PREVALENCE OF HEPATITIS B VIRUS GENOTYPES AMONG PATIENTS WITH LIVER DISEASE IN A TERTIARY CARE HOSPITAL MATHURA UTTAR PRADESH

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Abstract
Hepatitis B Virus genome evolves at an error rate of ~ 10 –3 to 10 –6 nucleotide substitutions/site/year, which is approximately 100 times higher than that of other DNA viruses. Accumulation of these mutations over a long period of time results in a large amount of genetic diversity. It is important to note that the rates of disease progression, clinical and treatment outcomes may different for each HBV genotype. Our aim was to detect the most prevalent genotypes of Hepatitis B virus among patients with liver disease in KD Medical College Mathura. A total of 280 patients with liver disease who were also positive for HBsAg by Elisa were enrolled. All sera were tested for liver function test, HBe Ag and HBV DNA viral load. HBV genotyping was done by RT-PCR. The median of HBV DNA viral load and ALT levels were 4.56 log IU/mL and 106.95 IU/L, respectively. HBV genotype D (38.18%) was the predominant circulating genotype, followed by genotypes A (21.81%), B (3.6%), C (0.9%), and Genotypes A/D (7.27%), D/C (4.54%), B/D (5.45%) and A/B (2.7%) were present. This study illustrated distribution of HBV genotype A, B, C, D. HBV in Mathura is comprised of a mixture of HBV genotypes. Genotyping can help practicing physicians identify those at risk of disease progression and determine optimal anti viral therapy. The study contributes in narrowing the existing gap of HBV molecular study in Uttar Pradesh India.

Keywords: HBeAg, HBsAg, ELISA, Genotyping, PCR.

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INTRODUCTION

Hepatitis B Virus affected 400 million people worldwide chronically as a result of developing liver cirrhosis and hepatocellular carcinoma [1]. Several studies showed that HBV genotypes are found to be asscociated with natural history, pathogenesis and evolution of disease outcomes and influence the efficacy of interferon-based antiviral treatment [2]. Due to its diversity in the entire HBV genomic sequence, 10 HBV genotypes (A to J) and several subtypes have been identified. Out of these I and J are newly identified genotypes. The geographic and ethnic distributions of HBV genotypes and subtypes are well characterized [3,6,7].Genotype A is highly prevalent in sub-Saharan Africa, Northern Europe, and Western Africa. Genotypes B and C are common in Asia. Genotype C mainly exists in East and Southeast Asia. Genotype D is prevalent in Africa, Europe, the Mediterranean region and India. Genotype E is restricted to be found in West Africa. Genotype F is found in Central and South America. Genotype G has been detected in France, Germany, and the United States. Genotype H is found in Central America.[4,5].Recently, genotype I, a novel inter genotypic recombination among genotypes A, C, and G, was isolated in Vietnam and Laos.[8]. The newest HBV genotype, J, was identified in the Ryukyu Islands in Japan. This genotype has a close relationship with gibbon/orangutan genotypes and human genotype C. [8] Geographic distribution of HBV genotype may correlate with the modes of transmission. Genotypes B and C are prevalent in highly endemic areas where perinatal or vertical transmission of Hepatitis B is seen whereas the remaining genotypes are frequently found in areas where horizontal transmission is the main mode of transmission. Therefore, HBV genotyping may serve as an epidemiology for the investigation of transmission, as well as geographic evolution of HBV.[9,10]

A Study from Southeast Asia have shown that Genotype C is associated with severity of liver diseases [8]. Genotype B is found to be associated with the development of HCC at younger age in Taiwanese patients [11]. Public health importance of HBV genotypes and sub-genotypes has distinct geographical distribution. Nowadays an important confounding factor is immigration and global HBV distribution substantially changing the geographic pattern of HBV genotypes.Inter-genotype recombination can further contribute to the virus's evolutionary history. It is also noted that the circulation of novel recombinants and variants justifies the need to improve immune prophylaxis, diagnosis and treatment strategies. HBV genotyping is an important tool that has been used to investigate the cause of outbreaks of hepatitis B and modes of transmission [12, 13,14].In view of presence of inadequate data on prevalence of HBV Genotypes in western Uttar Pradesh India we have done this study to narrowing this gap of inadequacy as this is the first study on prevalence of Genotypes of HBV in Mathura.

METHODS

This study was done in KD Medical college hospital and research center Mathura from April 2021 to December 2022. During this period all seropositive individuals for hepatitis B Virus were taken. Consents and history were taken at the time of collection of samples. All samples were stored at -70 ˚ C for further investigations.

Study Design

In this cross-sectional study, positive samples for HBs Ag antigen by ELISA were investigated for HBV DNA Viral load and HBV genotyping.

Inclusion Criteria-

The major inclusion criteria were seropositivity for HBsAg for over six months, seropositivity for HBe Ag with altered levels of AST and ALT and other liver function test.

Exclusion criteria

Patients who were positive for HCV and patients with other causes of liver diseases.

Sample Collection and storage-

5-10 ml of venous blood was collected from patients. Serum was separated and stored at -70˚C for further analysis and genotyping.

Serological investigation

HBs Ag and HBe Ag were carried out while using ECLIA Roche diagnostics on the COBAS e 411 immunoassay analyzer,

Biochemical Analysis-

Liver function tests- Total and Direct Bilirubin, Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Total Protein were analyzed in serum samples while using routine biochemistry analyzer COBAS Machine.

HBV Genotyping and DNA Extraction-
The quality and quantity of extracted DNA was determined after DNA extraction. Genotyping was carried out according to the instruction while using a commercially available kit professional biotech based on hybridization with type-specific probes immobilized. This kit adopts polymerase chain reaction (PCR) combined with Taqman fluorescence probe technology to genotype of HBV in the samples which can detect genotype A,B,C,D and mixed genotype. Two fluroscence probe FAM and HEX probes were used. The use of dUTP and UNG enzyme in the kit can effectively eliminate possible carry over template contamination**.**

Statistical Analysis
Statistical analyses were performed using SPSS Statistics software. Baseline variables, summary statistics employed frequencies data, mean, and standard deviation (SD) for continuous variables. Continuous variables were compared using unpaired t-tests and ANOVA. Descriptive Statistics were calculated to determine the prevalence of genotype with respect to data of demographic factors, serology, and other investigations.

RESULTS
Total of 280 HBs Ag seropositive patients were taken in our study. Out of which 154 were male and 126 were female with prevalence rate 55% and 45% respectively **Table1.**The highest prevalence of HBV infection was shown for the age group 30-40 followed by 40-50.**Table 2** Mode of transmission was also defined in which most of the patients have unknown with highest frequency rate 207(73.92%), followed by Intra venus drug users (IVDUs) 50(17.85%) **Table 3.** 140 (50%) patients positive for both HBs Ag and HBe Ag could be tested for viral load out of which 110 patients serum containing high viral load could be genotyped. **Table 4.** A, B, C, D and mixed genotype infections A/D, B/D, C/D. **Table 5, Figure 5.** It Illustrates the frequency of HBV genotypes A, A/D, B, B/D, C, D, and D/C, The prevalence of genotype A was found to be 21.81%, genotype A/B was 2.7%, genotype B was 3.6%, genotype B/D was 5.45%, genotype C was 0.9%, genotype D/C was 4.54 %. Genotype E and F were not detected, and undetermined genotypes was 15.45%. Among the referred genotypes, genotype D showed the highest occurrence (38.18%), which indicated that genotype D is the most prevalent HBV genotype in our study population. There was no significance difference between genotype and nationality, gender and age-group ($p > 0.05$).

Table 1. Sex wise distribution with the prevalence of hepatitis B virus infections examined patients (N = 280).

Sex	Frequency	Prevalence
Male	154	55%
Female	126	45%
Total	280	

Table 2- Age wise Distribution of HBV Seropositive patients

Age	Frequency	Prevalence
0-10	0	0
11-20	02	0.71%
21-30	60	21.42%
31-40	78	27.85%
41-50	52	18.57%
51-60	36	12.85%
61-70	29	10.35%
71-80	16	5.71%
81-90	07	2.5%

3– Frequency of mode of transmission Hepatitis B Virus

Mode of Transmission	Frequency	Percentage
Intra venus Drug Users (IVDUs)	50	(17.85%)
Sexual contact	15	(5.35%),
Vertical transmission	08	(2.8%)
Unknown	207	(73.92%),

Table 4- Demographic and Biochemical information of HBeAg positive patients n=280

Characteristic	Value
Sex	
Male	154
Female	126
Age years	35.01±15.32
HBe Ag	
Positive	140
Negative	208
Liver function Test	
ALT	106.95±65.09
AST	62.42±38.02
Serum Bilirubin	3.50±3.72
Serum Albumin	3.32±0.68
HBV DNA Level	
Negative	170
Positive	110
(Log10IU/ml	4.56±1.85

Table 5- Distribution of HBV Genotypes in the Study Population

GENOTYPE	TOTAL	PREVALENCE
A	24	21.81%
B	04	3.6%
C	01	0.9%
D	42	38.18%
B/D	06	5.45%
A/D	08	7.27%
D/C	05	4.54%
A/B	03	2.7%
UNDETERMINED	17	15.45%

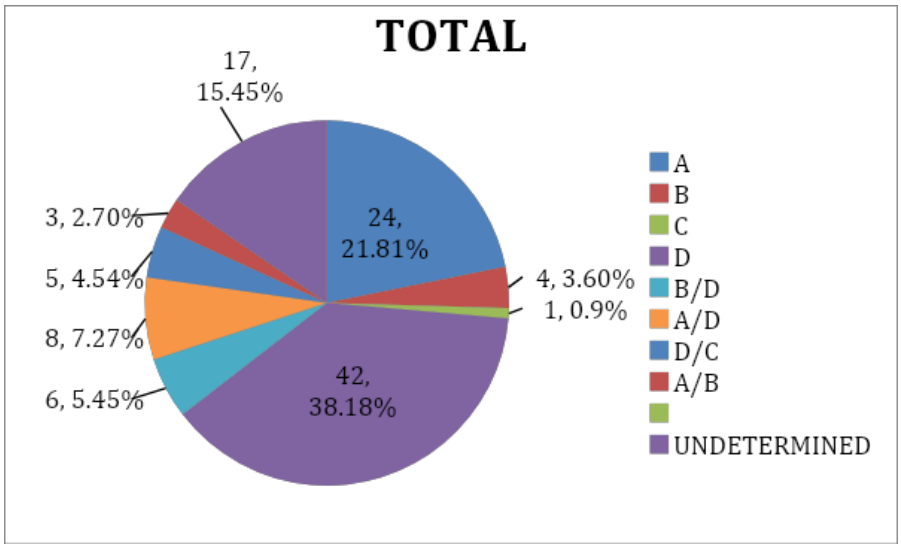

Figure 5. Prevalence of HBV genotypes among 110 patients under study

DISCUSSION

HBV infection is a serious and life threatening global problem that places a continuously increasing burden on developing countries. This study reports for the first time in Mathura, the correlation of HBV genotypes frequency with the demographic characteristics and hepatic biomarker. The results showed that there were no significant differences of genotype frequency in relation to the demographic characteristics as well as hepatic biomarkers. There was a significant increased risk of HBV infection in male as compared to females.49.7% of HBV positive patients had Relationship between genotype and age-group indicates that HBV prevails 27.85% in <31 years.

The frequency of mode of transmission was highly unknown (73.92%), followed by Intravenous Drug Users (IVDUs) (17.85%), sexual contact (5.35%), vertical transmission (2.8%) on the basis of patients history which has been taken at the time of collection of samples. Unfortunately any other mode of transmissions history such as dental and surgical procedures went unrecognized. In another study dental procedures and surgical operations account for 37.2% and 35.6%, respectively, of the HBV transmission routes followed by the blood transfusion [24.6%], the sexual contact and intervenors drug abuse were found least possible routes of transmission[13].India has an estimated 1.1 million injection drug users and prevalence of HBs Ag positivity was seen 2.7-10.8%.[14]In this study we found some significant difference in the HBV genotype prevalence with respect to some investigated variables such as frequency of HBV genotype is more related to males and the risk of HBV infection increased with age which is analogous.[15,16,17]

In present study Genotype D was found as most prevalent genotype followed by genotype A among patients. According to several studies in India and other countries Genotype D has been found most prevalent and significant also.[18,19] Genotype D is widely spread across Eastern Europe, Mediterranean region, including North Africa, Russia, Middle East, Indian subcontinent and across the arctic .Genotype E is found in West Africa. Although HBV genotype G has been found only in small areas of the world, in the United States, Vietnam and Southern Europe appears

primarily to be present as a co infection with another genotype, most commonly Genotype A. On other hand Genotype F and H are the New World genotypes found in indigenous populations in Alaska and Central and South America [20,21]. Most severe HBV infections in India are due to genotype D.[22,23]In India a study from Arunachal Pradesh was conducted by B.J Borkakotymet al. They found that the predominant genotype was genotype A (41.6%) Followed by Genotype C (27.8%) and D (11.1%).The higher prevalence of this genotype might be attributed to different factors, like the presence of high number of patients belongs to Western Uttar Pradesh that are known to have dominant D genotype, such as Aligarh ,Meerut.(23,24,25) These infected individuals as most of them belong to highly endemic regions with low educational and socio-economical backgrounds; they positively contribute to the transmission of the disease. Living in small houses and having unhygienic behaviors such as sharing same razors and toothbrushes put such workers at high risk of contracting HBV. As it has been already reported in some studies that a poor hygiene system in hospitals of such countries is known as a high-risk factor for HBV transmission, as the same syringe is used for vaccination of different people. [15,16]

Being quasi-species in nature, HBV infection indicates that variation and evolution of Hepatitis B virus has been influenced by the recombination between genotypes. Hence, a high prevalence of more than one dominant genotype in a certain region is not uncommon [21,26]. It is also reported that mixed infection with different HBV genotypes are common and increases a great virological and clinical interest. For example, a study done by Chen et al. (2004) showed that the prevalence of mixed HBV genotype infection was 16.3% for HBsAg positive and 34.4% in occult HBV-infected intravenous drug users.[26]

HBV is a non-cytopathic virus. A complex and important interaction between the virus and host causing HBV-related liver disease. Bilirubin, Direct Bilirubin, ALT, and AST are the most common liver enzymes found abnormal due to HBV infection [27]. Elevated ALT levels, elevated AST level, elevated serum bilirubin, and decreased serum albumin shows an indication of advanced liver diseases and even cirrhosis [28,29]. Present study does not show any type of significant association between HBV prevalence and liver function test. Each genotype showed variation depending on the mean and standard deviation of the liver function test associated with that genotype. This might be an indication of acute hepatitis. Genotype A and mixed genotypes B/D showed higher than the normal maximum level for ALT, AST; mixed genotypes A/D showed higher than the normal maximum level for Bilirubin, ALT, AST; genotype B showed higher than the normal maximum level for ALT and; genotype D showed higher than the normal maximum level for ALT, Genotype E and F were undetermined. In a study it has been reported that elevated liver enzymes levels in an asymptomatic hepatitis B patient is associated with high infectivity [28]. Recently, several studies have been done on the influence of HBV genotypes on the clinical features [30,31,32] and on the response to antiviral treatment (interferon and lamivudine) of patients infected with HBV. [33,34,35,36]

This study, like many studies, has some limitations like only 110 individuals could be genotyped, which is a relatively small sample and it may not represent an accurate picture of HBV prevalence at the population level. On other side, cases from other government and private health facilities were not included in this study which may contribute to lower prevalence. Some of the obtained data from patients were based on patient self-reporting of risk factors, which is subject to social desirability bias.

5. CONCLUSIONS

This study highlighted the importance of hepatic biomarker with genotypes, which can be used as a base for further studies to investigate such an association. . In order to further investigate we need a suitable approach and guidance. The advantage of this study was to provide a baseline study to draw a good estimate of HBV genotype distribution in Mathura

Ethical Approval

Ethical and research committees of KD Medical College Hospital and research center Mathura Ref id KDMCHRC/IEC/2021 All of the patients signed the informed consent form before participation.

Author Contributions

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Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES-

1. D. Lavanchy, "Hepatitis B virus epidemiology, disease burden, treatment, arid current and emerging prevention and control measures," Journal of Viral Hepatitis, vol. 11, no. 2, pp. 97–107, 2004.
2. Ganem D, Prince AM. Hepatitis B virus infection - natural history and clinical consequences. N Engl J Med. 2004; 350:1118–29.
3. McMahon BJ. The influence of hepatitis B virus genotype and sub genotype on the natural history of chronic hepatitis B. Hepatol Int 2009; 3(2):334–342
4. Kurbanov F, Tanaka Y, Mizokami M. Geographical and genetic diversity of the human hepatitis B virus. Hepatol Res 2010; 40 (1):14–30
5. Kao JH, Chen DS. HBV Genotypes: epidemiology and implications regarding natural history. Curr Hepatitis Rep 2006;5: 5–13
6. Phung TB, Alestig E, Nguyen TL, Hannoun C, Lindh M. Genotype X/C recombinant (putative genotype I) of hepatitis B virus is rare in Hanoi, Vietnam—genotypes B4 and C1 predominate. J Med Virol 2010;82(8):1327–1333
7. Tatematsu K, Tanaka Y, Kurbanov F, et al. A genetic variant of hepatitis B virus divergent from known human and a genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. J Virol 2009;83(20):10538–10547
8. Olinger CM, Jutavijittum P, Hübschen JM, et al. Possible new hepatitis B virus genotype, southeast Asia. Emerg Infect Dis 2008; 14(11):1777–1780
9. Lin CL, Kao JH, Chen BF, Chen PJ, Lai MY, Chen DS. Application of hepatitis B virus genotyping and phylogenetic analysis in intrafamilial transmission of hepatitis B virus. Clin Infect Dis 2005;41 (11):1576–1581.
10. Kao JH, Chen DS. HBV Genotypes: epidemiology and implications regarding natural history. Curr Hepatitis Rep 2006; 5: 5–13
11. Kurbanov F, Tanaka Y, Mizokami M. Geographical and genetic diversity of the human hepatitis B virus. Hepatol Res 2010; 40 (1):14–30
12. Cao GW. Clinical relevance and public health significance of hepatitis B virus genomic variations. World J Gastroenterol 2009;15(46): 5761–5769.
13. Tanwar S, Dusheiko G. Is there any value to hepatitis B virus genotype analysis? Curr Gastroenterol Rep 2012;14(1):37–46
14. Sandesh K, Varghese T, Harikumar R.et al. Prevalence of Hepatitis B and C in the normal population and high risk groups in north Kerala. Trop Gastroentrol.2006;27:80-83.
15. Kurien T, Thyagarajan SP,Jeyaseelan L,Peedicayil A,Rajendran P,Sivaram S,et al;STD study group. community prevalence of Hepatitis B infection and mode of transmission in Tamilnadu, India.Indian J Med Res 2005;121(5):670-5.
16. Aceijas C, Friedman SR, Cooper HL, Wiessing L, Stimson GV, Hickman M. Estimates of Injection drug users at a national and local level in developing and translational countries and gender and age distribution. Sex Transm infect.2006;82(suppl.3):10-17.
17. Vivekanandan P,Abraham P, Sridharan G, et al. Distribution of Hepatitis B virus genotypes in blood donors and chronically infected patients in a tertiary care hospital in southern India. Clin infect Dis. 2004;38(9):e81-e86.
18. Kumar A, Kumar SI, Pandey R, Naik S, Agrawal R. Hepatitis B virus genotype A is more often associated with severe liver disease in northern India than is genotype D. Indian J Gastroentrol.2005;24(1):19-22.
19. Thakur V, Guptan RC, Kazim SN, Malhotra V, Sarin SK. Profile, spectrum and significance of HBV genotypes in chronic liver disease patients in the Indian subcontinent. J Gastroenterol hepatol.2002; 17(2):165-170.
20. Toan NL, le Song H, Kremsner PG, Duy DN, Binh VQ, et al. Impact of the hepatitis B virus genotype and genotype mixtures on the course of liver disease in Vietnam. Hepatology. 2006; 43:1375–84.
21. Chu CJ, Keefe E, Han SH, Perrillo RP, Min AD, Soldeveila-Pico C et al. Hepatitis B virus Genotypes in the United States: results of a nationwide study. Gastroenterology2003; 125:444-451.
22. Kar P, Polipalli SK, Chattopadhyay S, Hussain Z, Malik A, Hussain SA, et al . Prevalence of Hepatitis B Virus genotype D in pre -core mutants among chronic liver disease patients from New Delhi, India. Dig Dis Sci 2007;52(2):565-9.

23. Thakur V, Guptan RC,Kazim SN, et al. Profile, spectrum and significance of HBV of HBV genotypes in chronic liver disease patients in the Indian subcontinent. J Gastroenterol Hepatol. 2002;17:165-70.
24. Kumar A. Dwivedi M,Mishra SP, et al Distribution of Hepatitis B virus genotypes and it's association with severity of liver disease in patients with chronic hepatitis B in Uttar Pradesh, India. Indian J Virol.2011;22:24-8.
25. Madan K, Batra Y, Sreenivas V, et al. HBV genotypes in India : do they influence disease severity? Hepatol Res. 2009;39:157-63.
26. Yin J, Zhang H, Li C, Gao C, He Y, et al. Role of hepatitis B virus genotype mixture, subgenotypes C2 and B2 on hepatocellular carcinoma: compared with chronic hepatitis B and asymptomatic carrier state in the same area. Carcinogenesis. 2008; 29:1685–91.
27. Hui, C.; Lau, E.; Monto, A.; Kim, M.; Luk, J.; Poon, R.; Wright, T.L. Natural History of Patients with Recurrent Chronic Hepatitis C Virus and Occult Hepatitis B Co-Infection after Liver Transplantation. Am. J. Transplant. 2006, 6, 1600–1608.
28. Limdi, J.K.; Hyde, G.M. Evaluation of abnormal liver function tests. Postgrad. Med. J. 2003, 79, 307–312.
29. Giboney, P.T. Mildly elevated liver transaminase levels in the asymptomatic patient. Am. Fam. Phys. 2005, 71, 1105–1110
30. H. Sumi, O. Yokosuka, N. Seki, et al., "Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease," Hepatology, vol. 37, no. 1, pp. 19–26, 2003.
31. .H. L.-Y. Chan, S. W.-C. Tsang, C.-T. Liew, et al., "Viral genotype and hepatitis B virus DNA levels are correlated with histological liver damage in HBeAg-negative chronic hepatitis B virus infection," American Journal of Gastroenterology, vol. 97, no. 2, pp. 406–412, 2002.
32. M.-F. Yuen, E. Sablon, H. -J. Yuan, et al., "Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications, and hepatocellular carcinoma," Hepatology, vol. 37, no. 3, pp. 562–567, 2003.
33. C.-J. Liu, J.-H. Kao and D.-S. Chen,"Therapeutic implications of hepatitis B virus genotypes," Liver International, vol. 25, no. 6, pp. 1097–1107, 2005.
34. K. Jia-Horng, W. Nan-Hu, C. Pei-Jer, L. Ming-Yang, and C. Ding-Shinn, "Hepatitis B genotypes and the response to interferon therapy," Hepatology, vol. 33, pp. 998–1002, 2000. 6 Hepatitis Research and Treatment
35. Erhardt A, Blondin D, Hauck K, Sagir A, Kohnle T, et al. Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. Gut. 2005; 54:1009–13.
36. C. A. Valdes, M. Buti, R. Jardi, et al., "The role of HBV genotype in the emergence of YMDD variants in chronic hepatitis B patients treated with lamivudine," Hepatology, vol. 38, supplement 2, p. 178, abstract no. 613