Preethi et al | Article 8754
J Pure Appl Microbiol. 2023;17(4):2410-2414. doi: 10.22207/JPAM.17.4.35
Received: 15 June 2023 | Accepted: 25 October 2023
Published Online: 29 November 2023

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Comparison between Quantitative Hepatitis B DNA and HBeAg Positivity to Detect Active Viral Replication among Patients with Hepatitis B infection in a Tertiary Care Hospital

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Abstract

To study the correlation between quantitative Hepatitis B DNA and HBeAg positivity among HBsAg positive patients in a tertiary care hospital. To detect HBsAg, HBeAg levels by CMIA (Chemiluminescent microparticle immunoassay). To perform quantitative Hepatitis B virus DNA assay using Real Time Polymerase Chain Reaction. The study took place between July and December 2022 for 6 months. Sixty four patients were included in the study for whom HBsAg was positive by CMIA and for whom HBeAg (detected by CMIA) and Hepatitis B Virus DNA by quantitative Real Time PCR was performed. Of the 64 HBsAg reactive patients, the 'e' antigen of Hepatitis B virus was positive in six patients. In all the six HBeAg positive patients, HBV DNA was detected with a range of 78-10,288 IU/ml with 66% having levels more than 2000 IU/ml and requiring treatment. Among the 58 patients who were negative for the 'e' antigen of Hepatitis B virus, the Hepatitis B Viral DNA was detected in 27 patients with a range of 10-1,76,000 IU/ml, with 21% of patients having levels more than 2000 IU/ml. HBeAg is a good serological test to detect viral replication. However, it alone might not be sufficient as quantitative HBV DNA levels are more accurate and it does identify more patients who may require treatment.

Keywords: Hepatitis B Infection, HBeAg, Quantitative HBV DNA

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Citation: Preethi V, Lakshmi SSJ, Leela KV. Comparison between Quantitative Hepatitis B DNA and HBeAg Positivity to Detect Active Viral Replication among Patients with Hepatitis B infection in a Tertiary Care Hospital. *J Pure Appl Microbiol.* 2023;17(4):2410-2414. doi: 10.22207/JPAM.17.4.35

INTRODUCTION

Despite having an effective vaccine and suppressive anti-viral treatment for many years now, Hepatitis B infection continues to be a global problem affecting millions of people, among which the majority are Asians.[1-3] According to the WHO, in 2019, two hundred and ninety six million people were suffering from Chronic infection with Hepatitis B and about 1.5 million people had newly acquired the infection in that year. In 2019 alone, chronic infection leading to cirrhosis and hepatocellular carcinoma led to an estimated 8,20,000 deaths and acute infection was responsible for claiming 36,000 lives, of which one third was in South East Asia.[4] Hepatitis B is transmitted through blood and body fluids. Though the spread is predominantly vertical, i.e. from mother to neonate, other modalities like horizontal transmission (between children), intravenous drug abuse (i.e. percutaneous) and sexual route do continue to play a major role in transmission.[1] Based on the modes of transmission, the prevalence rates of Hepatitis B in different geographical areas are variable and the regions are grouped as low (<2%), intermediate (2-8%) and high (>8%) prevalence areas. [1] The rate is highest in Africa and Western Pacific regions and long-lasting infection with Hepatitis B infection is of concern in the Asia Pacific region.[4,5]

Those with persistent Hepatitis B virus infection have a greater chance of developing complications like fibrosis, liver cirrhosis and sometimes cancer and therefore, monitoring the evolution of Hepatitis B infection becomes crucial.[2] The natural progression of persistent Hepatitis B typically has four phases namely the immune tolerant phase, immune clearance phase, low-replicative phase and HBeAg negative hepatitis.[6] These phases do not occur in all people and not always in the same order.[6-8] Serological markers like HBsAg (surface antigen), HBeAg (e antigen) and molecular markers like HBV DNA are useful in diagnosis and monitoring treatment. The surface antigen of Hepatitis B is used as an indicator of infection and its levels change during the various stages of infection and it can also be used to monitor treatment response to chronic infection with Hepatitis B virus.[1,9] It has been well established that the presence HBeAg could indicate active viral replication. With the advent of molecular testing, and the wider availability of quantitative HBV DNA levels, the infection status and viral replication can be measured more accurately.[1]

A few studies have found a positive relationship linking serum levels of Hepatitis B surface antigen and viral DNA levels in patients who are positive for 'e' antigen of Hepatits B.[2,3,10] Such correlation was not seen in patients who were negative for Hepatitis B 'e' antigen.[2,10] A few studies, however, found a similar correlation between the surface antigen and Hepatitis B DNA levels even in patients who are negative for HBeAg.[6] Such differences in the observation between studies regarding the association of surface antigen of Hepatitis B virus and HBV DNA levels could be explained by the dynamic nature of infection or the differences in genotypes.[1,9] HBeAg seroconversion has been considered an important turning point in deciding the end point of antiviral therapy. However, there have been reports of hepatic flares with elevated levels of HBsAg, high levels of copies of HBV DNA and raised liver enzymes in patients with persistent Hepatitis B, irrespective of their HBeAg status. These flares could occur spontaneously, during treatment or due to immunosuppression.[11] Quantitative Hepatitis B DNA testing has been found to be a useful, independent element in monitoring extent of liver injury and treatment response.[12,13] However, testing for Hepatitis B DNA is not universally followed due to economic constraints and the implementation of quantitative DNA testing varies from one region to another. Therefore, the presence of HBeAg to monitor infection and treatment response is considered worthwhile.[14]

HBsAg and HBeAg status are still traditionally used to monitor the disease progression and for management in low resource settings as they are less expensive and can be performed in most laboratories in developing countries. Molecular assays including HBV DNA detection are reserved for more advanced laboratories as these require specialized equipment, trained personnel, and technical expertise.[12] Hence, the correlation between HBeAg and HBV DNA was studied in our hospital to assess its ability to analyse active viral replication

Table. Hepatitis B DNA levels and its relation with HBeAg reactivity

	HBV DNA levels (<2000 IU/ml)	HBV DNA levels (2000-20000 IU/ml)	HBV DNA levels (>20000 IU/ml)
HBeAg Positive (n=6)	2 (33.33%)	4 (66.66%)	0
HBeAg Negative and HBV DNA detected (n=27/58)	22 (81.48%)	2 (7.41%)	3 (13.64%)

and whether HBeAg positivity would reflect HBV DNA levels and whether it would be sufficient to monitor patients with Hepatitis B infection in settings where resources are constrained.

METHODOLOGY

The study was conducted between the months of July and December 2022 in the Microbiology Department, SRM Medical College and Research Centre, Kattankulathur. Seven thousand five hundred and sixty five serum samples were received in the laboratory for screening for HBsAg. One hundred and twenty one patients tested positive for the surface antigen of the Hepatitis B virus by Chemi-luminescent MicroparticleImmuno-Assay(CMIA). During the study period, only 64 patients with Hepatitis B were referred for further testing of HBeAg and quantitative HBV DNA and, therefore, were considered for inclusion in the study. Of these, 40 were males and 24 were females.

The samples from these 64 patients were further tested for qualitative HBeAg by CMIA and quantitative Hepatitis B DNA levels by Real Time PCR.

HBsAg and HBeAg detection

HBsAg and HBeAg were detected qualitatively using Abbott Architect by Chemiluminescent microparticle immunoassay. The test was performed and interpreted according to manufacturer's instructions. Signal cut off value of < 1.00 was interpreted as non-reactive and a value of >= 1.00 was taken as reactive.

Quantitative HBV DNA detection

Viral DNA was first extracted using HiPurA Viral DNA Purification kit. (HiGenoMB, Himedia). Quantitative HBV DNA level detection in serum and plasma samples was performed by means of Co-Dx SARAQ Hepatitis B viral load kit. (Cosara Diagnostics) . The reaction was performed in agreement with the directions given by the manufacturer. A reaction volume of 20 microlitre was subjected to the following cycling conditions, a holding period of 95°C for 20 seconds, followed by 45 cycles of 95°C for 3 seconds and 55°C for 32 seconds. HBV standards 1-5 were run to obtain a standard curve. The concentration of the standards were measured in copies/microlitre. The concentration of the DNA in the sample were expressed as copies/ml and in IU/ml. The limit of detection of this kit was 8.4 IU/ml.

RESULTS

During the study period, of the 7565 serum samples received for Hepatitis B testing, 121 samples tested positive for HBsAg by CMIA, giving a positivity rate of 1.59%. Of these 121 patients, 4 patients were found to be also infected with Human Immunodeficiency Virus (HIV) infection and 1 patient had concurrent Hepatitis C virus (HCV) infection. Only 64 patients were tested further for their quantitative Hepatitis B DNA levels in addition to the 'e' antigen and hence were considered for inclusion in the study. The 'e' Antigen of Hepatitis B was detected by CMIA in six out of 64 patients. In all the 6 samples, HBV DNA was detected and the load ranged from 2640 to 3,45,700 copies/ml (78-10,288 IU/ml) with 66% of patients having DNA levels more than 2000 IU/ml.

Of the other 58 patients in whose samples HBeAg was not detected, 31 samples were also negative for HBV DNA, whereas in 27/58 samples HBV DNA could be isolated and the range was 350 to 59,75,000 copies/ml (10-1,76,000 IU/ml) with 21% patients having DNA levels more than 2000 IU/ml (Table).

DISCUSSION

The positivity rate for HBsAg in our hospital is 1.59%. This is slightly lower than the national prevalence which ranges from 2-4% in the general population with geographical variations and much higher rates among tribal population.[15,16] The study highlights the discrepancy in the number of patients with Hepatitis B who underwent only serological testing and those who underwent molecular testing for viral load monitoring. Of the 121 patients in the study period with Hepatitis B infection, only about half the patients, i.e. 64 patients had their DNA levels measured. Some cases were lost to follow up but the most common reason cited was the high cost of molecular assays which was not affordable for some patients. Hence, though molecular assays are the most reliable to monitor the viral load, other serological parameters are still being preferred for financial reasons.

The development of liver carcinoma may be related to persistent HBsAg positivity and HBeAg seropositivity.[17-19] As per the American Association for the Study of Liver Disease (AASLD Practice Guidelines) a titre of 20,000 IU in patients positive for HBeAg may be an independent risk factor for liver carcinoma.[17] In HBeAg negative patients, those patients who have compensated liver disease and a titre of over 2000 require treatment and if decompensated, any detectable DNA level is considered significant. However, there is no cut-off to distinguish HBeAg negative patients with chronic infection and chronic Hepatitis B carriers.[17] In our study, all six HBeAg positive patients had titres less than 20000 but 4 out of 6 had values above 2000 IU/ml. However, among the HBeAg negative group with detectable DNA levels, i.e. in 27 out of 58 patients, 3 patients had a titre of more than 20000 IU/ml, 2 patients were in 2000-20000 range and the other 22 patients had titres less than 2000 IU/ml.

Thus, in the group who were positive for Hepatitis B 'e' antigen, 66% of the patients had HBV DNA levels more than 2000 IU/ml requiring treatment and in the patients group who were negative for HBeAg, about 21% of patients had nucleic acid levels greater than 2000 IU/ml/. This indicates that though HBeAg is a good marker of increased viral replication, the monitoring of HBV DNA levels by PCR could still uncover more patients who may require antiviral treatment.[20-22]

CONCLUSION

HBeAg is a good serological test to detect viral replication. However, it alone might not be sufficient as quantitative HBV DNA levels are more accurate and it does identify more patients who may require treatment. Hence, both the 'e' antigen of Hepatitis B and HBV DNA levels together would be useful in the appropriate treatment of patients with Hepatitis B infection.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

The study was approved by the Institution Ethics Committee, SRM Medical College Hospital & Research Centre, Tamilnadu, India, with reference number SRMIEC-ST0123-339.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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