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Original Research Article

Correlation of HBV-DNA Viral Load and Serological Markers (HbsAg and HBeAg) in Chronic Hepatitis B Carriers

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Abstract:
Hepatitis B infection is the most widespread and important type of viral hepatitis. It increases the risk of developing liver failure, cirrhosis and hepatocellular carcinoma. Every year over 115000 Indians die of hepatitis B related complications. This study was undertaken to access serological markers HbsAg and HBeAg in chronic carriers of Hepatitis B infection and compare with viral load as determined by polymerase chain reaction. This study included 113 patients who attended OPD and IPD of Jhalawar Medical College and associated group of hospitals in the period of March 2023 to January 2024 and tested positive for HBsAg. 5ml blood was collected from patients after detailed history and written consent. After the confirmation of carrier status, HbeAg was obtained by ELISA. Real time polymerase chain reaction technique was utilized for detection of HBV DNA. Majority of patients (31.8%) belonged to age group 31-40 yrs. Family history was the most common risk factor. 10.6% patients had HBV DNA below detectable level and 31% were super carriers (> 20,000 IU/ml). 34.5% patients were HBeAg positive. In HBV DNA positive cases, the value ranged in between 51 IU/ml and 3.55 IU/ml x 10^7.

Keywords: HbsAg, HBeAg, HBV DNA, Chronic carriers.

Introduction

Hepatitis B is the most widespread and the most important type of viral hepatitis. Over 2 billion people worldwide show some serological evidence of past or current hepatitis B virus (HBV) infection, of which 350 million are chronic carriers. [1,2,3] In our country, India, the carrier rate is 4-7% and there are 45 million people infected with this virus. [4]

Investigations for HBV infection include serological assays for hepatitis B surface antigen (HBsAg), hepatitis B envelop antigen (HBeAg), antibodies to the hepatitis B core antigen. [2,5,6,7] HBeAg, previously regarded as the best biomarker of HBV infectivity, is negative in large population of HBV-infected individuals with detectable levels of HBV DNA. [8,9] Molecular biology and PCR revolutionized the study of this virus and now its role in this field is of paramount importance. It is used for diagnosis, in management, for therapeutic decision and also in identification of drug resistant strains.

Usually the general population has 2%-4% prevalence of hepatitis infection but high risk populations like professional blood donors, patients on hemodialysis and health care workers have a higher prevalence. [10] Route of transmission for virus can be horizontal and vertical. Horizontal transmission includes blood transfusion, unsterile injections, surgical equipments, needle prick as well as sexual transmission. Vertical transmission includes mother to fetal transmission in utero. unsafe injection practices are rampant in developing countries and are responsible for 20 million new Hepatitis B infections. [11]

The natural course of chronic hepatitis B virus infection has four phases in natural history which are the result of dynamic interplay between the HBV and its host. The important host factors include gender, alcohol consumption, infection with other viruses and the immune status of the indvidual.

Phase 1: Immune-tolerant phase

Phase 2: Immune-clearance phase

Phase 3: Chronic active phase

Phase 4: Chronic inactive phase

These patients if immunosuppressed can have a

reactivation of HBV replication.

Material and Methods:

A cross sectional study of 113 patients attending the OPD and IPD of Jhalawar Medical College and associated group of hospitals in the period of March 2023 to January 2024 was undertaken. Both male as well as female patients with HbsAg positive status were included in the study. Exclusion criteria were patients with co-existent HIV, HAV or HCV, patients with decompensated hepatic status, cirrhosis, hepatocellular carcinoma, history of recent drug hepatitis, auto immune liver disease, severe malnutrition and who did not give consent.

5 ml of blood was withdrawn after written informed consent with full aseptic precautions. 2ml blood was put in purple capped EDTA vial and 3 ml in red capped plane tube. After centrifugation, serum from red capped vacutainer was tested for HbsAg and IgG antibody. After the confirmation of carrier status, HbeAg was obtained by ELISA. Real time polymerase chain reaction technique was utilized for detection of HBV DNA. Results were expressed in IU/ml. Ethical clearance was obtained from institutional ethical committee.

Results

Study population included 113 chronic carriers of HBV who tested positive for HbsAg.

Table 1 : Distribution of age and gender of patients

Age		Gender		Total
		Female	Male	
	11-20 Years	0	3	3
	21-30 Years	8	16	24
	31-40 Years	11	25	36
	41-50 Years	7	14	21
	51-60 Years	8	9	17
	61-70 Years	3	6	9
	>70 Years	0	3	3
Total		37	76	113
		(32.7%)	(67.3%)	(100.0%)

Age wise distribution shows that majority of patients belong to age group of 31-40 years (31.8%) followed by 21-30 year age group. Range of age is 16 to 76 years. Mean age is 42.42 yrs. Males were 67.3% and females were 32.7%. Most frequent risk factor identified was family history followed by frequent injections, invasive procedures and life style.

Table 2: Distribution of HBeAg of patients

HBeAg	Frequency	Percent
Positive	39	34.5
Negative	74	65.5
Total	113	100.0

HBeAg positive was found in 39(34.5%) patients. Most of the HbeAg positive patients belong to less than 40 year age group. However p value was not significant. This could be because of a small sample size. No significant association was found between HBeAg positive and gender.

Table 3: Distribution of DNA viral load of patients

DNA viral load	Frequency	%
Upto 5000	21	18.6
5001-10000	16	14.2
10001-15000	20	17.7
15001-20000	9	8.0
> 20000	35	31.0
ND	12	10.6
Total	113	100.0

ND: Not detectable

10.6% people had viral DNA below detectable level. 18.6% had less than 5000IU/ml and 14.2% had 5001 - 10000IU/ml. 25.7% were 10001- 20000 IU/ml. 31.0% were >20000IU/ml. No significant association was found between HBeAg and viral load.

Table 4 : Distribution of DNA viral load according to HBeAg of patients

DNA viral load		HBeAg		Total
		Positive	Negative	
	Upto 5000	10	11	21
	5001-10000	3	13	16
	10001-15000	7	13	20
	15001-20000	4	5	9
	> 20000	9	26	35
	ND	6	6	12
Total		39	74	113

Chi sq = 6.222 p value = 0.285

Discussion

In our present study 113 chronic careers were enrolled. This study includes asymptomatic subjects who were detected during routine screening of blood donors, family contacts of HBV positive patients and during preoperative evolution of patients. The duration of study was from March 2023 to January 2024. Quantitative estimation of HBV DNA was done by PCR assay and HbsAg, IgG antibody and HBeAg obtained from laboratory. The results obtained were subjected to appropriate statistical analysis.

Profile of Study Group

The age of the patient in the study was range from 16 to 76 years with the mean age of 42.42 years. Most (46.7%) people belong to 31 to 40 years age group. In present study males were 76 (67.3%) and females were 37 (32.7%). There is a preponderance of males among HBV infected persons with detectable viral load has seen in our study. Although the reason is not clear, similar finding has also been documented in studies of Amidu N et al [12] and Onwuliri EA et al. [13] Okwuraine et al [14] had similar findings in Lagos and suggested it could be due to increased financial resource available to males to go to test as compared to females. In contrast, Onwuliri EA et al [13] and okonko et al [15] found more females with HBV infection among HIV patients and blood donors respectively. A well designed study may be needed to determine whether women abort the infection better than men.

Most of the HBeAg positive patients fall in the age group of 31-40 yrs. Similar findings were reported by Iregbu KC, Nwajiobi-Princewell PI [16], Okwuraiwe et al [14] and Uddin PK et al [17]. Dixit et al [18] also found that HBeAg positive patients tend to be younger than HBeAg negative patients. However, a study by shakeri et al [19] found the lowest prevalence of HBeAg in 35-40 years age group.20 This may be related to the higher incidence of activities associated with HBV acquisition or reactivation of existing infection in this age group.

Serological Profile

The present study consists of chronic careers of hepatitis B virus . The serological marker HBeAg was obtained in all the patients. In our study 34.5% patients were found to be HBeAg positive and 65.5% were HBeAg negative. Our findings are consistent with those observed in the study by shammugham et al [20]. Their study reported replicative carriers to be 23.4%.

Quantitative detection of HBV DNA was done in plasma of all patients by polymerase chain reaction assay. In the present study, the analysis of HBV DNA load showed that most cases (89.4%) were positive for viral DNA in their plasma but it was not detected in remaining 10.6% cases. The study by Behnava et al [21] also showed increase number which is in accordance with our results. But in the study conducted by Hasan N K et al [22] and Rabbi et al[23] the results showed HBV DNA positivity in 44.8% and 40.2% cases respectively. Our findings are also not in accordance with Iregbu KC, Nwajiobi-Princewell [16]. In HBV DNA positive cases, the value ranged in between 51 IU/ml and 3.55 IU/ml x 10^7.

Serology and Virological Profile: The level of HBV DNA was compared with serological profile

HBeAg positive and HBeAg negative in our study. The result showed that 84.6% of HBeAg positive cases were also positive for HBV DNA and most of them had high viremic levels >20000 IU/ml. This is almost in accordance with the studies by Widita H et al. [24] Viral DNA level was negative in 8.1% of the patients with negative HBeAg. The result of various studies by Rabbi et al [23], Hasan NK et al [22] and shammugham et al [20] showed that HBeAg negative group had DNA positive status in 31.5%, 7.6% and 7% respectively. There is no association between HBeAg and viral DNA load in our study. X liu et al [25] in their study also did not find association between HBeAg and HBV. Akther S et al [26] observed a positive correlation among HBeAg and HBV DNA in chronic carriers. Thus, HBeAg may be useful in diagnosis and treatment of hepatitis B infection but it cannot be replaced by HBV DNA specially when we considered HBeAg negative CHB patients. It can be used as a complementary test. HBV DNA quantitation by PCR is a reliable, accurate and reproducible test which can be used to diagnose, understand the natural history and progression or regression of the disease and also actively guide and monitor the therapy.

Conclusion

India has a large pool of Hepatitis B patients and asymptomatic carriers are the one of the main reservoir responsible for the transmission of infection in the community. The aim of this study was to detect HBeAg in HbsAg positive patients and correlate the information with their viral load. The results of present study lead to following conclusions.

- The majority of patients were middle aged males. Males were 76 (67.3%) and females were 37 (32.7%).
- In our study 34.5% patients were found to be HBeAg positive and 65.5% were HBeAg negative. Most of the HBeAg positive patients fall in the age group of 31-40 yrs.
- In HBV DNA positive cases, the value ranged in between 51 IU/ml and 3.55 IU/ml x 10^7.
- There is no association between HBeAg and viral DNA load in our study.
- There is no significant association between viral load and age and gender of patients (p >0.05). It means there is no effect of age and gender in viral load.

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