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Predominant Hepatitis B virus genotype in association with clinical complictions observed among Bangladeshi chronic carriers

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ABSTRACT

Background: *Hepatitis B virus (HBV) infection is one of the major causes of chronic liver diseases, including liver cirrhosis (LC) and hepatocellular carcinoma (HCC) and affects over 240 million people worldwide. HBV is frequently variable and categorized into ten genotypes (A-J) based on nucleotide divergence of more than 8% of the complete genome which is important for clinical outcomes and disease progressions.* ***Objectives:*** *To explore the prevalence of Hepatitis B virus genotype among Bangladeshi chronic carrier and their association with the clinical complications.* ***Methods:*** *A small cohort was performed between March 2014 and October 2016 with 172 HBV DNA positive patients from the Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. From them, 29 HBV DNA samples were isolated for sequencing by Sanger method. HBV genotype was determined by phylogenetic analysis of the 750 nucleotide fragment from the Polymerase gene.* ***Results:*** *The results of our study showed genotype C in 17 (58.6%) HBV isolates, genotype D in 10 (34.5%) and genotype A in 2 (6.9%).* ***Conclusion:*** *We conclude that genotype C is predominant in Bangladesh. This is followed by genotype D, and genotype A is the least dominant. Genotype C and A strains are found to be related with more complication. Therefore, patients infected with these HBV strains need to be monitored carefully to assess their clinical outcomes in future.*

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INTRODUCTION

Hepatitis B virus (HBV) is a DNA virus of the Hepadnaviridae family that infects hepatocytes and causes infectious hepatitis. HBV show a variety of clinical pictures ranging from asymptomatic infection to complete resolution or acute fulminant or chronic hepatitis that may lead to life threatening conditions, such as, liver cirrhosis and hepatocellular carcinoma (HCC).[1] Globally, HBV poses a major public health hazard where more than 240 million people are chronically infected with this virus.[2] The highest prevalence of HBV is found in Sub-Saharan Africa and South-East Asia. In the Indian subcontinent and the Middle East region, about 2-5% population are chronically infected with this virus.[2] Bangladesh belongs to the intermediate prevalence region for HBV infection.[3] About 5%-6% of apparently healthy individuals are HBV carriers in Bangladesh and most of them are unaware of their disease.[4-6] About 6-8 million populations are chronically infected with HBV and most of them are younger.[7,8] The lifetime risk of acquiring HBV is from 20% to 60%.[9] Various studies have shown that HBV is responsible for 31.25% cases of acute hepatitis and 76.3% cases of chronic hepatitis, 61.15% cases of cirrhosis of liver and 33.3% cases of HCC in Bangladesh.[3,10,11]

Many HBV genotypes, sub-genotypes, different HBV genomic mutants and recombinants emerge over time because the reverse transcriptase (RT) of HBV does not have proof reading ability. HBV is classified into ten genotypes (A to J) according to 8% of its genomic divergence.[12] About 4-8% nucleotide sequence variation is required for subgenotyping of HBV.[13] There are distinct geographical distribution of different genotypes with important roles for tracing the evolution and transmission of the virus. Different genotypes play important roles for the course, severity and complications of disease and treatment responses.[14,15] The HBV genotypes appear to influence not only the natural history of HBV related liver disease but also the response to HBV treatment. Genotype A has a tendency for chronicity, While, viral mutations are frequently encountered in genotype C. Both chronicity and mutation frequency are common in genotype D. HBV genotypes are also linked with both core promoter and basal core promoter (BCP) mutations. Genotypes A and B appear to have higher rates of spontaneous HBeAg seroconversion. More advanced liver disease and progression to HCC is more often seen in chronic infection with genotypes C and D, in contrast to genotypes A and B. More specifically, sub-genotypes A1, C, B2-B5 and H appear to be associated with more serious complications than genotypes A2, B1 and B6. Genotypes A and B have higher response rates to interferon based therapy than genotypes C and D.[16] Therefore, knowledge of HBV genotypes enable to clinicians to identify patients at increased risk of disease progression whilst aiding the selection of appropriate antiviral therapy. Genotyping and subtyping can also provide useful information for epidemiological studies.[13] Bangladesh is a country with intermediate HBV carrier prevalence; however little is known about the incidence of HBV genotypes in circulation. Therefore, we investigated the HBV genotype distribution in Bangladesh and then observed its clinical complications.

METHODS

Patients

This small Bangladeshi cohort was done in between March 2014 and October 2016 with chronic hepatitis B (CHB) patients who came to the department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh for their HBV DNA routine laboratory tests. The basis of selection criteria were HBsAg positivity for >6 months and HBV DNA positive detected by real-time PCR. A total of 172 HBV DNA positive CHB patients were interviewed at BSMMU, who were native residents of different cities of Bangladesh. From them, a total of 29 patients were randomly selected in this study. Blood samples were collected into a 5% EDTA containing microcentrifuge tube. The plasma samples were separated by centrifugation and stored at -20°C until analysis. This study had ethically approved from the Institutional Review Board of BSMMU and written informed consent was obtained from all study patients.

HBV DNA preparation and amplification

Viral DNA was extracted from 200 µl of plasma by D Neasy Blood and Tissue Kit (QIAGEN, Venlo, Limburg, The Netherlands). A nested PCR protocol was followed to amplify 1014-bp region of the partial *Pol* gene of HBV DNA and the primers were: 3079-3099 (5′- AGC CCT CAG GCT CAG GGC ATA-3′) / 1163-1140 (5′- CGT TGC CKD GCA ACS GGG TAA AGG-3′) as external primers and 3192-

3211 (5′- TCA TCC TCA GGC CAT GCA GT-3′) / 991-972 (5′-GAC ACA CTT TCC AAT CAA TNG G-3′) as internal primers (Biobasic, Canada). Finally, 1014-bp fragment of HBV DNA was obtained and detected by ethidium bromide staining in an agarose gel.

HBV DNA purification and sequencing

Nested PCR Products were cleaned by ExoSAP-IT (USB Corp, Cambridge). Internal primers and BigDye®Terminator v3.1 Cycle Sequencing Kit (California) were used for cycle sequencing. The products from cycle sequencing reactions were purified by BigDye® XTerminator™ Purification Kit and sequenced by an automatic sequencer (ABI PRISM® 3500xL Genetic Analyzer).

Phylogenetic analysis and HBV genotyping:

For sequence alignment as well as phylogenetic analysis, we selected the GenBank sequences with the best and the high scoring matches with our sequences in a NCBI BLAST search. Sequences were edited using Chromas 2.3 (Technelysium) and pairwise aligned by ClustalW program. The750 nucleotide sequences in the partial *pol* gene of HBV were analyzed using BioEdit 7.0.9.0 suite of programs.[17] Genotyping was done by phylogenetic analysis. Phylogenetic trees were constructed by the neighbor joining (NJ) method by MEGA 6.06 package.[18,19] Final dataset contains 250 amino acid positions. Genotyping was also determined by using the three online tools.[20] Statistical analysis was done by SPSS software package 17.0 (IBM SPSS Statistics for Windows, Armonk, NY, USA).

RESULTS

A total of 29 patients were selected for isolation and sequence analysis of HBV DNA. Among them, 25 (86.2%) patients were male and 4 (13.8%) patients were female with a mean age of 29.8±12 years and age range of 4 to 50 years. According to the treatment history of the study patients, 15/29 (51.7%) patients untreated and n-14 (48.3%) patients were treated with antiviral drugs. Their viral load values varied from 9.1×10^2 to $7.0\ \times10^8$ IU/ml, with mean value of 5.5×10^7 (SD±1.5×10^8) IU/ml. ALT values varied from 15 to 419 U/l, and mean ALT level was 89.8 (SD±74.9) U/l. Among the study population, 22 (75.9%) patients were HBeAg positive and 7 (24.1%) patients were HBeAg negative.

Phylogenetic analysis of 250 aa sequences in the *Pol* protein of revealed that only three major HBV genotypes were detected in this study, genotype C was detected in 17 (58.6%) patients, genotype D was detected in 10 (34.5%) patients, while genotype A was detected in only 2 (6.9%) patients (Figure 1).

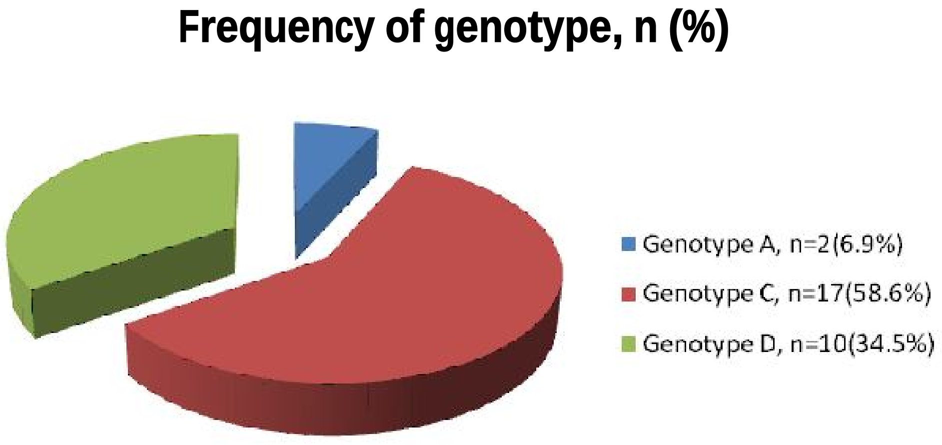

Figure 1: Frequency of genotype, n (%)

Present study showed that the mean HBV viral load of genotype C of HBV positive patients was $6.2\times10^{7}\pm1.7\times10^{8}$ IU/ml with mean ALT level was 102.2±94.1 U/l. Whereas, the mean HBV viral load of genotype D of HBV positive patients was $6.1\times10^{7}\pm1.4\times10^{8}$ IU/ml with mean ALT level was 76.9±27.1 U/l (Table I). In addition, mean viral load of two patients infected with genotype A strains was $8.1\times10^{5}\pm9.6\times10^{5}$ IU/ml with mean ALT level was 50±36.8 U/l. After observation of the study patients from March 2014 to October 2016, we observed that HCC was developed in 2 patients infected with genotype C strains. Both of the genotype A (adw2) positive patients died: one patient from HCC and another patient by LC (Table I).

Table I: Laboratory data and observed clinical complications of Chronic Hepatitis B patients infected with different genotypic strains of HBV

Genotypes of HBV (2014)	Viral load, Mean±SD (IU/ml) (2014)	ALT, Mean±SD (U/l) (2014)	Observed Clinical complications = Number of patients (2014-2016)
Genotype C	$6.2\times10^{7}\pm1.7\times10^{8}$	102.2±94.1	HCC=2
Genotype D	$6.1\times10^{7}\pm1.4\times10^{8}$	76.9±27.1	ND
Genotype A	$8.1\times10^{5}\pm9.6\times10^{5}$	50±36.8	HCC=1 LC=1

Note: HBV viral load in plasma; ND: not dictected; HCC: Hepatocellular Carcinoma; LC: Liver Cirrhosis.

DISCUSSION

The extent of HBV replication among Chronic Hepatitis B (CHB) patients is considerable, reaching $>10^{8}$ to 10^{11} viral particles per day.[21] As *Pol* is a Reverse transcriptase (RT) that lacks proof reading capacity, HBV replication is also associated with a high mutational rate of 10^{5} substitutions /base /cycle.[22] Thus, all possible single base changes in the HBV genome are generated daily, therefore, many genotypes, sub-genotypes and subtypes of HBV and also different type of genomic mutations emerge. The genomic changes associated with antiviral resistance and stable mutations in HBsAg and these resistant viruses are transmitted to other individuals.[23]

On the analysis of our results we found, only three HBV genotypes in the present study, genotype C, genotype D and genotype A. Genotype C (58.6%) was the predominant genotype, followed by genotype D (34.5%), while genotype A was the least dominant genotype observed in only 6.9% of patients. This finding showed similarity with the previous study for HBV genotyping in Bangladesh, the author Rahman et al.[24] reported that the most prevalent genotype was genotype C (48.7%) followed by genotype D (28.2%) and genotype A (23.1%). On the contrary, other two previous Bangladeshi studies found that the prevalent genotype was genotype D, followed by genotype C and genotype A.[25,26] Whereas, the neighbouring country, Eastern India, Kolkata showed presence of genotype C in addition to genotypes A and D among the HBV carriers.[27] Whereas, Western and Northern India showed that genotypes D and A were more prevalent.[28,29] Prevalent genotypes in Srilanka were B (36%), C (16%), D (12%) and in Pakistan were genotypes D (91.1%) and C (9.8%).[30,31]

Earlier studies suggested that HBV genotype C is associated with delayed HBeAg seroconversion, more active hepatitis, lower response to antiviral therapy, more advanced liver disease and a higher risk of hepatocellular carcinoma.[32-35] On observation of our present study showed most of the genotype C positive patients were associated with elevated ALT that indirectly reflects more liver damage. On further observation, we found that two of our patients infected with genotype C strains had died due to HCC. Interestingly, we found that only two patients were infected with genotype A strain and both patients died: one patient due to HCC and another patient by LC.

Limitations

The present study was based on a small cohort due to limitation of fund, thus large sample size is needed to confirm the validity of these findings.

CONCLUSION

In conclusion, the most prevalent HBV genotype is genotype C, followed by genotype D, while genotype A is the least dominant among Bangladeshi CHB patients. Genotype C and A strains are found to be related with more complications, therefore, patients infected with these HBV strains need to be monitored carefully to assess their clinical outcome in future.

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Conflict of interest: No conflict of interest to declare.

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