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Correlation between serum HBV DNA and HBsAg levels in non-cirrhotic HBeAg positive and negative chronic hepatitis B patients

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Abstract
Background: Quantification of serum HBV DNA is a laborious, time-consumed and expensive method requiring special machines and technicians. Quantification of serum HBsAg levels is recently available and a more favorable test to perform than HBV DNA quantification especially for developing countries like India.
Aim: To evaluate the correlation between serum HBsAg and HBV DNA levels in the treatment naïve HBeAg-positive and -negative non-cirrhotic chronic hepatitis B (CHB) individuals with HBV DNA greater than 2000 IU/ mL and ALT greater than twice the upper limit of normal.
Material and Methods: A descriptive-analytical (cross-sectional) study was carried out in 30 Indian non-cirrhotic patients of CHB with evaluation of serum HBsAg, HBeAg, Anti-HBe and HBV DNA levels through standardized methods.
Result: Non-association of HBsAg and HBV DNA levels in HBeAg-positive and -negative patients ($p>0.05$) was observed.
Conclusion: The correlation between HBsAg and HBV DNA levels was insignificant. In our study individuals, a low level of HBsAg indicates a low HBV DNA level, whereas a high HBsAg level did not correspond to a high viral load in most of the cases.

Keywords: hepatitis B surface antigen quantitative, HBV DNA, hepatitis B, HBeAg, viral load

Introduction

Hepatitis B is a global healthcare burden with more than 350 million people infected worldwide [1]. As per recent predictions, 65 million of those chronically infected will die from hepatitis B virus (HBV) induced end-stage diseases such as hepatic decompensation, cirrhosis of the liver and hepatocellular carcinoma [2]. Undesirable side effects, resistance, cost and long term therapy with conventional antiviral agents, and reluctance of community toward transmission and vaccination continued the chronic hepatitis B to be a healthcare burden [2, 3].

As per American Association for study in liver diseases (AASLD) guidelines 2018, chronic hepatitis B (CHB) is defined as 1) positive HBsAg for ≥6 months; 2) Serum HBV DNA varies from undetectable to several billion IU/mL; 3) Subdivided into HBeAg positive and negative. HBV-DNA levels are typically >20,000 IU/mL in HBeAg-positive CHB, and lower values (2,000-20,000 IU/mL) are often seen in HBeAg-negative CHB; 4) Normal or elevated ALT and/or AST levels; and 5) Liver biopsy results show chronic hepatitis with variable necroinflammation and/or fibrosis [3].

Serum HBV DNA level is an important marker of active replication of the virus, infectivity and strongly associated with the serious sequelae of HBV infection [4]. Risk for cirrhosis and HCC notably increases at HBV-DNA levels ≥10 000 copies/mL. Sufficient evidence has been present to denote notable relation of high viral load and development of cirrhosis and hepatocellular carcinoma [5] but a lower HBV DNA level doesn't eliminate the risk of HCC development [4]. The HBV DNA level varies considerably with natural progression of the disease under influence of patient age, environmental factors (e.g., aflatoxin exposure, alcohol use), host genetic factors, genetic variation in HBV, etc [4].

Quantification of serum HBV DNA is an expensive time-consuming laborious method requiring special technician and standardization but a crucial component in the evaluation of patients of CHB and response to antiviral treatment. Most HBV-DNA assays used in clinical practice utilize real-time polymerase chain reaction technology with a sensitivity of 5-10 IU/mL and a dynamic range up to 7 log10 IU/mL [3].

On the other hand, qualitative serum HBsAg testing is primarily a method for routine screening of HBV infection while quantitative measurement is used as a predictor of early viral response during antiviral therapy, distinguishing disease status in chronic infection and also used as a surrogate marker of viral covalently closed circular DNA and intrahepatic HBV DNA [6].

Various studies have been attempted in different phases of the natural progression of chronic hepatitis B infection to correlate serum levels of HBsAg with HBV DNA, so that HBsAg quantification can be used as a marker which is less time-dependent and easier to perform instead of expensive HBV DNA quantification [7]. Earlier studies have implied that quantitative HBsAg can be a surrogate marker to monitor CHB patients on antiviral therapy, and HBsAg levels well correlate with HBV DNA levels [8, 9].

In the present study authors aimed to provide a true correlation between serum HBsAg and HBV DNA levels in treatment naïve HBeAg-positive and -negative non-cirrhotic CHB individuals with HBV DNA greater than 2000 IU/ mL and ALT greater than twice the upper limit of normal.

Material and Methods

Ethical consideration

Informed consent was obtained from study participants, and the study was approved by the Institutional Ethics Committee of Jamia Hamdard. Clinical trial registration no.: CTRI/2017/11/010386. The study protocol conforms and implemented to the ethical guidelines of the Declaration of Helsinki (1975).

Study design and participants eligibility

This was a descriptive-analytical (cross-sectional) study conducted in the department of moalajat (medicine), Majeedia Unani Hospital, Jamia Hamdard New Delhi, India during 2016-2017. 170 Indian patients with reports of positive hepatitis B surface antigen (HBsAg) were evaluated and enrolled as per the following inclusionary criteria's 1) positive HBsAg ≥ 2000 IU/mL; 2) ALT >2 times of upper limit of normal; 3) both HBeAg positive and negative patients; 4) patients age between 18-60 years and 5) patients of any sex. The exclusionary criteria's were 1) pregnant women and lactating mothers; 2) mentally retarded person; 3) patients who fail to give informed consent; 4) patient with cirrhosis, portal hypertension/ ascites and obstructive jaundice; 4) patients of diabetes and hypertension; 5) patients with kidney and heart disease, and 6) patients with neurological disorder. 30 CHB patients qualified for the above inclusion and exclusion criteria and being studied. Among 30 patients, 14 patients were HBeAg positive while 16 patients were HBeAg negative.

Outcome measures

All patients were tested for routine hepatitis B serological markers (HBsAg, HBeAg, anti-HBe) by commercial standardize methods. Serum HBsAg assay was performed through mini VIDAS®, a compact automated immunoassay system based on the enzyme-linked fluorescent assay (ELFA) technology. Serum HBeAg assay was performed through fully automated bidirectionally interfaced chemiluminescent immunoassay (CLIA) technology. Serum HBV DNA was quantified by real-time polymerase chain reaction (PCR) assay, the Taq-Man HBV quantitative test, which had a lower limit of quantification of 10^3 copies/mL (200 IU/mL). 1 IU/ mL was equivalent to 5.82 copies/ mL.

Statistical analysis

Spearman rank correlation coefficient was used to estimate the correlation between HBsAg and HBV DNA levels. Variables with a normal distribution, mean value and standard deviation were calculated using SPSS software, version 13.0 (SPSS, Inc., Chicago, IL). A value of $P < 0.05$ was considered statistically significant.

Results

The baseline characteristics of HBeAg positive and negative patients. [Table 1]

Correlation value between serum HBV DNA and HBsAg levels. [Figure 1, 2]

HBeAg-positive (n=14): r = -0.00909, P (2-tailed) = 0.97884.

HBeAg-negative (n=16): r= -0.12088, P (2-tailed) = 0.68061

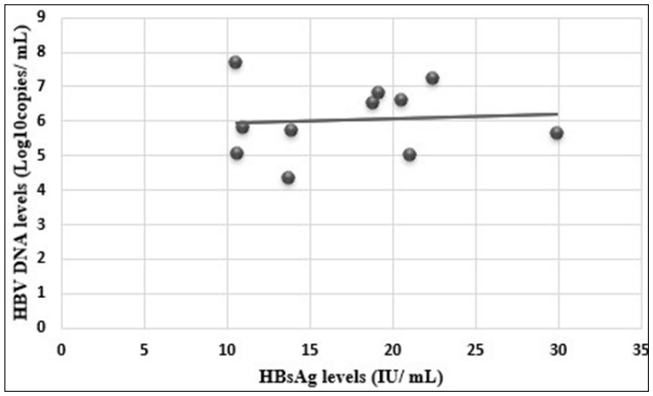

Fig 1: Correlation between HBsAg and HBV DNA levels in HBeAg positive individuals

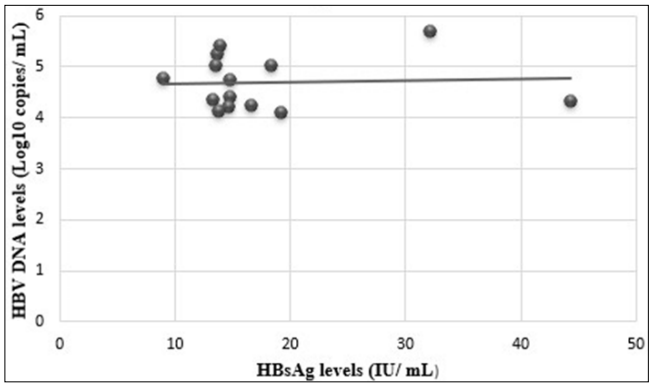

Fig 2: Correlation between HBsAg and HBV DNA levels in HBeAg negative individuals

Table 1: Baseline characteristics of study participants Mean ± SD

Variables	HBeAg Positive (n=14)	HBeAg Negative (n=16)
Age (Years)	29.64±11.47	30.68±11.31
Sex (Male: Female)	12:2	9:7
BMI (kg/m^2)	20.01±2.98	21.71±2.95
ALT (IU/mL)	350±576	204±341
AST (IU/mL)	296.8±451	156±297
Total Bilirubin (mg/dL)	3.14±2.56	1.25±1.71
Mean HBV DNA Level (Log_{10} copies/mL)	5.88±1.07	4.68±0.5
Mean HBsAg levels (IU/ mL)	17.42±6.07	18.03±9.22

Discussion

We were expecting a strong correlation between HBsAg and HBV DNA quantification as per the finding of Alghamdi *et al*., (2013) [8] in a cross-sectional study which has strongly correlated HBsAg quantitation and HBV DNA levels in treatment-naïve patients with HBeAg-negative HBV/D in a Saudi Arabian population $r = 0.383$, $P < 0.05$ [8].

The present study evidenced a non-association of serum HBsAg levels with serum HBV DNA levels, similar to the study of Ganji *et al*., (2011) [10] in in HBeAg-positive (P =

0.053 and r = -0.57) and -negative patients (P = 0.605 and r = 0.057) [10] with baseline characteristics similar to our study patients and reports of Wiegand *et al*., (2008) [11]. In another study of HBeAg-negative CHB, HBsAg correlated poorly with serum HBV DNA and did not correlate with intrahepatic CCC DNA or total HBV DNA [12].

However, HBV DNA quantification is an important marker for disease activity and evaluation of response to antiviral therapy. Despite non-association between HBV DNA and HBsAg levels, the quantification of HBsAg represents the cornerstone of HBV infection diagnosis and used to manage and monitor patients as well. Recent studies show that the HBsAg level well correlates with covalently closed circular DNA (cccDNA) level in the liver and reflects the amount of cccDNA inside the hepatocytes [5]. Also besides, HBsAg quantification indirectly reflects the number of infected hepatocytes. Today HBsAg level is used for differentiation between inactive carriers and patients with active disease [13].

Conclusion

No correlation of HBV DNA was observed with HBsAg levels in noncirrhotic HBeAg-positive and -negative CHB patients in our study but our sample size was likely too small to observe any significant correlation between HBsAg and HBV DNA levels. In our study individuals, a low level of HBsAg indicates a low HBV DNA level, whereas a high HBsAg level did not correspond to a high viral load in most of the cases in both HBeAg-positive and -negative group.

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