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Prevalent Hepatitis B Virus Genotypes Circulating among HIV Co-Infected Patients in an Urban City, South-South Nigeria

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Abstract

Hepatitis B Virus (HBV) has been classified into genotypes A-J which characteristically has different geographic origins, disease progression and responses to antiviral treatment. Co-infection caused by Human immunodeficiency virus (HIV) and HBV is common because they both share similar routes of transmission. In this study, we report the prevalent HBV genotypes circulating among HBV-HIV co-infected patients in Port Harcourt, south-south Nigeria. DNA was extracted from stored serum samples obtained from 25 HBV-HIV co-infected patients selected out of 535 HIV I/II sero-positive patients confirmed to be HBsAg positive from University of Port Harcourt Teaching Hospital (UPTH) and Obio Cottage Hospital both in Port Harcourt City. HBV genotyping was carried out using a nested Polymerase Chain Reaction (PCR) approach followed by Big-dye termination sequencing. The mega blast search in the National Centre for Biotechnology Information (NCBI) database, revealed a close relatedness to HBV genotype B and E. Of the 25 samples, 15 (60.0%) were successfully genotyped. HBV genotype E was found in 10 (66.7%) of samples whereas, B was found in 5 (33.3%) participants. HBV genotypes B and E were found among HIV co-infected patients though genotype E was more predominant in this study. Early detection and identification of HBV genotype particularly in HIV co-infected patients could reduce the rate of liver degeneration and enhanced treatment.

Keywords: HBV-HIV; Genotypes; Co-infection

Introduction

The arrangement and sequence of the amino acids contained in the genome of every virus is the foundation for grouping them into a genotype. HBV genotypes were classified into ten which are specific and noticeable in their communities of origin. The classification was essentially done based on the fact there is higher than 8% in the diversity of the DNA sequence that exist in the full length of the genome of the virus [1,2]. Furthermore, based on the presence of distinct virology and epidemiology uniqueness which are properly defined, and varieties which are already in place among HBV, most genotypes have been classified into sub genotypes. HBV genotype E has been successfully genotyped and sequenced in two separate studies carried out in southwestern Nigeria [3,4].

Relationship between the virus genotype and the damage to the liver has been highlighted, for instance, genotype C has a higher frequency than B among individuals with chronic HBV infection whereas chronic infection could lead to liver damage [5,6]. Also, in individuals infected with genotype B, there is sudden seroconversion from HbeAg into anti-HBeAg than it is observed in patients infected with genotype C [7].

Furthermore, clearance and resolution of HBsAg take place more often in those infected with genotype A than genotype D, while death arising from liver damage occurs in higher frequency in patients whose infection is caused by genotype F [8]. The implication is that the consequence of chronic HBV infection differs based on the genotype that is incriminated in such infection in the patients [6].

There are documented cases of HBV and HIV co-infection which may result in accelerated degeneration to liver cirrhosis and hepatocellular carcinoma in humans worldwide. HBV appears to be more damaging in individuals who are positive to HIV than is found in mono-infection, with associated elevation in rates of carrier of HBV, higher levels of vireamia, more recurrent incidents of initiation, and accelerated rate of degeneration to cirrhosis [9,10]. HBV and its genotypes play a significant role in promoting the advancement of the disease condition particularly in HIV infected patients. Despite available data on the prevalence of HBV infection in Port Harcourt, Nigeria, there is a dearth of information about the study of molecular status of the prevalent HBV genotypes among this category of patients in Port Harcourt and this necessitated the need for this study.

Materials and Methods

Patients

The target populations were patients who presented at the anti-retroviral clinics of the University of Port Harcourt Teaching Hospital and Obio Cottage Hospital. Ethical and administrative approvals were obtained from these hospitals.

Patients' sera samples were screened and confirmed positive to both HIV and HBsAg. Twenty-five serum samples that were positive for both HIV and HBsAg were used in this study.

HBV DNA extraction

HBV DNA was extracted from 100 µl of serum samples using the Quick-gDNA Blood MiniPrep extraction kit (Zymo Research Corporation, USA) according to the manufacturer's instructions. An elution volume of 50 ul was used.

DNA amplification by polymerase chain reaction

The extracted DNA was directly amplified after extraction. The resulting pellet from the extracted DNA (1 µl) was re-suspended in RNase-free water and then subjected to nested PCR. This was described as follows; The HBV genome was amplified by nested PCR using the universal primers P1b (universal, sense)-5′-TCA CCA TAT TCT TGG GAA CAA GA-3′ nt 2823-2845, and S1- (universal, antisense)-5′-CGA ACC ACT GAA CAA ATG GC-3′ nt 685–704 for the outer primers [11]. This was followed by two different mixtures containing type-specific inner primers B2 (specific, and sense)-5′-GGC TCM AGT TCM GGA ACA GT-3′ nt 67-86, types A to E, B2R (specific, antisense)-5′-GGA GGC GGA TYT GCT GGC AA-3′ nt 3078-3097, types D to F.

The first PCR was carried out in a tube containing 40 ml of a reaction buffer made up of the following components: 50 ng of each outer primer, a 200 mM concentration of each of the four deoxynucleotides, 1 U of AmpliTaqGold DNA polymerase (Perkin-Elmer, Norwalk, Conn) and 13 PCR buffer containing 1.5 mM MgCl2. AmpliTaq Gold DNA was used to obtain an automatic hot-start reaction.

The thermocycler (GeneAmp PCR system 9700; Applied Biosystems) was programmed to first incubate the samples for denaturation in 10 minutes at 95°C, followed by 40 cycles consisting of primer annealing at 94°C for 20 seconds, then extension at 55°C for 20 seconds, and final extension at 72°C for 1 minute.

HBV genotyping, sequencing and phylogenetic analysis

Genotypes of HBV for each sample were determined by identifying the genotype-specific DNA bands. The two different second-round PCR products from one sample were subjected to electrophoresis separately on a 2% agarose gel, stained with ethidium bromide, and evaluated under UV light. The sizes of PCR products were estimated according to the migration pattern of a 100-bp DNA ladder (Zymo Research Cooperation, USA).

To test the validity of our PCR genotyping system, genotypes of HBV were also determined by phylogenetic analysis of pre-S through S genes in 40 samples. Amplified PCR products were subjected to direct sequencing and then phylogenetic analysis was performed. Mix A allowed for the specific detection of PCR products for types A, B, and C, and mix B allowed for detection of types D, E, and F.

Genotypes were determined according to differences in the sizes of amplified DNA which is specific for each genotype; Genotype B was amplified at 375 bp while genotype E was amplified at 175 bp.

Sequences were edited using the bioinformatics software Trace edit for removal of sequencing errors from both ends. Similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) database the BLAST algorithm and were aligned using Clustal X.

The evolutionary history was inferred using the Neighbor joining method in MEGA 5. The bootstrap consensus tree inferred from 500 replicates was taken to represent the evolutionary history of the taxa analyzed [12].

Results

The results of the HBV genotyping done by agarose gel electrophoresis showed that out of the 25 HBV positive samples screened in this study, 15 were successfully amplified with distinct bands of HBV genotypes B (375 bp) and E (175 bp). HBV DNA bands 2, 4, 13, 19, and 24 represent HBV genotype B (375 bp) while 3, 5, 7, 10, 12, 14, 22, 25, 27 and 29 represent genotype E (175 bp).

Bands 1, 8, 15, 16, and 23 represent the 100 bp molecular ladder (Figure 1).

Therefore, genotype B accounted for 5 (33.3%) while genotype E accounted for 10 (66.7%) of the HBV genotypes recorded in this study (Table 1).

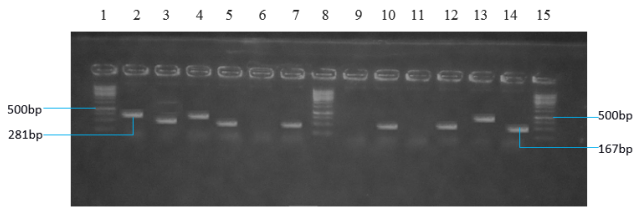

Figure 1: Agarose gel electrophoresis micrograph of DNA bands of the HBV subtypes: 1, 8 and 15 represent the 100 bp molecular ladder. 2, 4 and 13 represents the HBV genotype B bands (375 bp) while 3, 5, 7, 10, 12 and 14 represent the genotype E bands (175 bp).

HBV Genotype/Subtype	Frequency (%)
Type E	10 (66.7)
Type B	5 (33.3)
Total	15 (100.0)

Table 1: Percentage distribution of HBV genotypes in HBV-HIV Co-infected patients in Port Harcourt

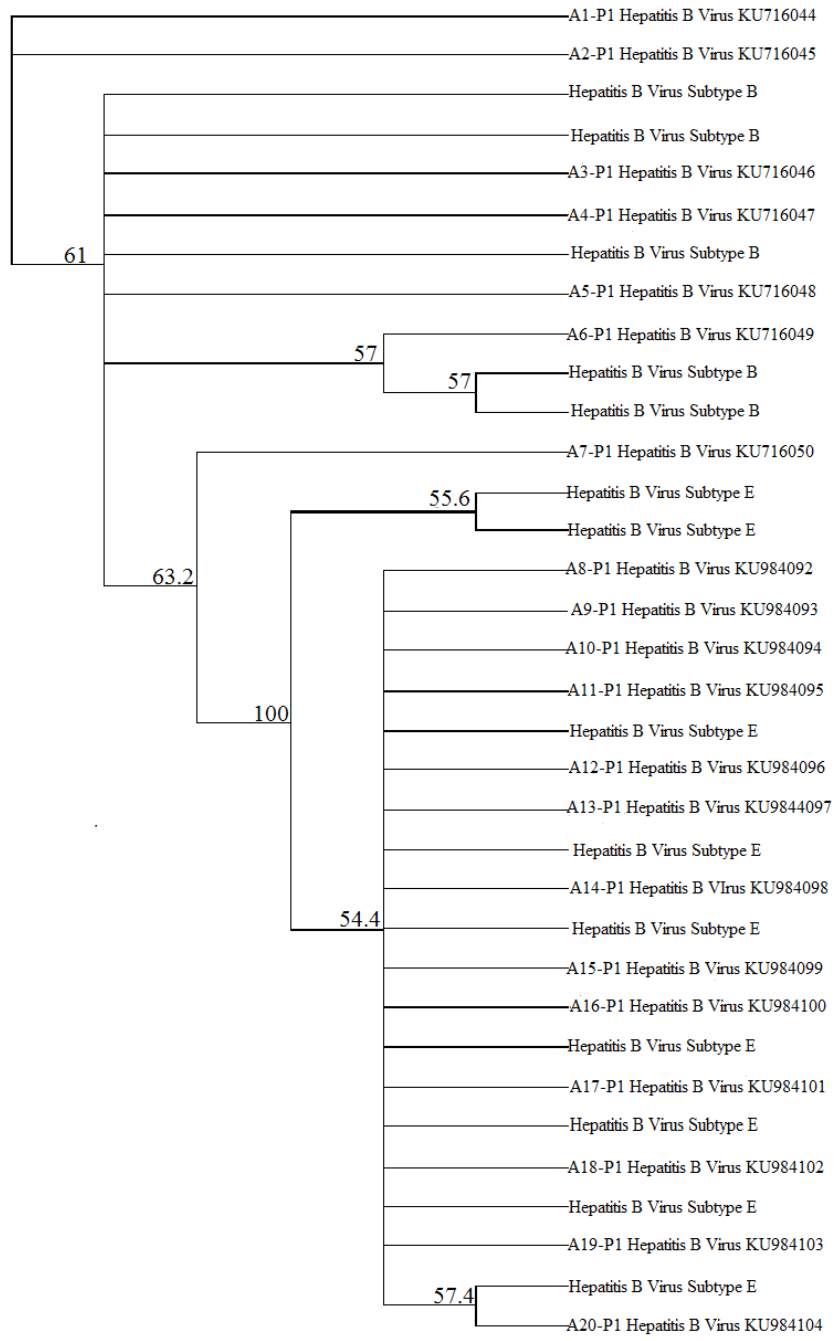

Figure 2: Phylogenetic analysis of hepatitis B Virus (HBV) isolates and HBV genotypes (B and E) from HBV-HIV Co-infected patients in Port Harcourt

The HBV genotyping results obtained from sequencing were as designated on the phylogenetic tree (Figure 2). The HBV genotyping results obtained from the agarose gel electrophoresis were like those sequenced. Sequenced DNA and genotypes B and E were aligned using ClustalX software version 2.1. The HBV sequenced were deposited with NCBI while the accession numbers for the HBV references were: KU716044, KU716045, KU716046, KU716047, KU716048, KU716049, KU716050, KU984092, KU984093, KU984094, KU984095, KU984096, KU984097, KU984098, KU984099, KU984100, KU984101, KU984102, KU984103, KU984104.

Discussion

Hepatitis B Virus DNA was detected in 15 out of the 25 serum samples of HBV/HIV co-infected patients since all the subjects were originally sero-positive to HBsAg. There are instances when serological methods of diagnosing viral infections becomes confusing and not clear enough or inconclusive, assessment of the HBV DNA level will be valuable for disease progression monitoring and predicting the risk of development of hepatocellular carcinoma [13]. The use of molecular assays will then become very useful in such cases [14]. Nucleic acids tests are carried out in three formats which include non-amplified, amplified and advanced methods, but amplified based methods have been recognized as the most vital for applicable detection of viral hepatitis [15]. However, research involving detection of DNA generally and HBV DNA in particular, is yet to be embraced by most researchers in this locality because of lack of access to molecular laboratory facilities hence, there is scarcity of literature regarding the prevalence of HBV DNA among HBV-HIV co-infected patients in the study area.

Out of the 25 samples that were obtained from HBV-HIV co-infected patients, 15 (60.0%), were successfully genotyped, while 10 (40.0%) were not genotyped. The genotypes of the HBV responsible for infection in the studied patients were determined. Ten, amounting to 66.7% were HBV genotype E while 5 (33.3%) were HBV genotype B (Figure 2).

The relevance of genotypes is not limited to the frequency differences of hepatitis B virus mutants found in different geographic regions, but also to the differences seen in the clinical consequence and reaction to treatment by antiviral agents in different population groups [16]. Studies highlighting the connection between the genotypes and gravity of liver degeneration have been reported. For instance, in a study carried out in China and Japan, genotype C was more involved in chronic HBV than genotype B in patients with cirrhosis and hepatocellular carcinoma [5,6,17].

There is an ongoing reinforcement of spontaneous sero conversion rate of "e" antigen to "e" antibody which is found to be appreciably reduced in patients who are infected by genotype C than with genotype B [7]. The studies also provided clear proof that genotype B relates to reduced active and slowly progressive degeneration in the liver when compared with genotype C. Patients who contract HBV genotype B have less chance of advancing to degenerated liver damage, based on the aforementioned observations. Also, it has been documented that genotypes A and B infected patients have better responses to treatment using interferons than individuals with genotypes C and D [18-20].

The occurrence of genotype E in this study is consistent with different studies done in Nigeria and Botswana in which HBV genotype were reported [3,21,22]. HBV genotype E is regarded as being restricted to West Africa including Nigeria where it is predominant [3]. Genotype E is predominant in the sub-Saharan Africa, and has been discovered in Central African Republic, Senegal, Namibia, and in East Africa [23]. Genotype E has widespread geographical distribution but a very low genetic diversity. In a research conducted in a highly endemic region of Nigeria on 20 chronic and acute hepatitis patients, all the subjects studied were discovered to be infected by HBV genotype E following the analysis of the phylogenetic tree generated from the complete pre-S2/S genes [3]. They further suggested that genotype E has not been identified or found in any other area including Americas except in Nigeria, and based on this observation, it was opined that this genotype could be a recent one and the reason behind its relatively low genetic diversity. The identification of HBV genotype E in our study therefore is in line with the finding and in consonance with the suggestion that genotype E is indigenous to West Africa and Nigeria in particular [3]. Genotype E is very difficult to treat requiring a longer duration. Furthermore, the mean decrease in the concentrations of the hepatitis B surface antigen was lowest among patients infected by genotype E when compared with B and D, whereas, HBsAg relapse was documented for individuals with genotypes B and E [17]. It is therefore imperative for more research to be conducted to establish the pace of relapse of infection following treatment and to develop more potent antiviral agents to effectively treat the infection by genotype E. To detect the circulation of hepatitis B virus immune escape mutants among asymptomatic community dwellers in Ibadan, southwestern, Nigeria, HBV genotype E was documented while, a novel strain of this genotype was reported [4,24]. This lay credence to the idea muted that the HBV genotype is indigenous to West Africa and Nigeria in particular [3].

Genotype E was predominant in this study accounting for 66.7% of the successfully genotyped samples obtained in Port Harcourt, Nigeria. The same genotype had the highest frequency in the study, accounting for 87.5% of the samples genotyped [21]. However, it has a low prevalence of 1.4% [22-24].

The degenerating impact of HBV on the liver of HIV co-infected patients can be avoided if patients are diagnosed early, particularly to the molecular level and treatment initiated immediately [13,25,26].

Conclusion

HBV isolated in this study were genetically characterized and identified. The sequences of the isolates from the serum of the HBsAg sero-positive patients in this study had an exact match with similar sequence during the mega blast search from the NCBI database.

For the first time, HBV was sequenced, genetically identified and characterized in Port Harcourt. These have been deposited with the NCBI gene bank and accession numbers series KU716044, KU716045, KU716046, KU716047, KU716048, KU716049, KU716050, KU984092, KU984093, KU984094, KU984095, KU984096, KU984097, KU984098, KU984099, KU984100, KU984101, KU984102, KU984103, KU984104 assigned to them.

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