Research Article

Correlation of hepatitis B virus viral load and HBeAg status in HBsAg positive patients

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Abstract

Background: Hepatitis B virus (HBV) infection is a major cause of chronic liver disease and hepatocellular carcinoma. About a one third of the world's population is infected with the HBV. To control the spread of HBV it is important to understand the molecular epidemiology of HBV. **Objective:** Present study was aimed to detect HBV viral load by Real Time PCR in HBsAg positive patients and to correlate with HBV markers. **Material and methods**: A total of 100 blood samples were obtained from HBsAg positive patients. DNA was extracted by Roche High Pure System COBAS TaqMan kit and quantified by COBAS TaqMan HBV Test Kit on COBAS TaqMan 48 analyzer (Roche). **Results:** Majority (48%) of the patients included in the present study were in the age group 20-40 years and most of the patients (60%) were found to be asymptomatic. Out of 100 patients, 80 (80%) were males and only 20 (20%) were females and 28 were HBeAg positive and 72 were HBeAg negative. Of above 28 HBeAg positive samples 16 (57.15%) were having viral load >20000 IU/mL and 12 (42.85%) were having <20000 IU/mL Out of 72 HBeAg negative samples 20 (27.77%) were having viral load >20000 IU/mL and 52 (72.23%) were having <20000 IU/mL. Only 6% (6/100) of the patients, the target was not in detectable (TND) range and these all were HBeAg negative. **Conclusion:** It's important to carry out HBV viral load testing in patients who are HBsAg positive on regular basis not only in HBeAg positive cases but in negative cases too. As on our study we found HBeAg negative patients had viral load more than 20000 IU/mL which mandates initiation of treatment.

***Keywords*:** Hepatitis B virus, viral load, HBeAg, HBsAg, PCR

Introduction

Hepatitis B virus (HBV) infection is still an important public health problem. More than 350 million people worldwide have chronic hepatitis B (CHB). The serum HBV DNA level is an important marker of the viral activity. It is proved that a high viral load plays an important role in the development of CHB complications. A lower HBV DNA level is associated with a lower risk of a hepatocellular carcinoma (HCC) development than a higher level (Chan, 2012).

The HBV DNA level varies considerably with time, so for dynamic observation the measurement must be repeated. However, the viral load measurement is an expensive method which requires specialized equipment. As a result in clinical practice HBV infected patients are being monitored with Hepatitis B e antigen (HBeAg) only which is an indicator of active viral replication. High viral load has been found to correlate significantly with HBeAg status (Maria et al., 2015; Parizad et al., 2016). However there are reports that in many patients with pre core mutations HBeAg may be negative and patient may have high HBV DNA levels (Seeger et al*.,* 2007; Taghavi et al., 2010).

HBeAg-negative CHB is associated with the lower HBV DNA viral load, more significant intrahepatic necroinflammatory damage, more progressive disease and higher number of cirrhosis and/or hepatocellular carcinoma compared to HBeAg-positive CHB. HBeAg-negative HBV patients are more difficult to treat than HBeAg positive due to lower sustained responses (Pasdar et al., 2017).

Hepatitis B surface antigen (HBsAg) detection represents the cornerstone of HBV infection diagnosis. Recent data show that HBsAg and HBeAg detection correlates with covalently closed circular DNA (cccDNA) level in the liver and reflects

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the amount of cccDNA inside hepatocytes (Brunetto, 2010; Chan et al., 2010).

Therefore, the present study was undertaken to investigate the correlation between the HBV DNA level and HBeAg status in patients attending SMS Hospital.**Material and Methods**

The present study was conducted on 100 HBsAg positive patients, whose blood samples were received for HBV DNA viral load in the Advance Research Laboratory in the Department of Microbiology and Immunology, SMS Medical College, Jaipur from different Wards and OPDs of SMS hospital and allied hospitals.

Biochemical estimation

Blood (plasma/serum): A total 5 mL of blood was collected in an EDTA (ethylene diamine tetra acetic acid) vial for PCR and in plain vial for serology. The blood was centrifuged and plasma/serum was transferred to a sterile eppendrof tube for storage. Samples were stored in freezer at -20^{0}C till they were tested.

HBeAg detection: Antigen was detected by enzyme-linked Immunosorbent assay (ELISA) using Dia.Pro ELISA kits as per manufacturer's instructions.

Extraction and Quantification of HBV DNA

DNA was extracted by Roche High Pure System COBAS TaqMan kit and quantified by COBAS TaqMan HBV Test Kit on COBAS TaqMan 48 analyzer (Roche) as per manufacturer's instructions. The HBV DNA titre is expressed in International Units (IU)/mL.

Initial activation of UNG (Uracil-N-glycosylase) was done at 50^{o}C for 12 min followed by Activation of Taq Polymerase & inactivation of UNG at 95^{o}C for 10 min. Cycling profile for PCR was 30 cycles of denaturation at 95^{o}C for 16 s and annealing at 60^{o}C for 1 min.

Results and discussion

Majority (48%) of the patients included in the present study were adults and most of the patients (60%) were found to be asymptomatic. Out of 100 patients, 80 (80%) were males and only 20 (20%) were females. Out of 100 samples 28 were HBeAg positive and 72 were HBeAg negative. Out of 100 patients, in 86% (86/100) the mode of transmission was unknown and majority of patients belonged to the asymptomatic group. The mode of transmission by HRSC, blood transfusion and perinatal route were 2%, 10% and 2% respectively (Table 1).

Of above mentioned 28 HBeAg positive samples 16 (57.15%) were having viral load >20000 IU/mL and 12 (42.85%) were having viral load <20000 IU/mL. Out of 72 HBeAg negative samples 20 (27.77%) were having viral load >20000 IU/mL and 52 (72.23%) were having viral load <20000 IU/mL. In 26% (26/100) of the patients, the target was not in detectable/ very low (below 29 IU/ML) range and these all were HBeAg negative (Table 2).

In present study we studied correlation of serum HBeAg and HBV DNA levels as both are used biomarkers to monitor the disease course and response to treatment in HBV positive patients.

In present study, 82% of patients were adults. Similarly various studies have reported that adults men or older were most commonly affected with HBV (Attaullah et al., 2011; Karki et al., 2008). The prevalence of HBV has been reported to be significantly elevated in males then females, similar findings have been reported from India (Chattopadhyay et al., 2006; Perumal et al., 2004; Attaullah et al., 2011). This could be due to higher prevalence of infection in adult males as they are more exposed to the risk factors.

Table 1. Age wise, sex wise and mode of transmission wise distribution of patients

Groups	Total patients	Asymptomatic	Symptomatic
Children	18	10	8
Adults	82	50	32
Male	80	52	28
Female	20	10	10
HRSC (high risk sexual contact)	2	0	2
BT (blood transfusion)	10	4	6
Unknown (incidentally detected)	86	38	28
Perinatal	2	2	0

Table 2. Distribution of viral load and correlation with HBV markers

Viral load (IU/mL)	Total	HBeAg +ve		HBeAg –ve	
		Total	%	Total	%
TND (target not detected)	6	-	-	6	100
<6	14	-	-	14	100
6-2.9*10^1	6	-	-	6	100
>2.9*10^1 - 1.1*10^8	62	20	30	42	70
>1.1*10^8	12	8	60	4	40

In present study we observed that the HBV DNA load was high in HBeAg positive cases, and good correlation was seen between the two, but in 26% of HBeAg negative cases the HBV DNA levels were > 20,000 IU/mL which is the level at which treatment is started, monitoring these patients only on basis of HBeAg can be misleading in such cases. Such findings are generally observed in patients with precore mutations (Ghabeshi et al., 2013). Moreover 46 (62.16%) HBeAg negative patients had HBV DNA level more than 29 IU/mL. Therefore only HBeAg cannot be used as marker for monitoring CHB patients. However as in these patients HBeAg status was done only once, which can be misleading, it is recommended to do HBeAg testing at least twice to predict response of CHB patients. Another limitation of the study was that sequencing was not done to know the presence of precore mutations.

Certain studies have even used HBsAg quantitation by chemiluminescence, Architect QT (Abbott Laboratories) and Elecsys HBsAg II quant (Roche Diagnostics) to monitor response to treatment and correlate level with HBV DNA though some authors have reported contradicting results also, whether HBsAg and HBeAg detection correlates with HBV DNA (Kuhns et al., 2004; Wiegand et al., 2008), but most studies have shown significant correlation between these values (Manesis et al., 2007; Moucari et al., 2009; Brunetto et al., 2009).

Several studies show that HBsAg and HBV DNA levels change during different phases of CHB (Nguyen et al., 2010; Jaroszewicz et al., 2010; Chan et al., 2011). Without treatment, the serum HBsAg level changes slower than the HBV DNA level (Chan et al., 2010; Nguyen et al., 2010). Recently HBsAg level has been used for differentiating between inactive carriers and patients with active disease. Most inactive carriers of HBV infection have a low HBsAg level (Chan et al., 2010).

There have been many limitations in our study, we had not done HBsAg quantitation by chemiluminescence which would have helped understand correlation of HBsAg HBeAg and HBV DNA levels, moreover sequencing of precore mutations would also help us understand the correlation better.

Conclusion

It's important to carry out HBV viral load testing in patients who are HBsAg positive on regular basis not only in HBeAg positive cases but in negative cases too. As in our study, we found that 26% HBeAg negative patients had viral load more than 20000 IU/mL which mandates initiation of treatment and only 26% patients had viral load below 6 IU/mL or TND in HBeAg cases.

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Conflicts of interest

The author declares no conflict of interest.

References

Attaullah S, Rehman SU, Khan S, Ali I, Ali S, Khan SN. 2011. Prevalence of Hepatitis B virus genotypes in HBsAg positive individuals of Afghanistan. Virology Journal 8:281.

Brunetto MR, Moriconi F, Bonino F, Lau GK, Farci P, Yurdaydin C, Piratvisuth T, Luo K, Wang Y, Hadziyannis S, Wolf E, McCloud P, Batrla R, Marcellin P. 2009. Hepatitis B virus surface antigen levels, a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. Hepatology 49:1141-1150.

Brunetto MR. 2010. A new role for an old marker, HBsAg. Journal of Hepatology 52:475-477.

Chan HL, Thompson A, Peignoux M, Piratvisuth T, Cornberg M, Brunetto MR, Tillmann HL, Kao JH, Jia

JD, Wedemeyer H, Locarnini S, Janssen HL, Marcellin P. 2011. Hepatitis B surface antigen quantification, why and how to use it in a core group report. Journal of Hepatology 55:1121-1131.

Chan HL, Wong VW, Wong GL, Tse CH, Chan HY, Sung JJ. 2010. A longitudinal study on the natural history of serum hepatitis B surface antigen changes in chronic hepatitis B. Hepatology 52:1232-1241.

Chan HL. 2012. Identifying hepatitis B carriers at low risk for hepatocellular carcinoma. Gastroenterology 142:1057-1060.

Chattopadhyay S, Das B, Kar P. 2006. Hepatitis virus B genotypes in chronic liver disease patients from New Delhi World. Journal of Gastroenterology 12:6702-6706.

Esmaeelzadeh A, Saadatnia H, Memar B, Amirmajdi EM, Goshayeshi L, Meshkat Z, Pasdar A, Vosoughinia H, Farzanehfar M, Tehranian S, Ghaffarzadehgan K, Rajabzadeh F, Ahadi M. 2017. Evaluation of serum HBV viral load, transaminases and histological features in chronic HBeAg-negative hepatitis B patients. Gastroenterology and Hepatology Bed Bench 10(1):39–43.

Ghabeshi S, Sharif Z, Hosseini SM, Shooshtari MM. 2013. Correlation between viral load of HBV in Chronic Hepatitis B Patients and Precore and Basal Core Promoter Mutations. Hepatitis Monthly 13(2):e7415.

Jaroszewicz J, Serrano B, Wursthorn K, Deterding K, Schlue J, Raupach R, Flisiak R, Bock CT, Manns MP, Wedemeyer H, Cornberg M. 2010. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection, a European perspective. Journal of Hepatology 52:514-522.

Karki S, Ghimire P, Tiwari BR, Rajkarnikar M. 2008. HBsAg sero surveillance among Nepalese blood donor. Annals of Tropical Medicine and Public Health 1:15-18.

Kuhns MC, Kleinman SH, McNamara AL, Rawal B, Glynn S, Busch MP. 2004. Lack of correlation between HBsAg and HBV DNA levels in blood donors who test positive for HBsAg and anti-HBc, implications for future HBV screening policy. Transfusion 44:1332-1339.

Manesis EK, Hadziyannis ES, Angelopoulou OP, Hadziyannis SJ. 2007. Prediction of treatment-related HBsAg loss in HBeAG-negative chronic hepatitis B, a clue from serum HBsAg levels. Antiviral Therapy 12:73-82.

Maria B, Viktor A, Sergey F, Yakovlev A. 2012. HBsAg level and hepatitis B viral load correlation with focus on pregnancy. Annals of Gastroenterology 28:379-384.

Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Peignoux MM, Dauvergne A, Asselah T, Boyer N, Bedossa P, Valla D, Vidaud M, Nicolas-Chanoine MH, Marcellin P. 2009. Early serum HBsAg drop, a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology 49:1151-1157.

Nguyen T, Thompson AJ, Bowden S, Croagh C, Bell S, Desmond PV, Levy M, Locarnini SA. 2010. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B, a perspective on Asia. Journal of Hepatology 52:508-513.

Parizad EG, Parizad EG, Khosravi A, Amraei M, Valizadeh A, Davoudian A. 2016. Comparing HBV Viral Load in Serum, Cerumen, and Saliva and Correlation with HBeAg Serum Status in Patients with Chronic Hepatitis B Infection. Hepatitis Monthly 16 (5):e30385.

Perumal V, Abraham P, Shridhran G, Chandy G, Daniel D, Raghuraman S, Daniel HD, Subramaniam T. 2004. Distribution of Hepatitis B Virus Genotypes in blood donor and Chronically Infected Patients. Clinical infectious disease 38:81-6.

Seeger C, Zoulim F, Mason WS. 2007. Hepadnaviruses. In: Howley P. Knipe D. editors. Fields Virology, Lippincott 2977–3029.

Taghavi SA, Tabibi M, Eshraghian A. 2010. Prevalence and clinical signifcance of hepatitis B Basal core promoter and precore gene mutations in southern Iranian patients. Hepatitis Monthly 10 (4):294-7.

Wiegand J, Wedemeyer H, Finger A, Heidrich B, Rosenau J, Michel G, Bock CT, Manns MP, Tillmann HL. 2008. A decline in hepatitis B virus surface antigen (hbsag) predicts clearance, but does not correlate with quantitative HbeAg or HBV DNA levels. Antiviral Therapy 13:547-554.