat Mon 11:342-345.
16. Sonneveld MJ, Rijckborst V, Boucher CA, Zwang L, Beersma MF, et al. (2011) A comparison of two assays for quantification of Hepatitis B surface Antigen in patients with chronic hepatitis B. J Clin Virol 51:175-178.
17. Ganem D, Prince AM (2004) Hepatitis B virus infection-natural history and clinical consequences. N Engl J Med 350:1118-1129.
18. Marquardt O, Heermann KH, Seifer M, Gerlich WH (1987) Cell type specific expression of pre S 1 antigen and secretion of hepatitis B virus surface antigen. Brief Report. Arch Virol 96:249-256.
19. Mühlbacher A, Weber B, Bürgisser P, Eiras A, Cabrera J, et al. (2008) 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.
20. Thomas HC, Lemon S, Zuckerman AJ (2005) Viral Hepatitis. In: Kann M, Gerlich WH, editors. Structure and molecular virology. 3rd ed. Oxford: Blackwell Publishing pp:149-180.
21. Tong S, Kim KH, Chante C, Wands J, Li J (2005) Hepatitis B virus e antigen variants. Int J Med Sci 2:2-7.