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Molecular Genetic Diversity of Hepatitis B Virus in Kenya

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Key Words

Genotypes · Hepatitis B virus · Kenya · Nuclear acid testing

Abstract

Eight genotypes of hepatitis B virus (A-H) and subgenotypes have been recognized worldwide. However, there is limited information on prevalent genotypes in many countries in Africa. This study was undertaken to determine the hepatitis B virus (HBV) genotypes in Kenya. Seropositive HBV blood samples from a blood donor setting were used in the study. HBV genotypes were determined in 52 nucleic acid-positive samples using specific primer in a nested PCR and sequencing employed in the HBV genotyping. This study shows presence of HBV variants with genotypes A (88%), E (8%) and D (4%). In conclusion, we found that HBV genotype A is the most predominant genotype in Kenya with both subgenotype A1 and A2 present. Genotype D and E are also present in our population. This demonstrates that there could be a high genetic diversity of HBV in Kenya.

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Hepatitis B virus (HBV), a well-known agent of acute and chronic hepatitis, is endemic in many parts of the world, especially Asia and Africa. More than 2 billion people have had contact with the virus and more than 350 million are chronic carriers globally [1, 2], making the infection a public health problem.

HBV is the prototype member of the genus *Orthohepadnaviridae* of the family *Hepadnaviridae* and the viral genome is about 3.2 kb long. It circulates in the serum as a Dane particle which is a round structure consisting of an envelope and an inner core of nucleocapsid protein, enclosing both a polymerase and the partly double-stranded circular viral DNA [3].

The highly compact genome contains the four major open reading frames (ORFs) encoding the envelope (preS1, preS2 and surface antigen HBsAg), core (preCore precursor protein, HBeAg and HBcAg), polymerase (HB-Pol) and X (HBX) proteins, respectively [4]. Earlier, before the genotype definition, HBV strains were distinguished into 9 hepatitis B surface antigen (HBsAg) subtypes designated *ayw1*, *ayw2*, *ayw3*, *ayw4*, *ayr*, *adw2*, *adw4q–*, *adrq+*, and *adrq–* by serological analysis [3].

The new classification based on a comparison of the complete genomic sequence classifies HBV into 8 geno-

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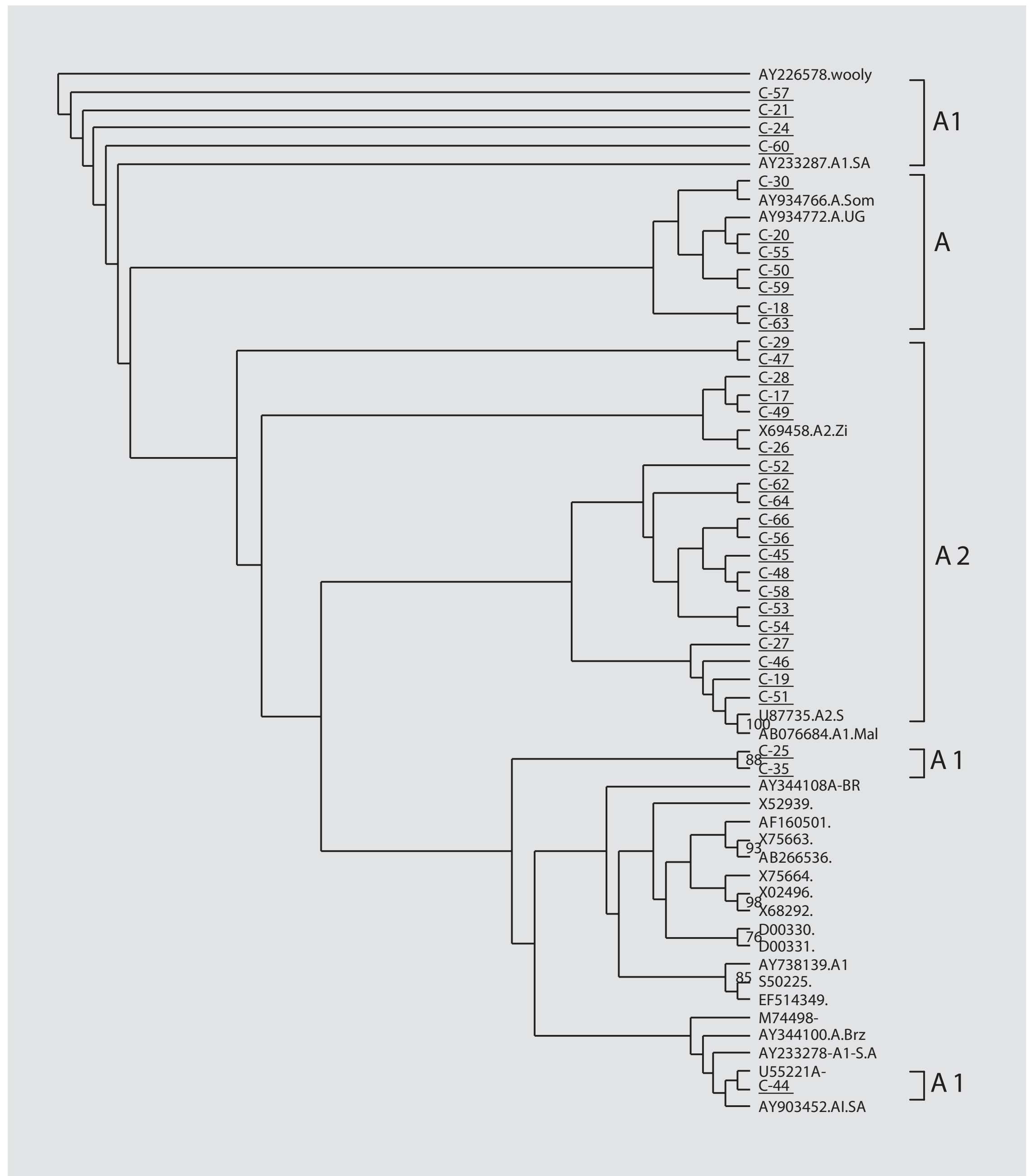

Fig. 1. Phylogenetic analysis of HBV strains in Kenya based on preS1 region. Kenyan isolates underlined.

types, A–H, with each genotype differing by more than 8% at the nucleotide level when compared to each other [1] and less than 4% intragenotype divergence. Some genotypes are further classified into subgenotypes: genotypes A and F into 2 subgenotypes each, and genotypes B, C and D into 4 subgenotypes each [5].

Africa is one of the highly endemic regions of HBV, with 5 genotypes A–E identified. Genotype D in Tunisia, genotype A–D in South Africa and genotype E in Nigeria are the predominantly reported genotypes in these countries [6, 7]. Little information is available on HBV genetic diversity in Kenya, yet the country is considered among the endemic countries for HBV infection.

To determine the HBV genotypes in Kenya, blood samples positive for HBV were collected from a blood transfusion center. Plasma from the samples was separated by centrifugation and unlinked anonymous testing carried out for all the samples using ELISA and particle agglutination tests for HBV surface antigen (HBsAg) which included; Hepanostica HBsAg test kit (Organon Technika) and KEMRI HEPCELL II (Kenya Medical Research Institute (KEMRI), Nairobi, Kenya). Viral DNA was extracted from 100 μl of plasma using SMITEST R&D RNA/DNA (Genome science Co. Ltd., Tokyo, Japan) according to the manufacturer's instructions. The extracted nucleic acid was resuspended in 20 μl of RNAse/DNAse free water and stored at –80° till use.

For the detection of HBV DNA, the extracted viral DNA was subjected to nested PCR with an AmpliTaq Gold PCR kit (Applied Biosystems, Foster City, Calif., USA). A part of preS1 gene corresponding to nt 2850–3246 was amplified with the primers, HBPr1 (5′-GGGTCACCATATTCTTGGG-3′, sense)/HBPr135 (5′-CA(A/G)AGACAAAAGAAAATTGG-3′, antisense) in the first round and HBPr2 (5′-GAACAAGAGCTACAGCATGGG-3′, sense)/HBPr3 (5′-CCACTGCATGGCCTGAGGATG-3′, antisense) in the second round [4]. Amplification was done with one cycle of 94° for 10 min and 35 cycles of 94° for 30 s, 50° for 30 s and 72° for 30 s with a final extension of 72° for 10 min in both rounds. PCR amplification was confirmed by visualization with ethidium bromide staining of the gel.

Parts of the amplified products were cloned with the TOPO TA cloning kit (Invitrogen, Carlsbad, Calif., USA) and sequenced as described previously [7]. At least 4 clones per sample were analyzed to obtain a consensus sequence. The rest of the samples were directly sequenced in an ABI prism genetic analyzer. The newly analyzed sequences were aligned with subtype reference sequences from the Los Alamos database by CLUSTAL W (version 1.81) with subsequent inspection and manual modification. The frequency of nucleotide substitution in each base of the sequences was estimated by the Kimura two-parameter method [8]. A phylogenetic tree was constructed by the neighbor-joining method [9], and its reliability was estimated by 1,000 bootstrap replications. The profile of the tree was visualized with Tree View PPC version 1.6.5.

In this study, of 80 HBsAg seropositive samples, 52 were positive for nuclear acid testing (NAT). The rest of the samples could not amplify specifically; these were considered to be either false-positives or had very low DNA undetectable by PCR. To determine the genotypes, the 52 NAT-positive samples were then sequenced and analyzed phylogenetically. Reference sequences from Gene Bank including all genotypes reported and especially those from Africa were included in the analysis.

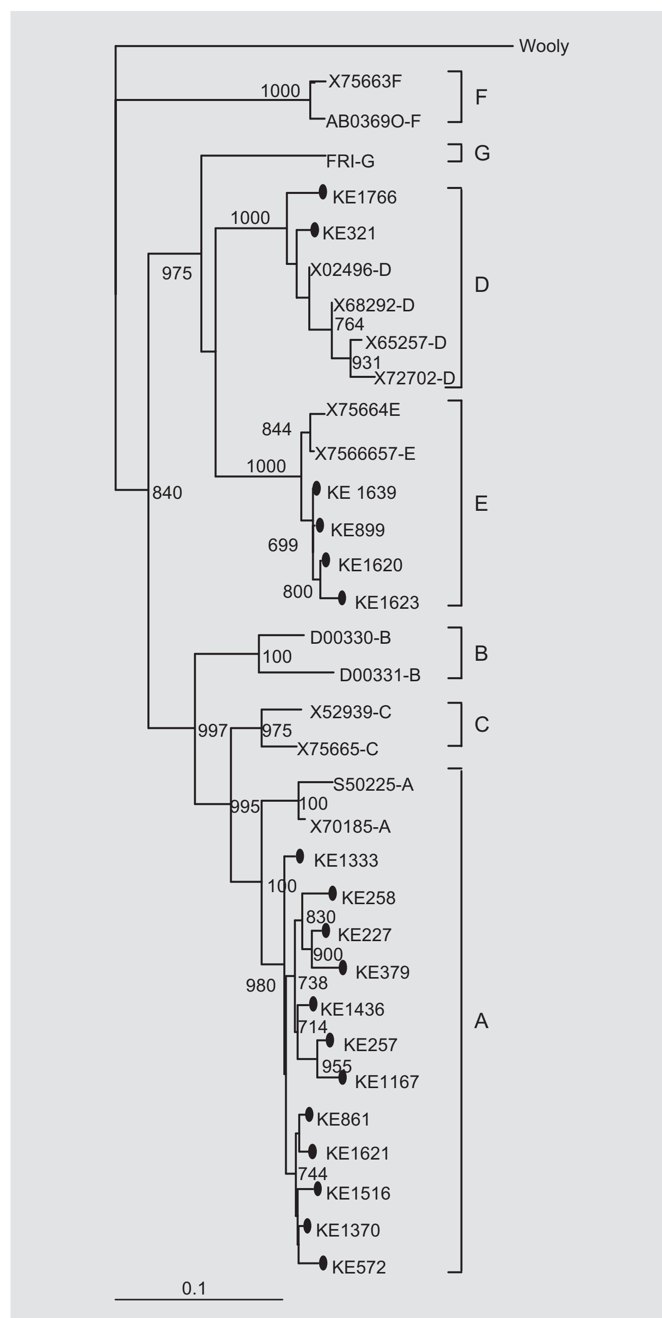

Fig. 2. Phylogenetic analysis of HBV strains in Kenya based on preS1 region. Kenyan isolates bulleted.

Upon sequencing, the 52 preS1 sequences obtained segregate HBV strains in Kenya into 3 genotypes, A, D

and E. The analysis revealed that 12 HBV strains of the samples that were cloned belonged to genotype A, 2 to genotype D and 4 to genotype E (fig. 2). Those obtained through direct sequencing revealed segregation of subtypes into A genotype, with both subgenotypes A1 and A2 present. 18 of these sequences clustered closely with reference A2 and A1 strain from South Africa, 6 with reference A from Uganda, 6 with A2 from Zanzibar and 1 with reference A from Somalia and 4 sequences clustering closely with reference A from Brazil. The nucleotide sequences of the 52 isolates obtained in this study have been deposited in the GeneBank. The accession numbers for the sequences reported are as follows: DQ460641 to DQ460665 for HBV *preS1* cloned samples and direct sequencing; EU514582 to EU514615.

There are 360 million people in the world with chronic HBV infections, 65 million (18%) of those infected live in Africa; however, there has been limited information into the type of HBV genotypes circulating in the region. In Kenya, particularly, very little information is available on the molecular epidemiology of HBV. In our study, we found that HBV genotype A (88.5%) was the dominant strain in Kenya, followed by genotype E (7.7%) and D (3.8%). HBV genotype A has been reported to be the most predominant subtype in sub-Saharan Africa [10], subtype E restricted to Africa [11] and subtype D in the Mediterranean countries. Our findings in this study suggest that the majority of infections in Kenya could be of subtype A and its variants. The clinical significance of HBV genotypes is a subject of discussion. It has been suggested that infection by HBV genotype A could be more frequently associated with chronic infection than genotype D [12, 13]. While genotype A appears to respond better to interferon treatment, compared to genotype D, it also generates a higher rate of viral resistance during treatment [14]. This genotype has also been shown to be the most prevalent among patients with acute hepatitis B [15]. Thus, the findings of our study suggest that most people in Kenya may develop chronic HBV infections and high viral resistance to treatment.

Hepatitis B screening is not a readily available test in Kenya for routine patients; most testing is done for safety of blood for transfusion. Normally, donors detected as positive for HBV infection during blood screening are not followed up and therefore no management of the infection is sought. Currently, interferon-a (IFN-a), lamivudine and adefovir have been licensed globally for the treatment of HBV. Thymosin-a1 has also been approved in more than 30 countries, mainly in Asia. Peginterferon-α-2a has been granted approval in some Asian and European countries and the approval process is underway in other countries [16]. Thus, even if treatment could be available, access to the treatment is limited due to lack of testing.

Most genotype A strains belong to subgroup or subgenotype A1; however, in our study 14 isolates (fig. 2) clustered closely with A2 subgenotype from South Africa suggesting that subgenotype A2 could be a prevalent subgenotype in Kenya. Six isolates clustered closely with subgenotype A2 reference from Zanzibar probably reflecting the effect of the coastal migratory route. Six genotypes clustered closely with genotype A from Uganda and the rest of the sequences (fig. 1, 2) could not be immediately placed to either subgenotype; however, some of these isolates clustered closely with A reference strain from out of Africa (D, E and some A genotypes). These isolates could not cluster with references from African countries, thus suggesting a distant link with Africa.

Genotype D was also detected in our study, this suggests that a significant proportion of this genotype could be circulating in Kenya. Genotype D is the most widely distributed genotype and has been found universally [13, 17] with highest prevalence stretching from Southern Europe and North Africa [18, 19] to India, in West and South Africa [20] and among intravenous drug users on all continents [21–23]. Thus, the finding of our study further seems to confirm this observation. Nevertheless, further studies are required to highlight HBV subtypes distribution in Kenya.

In our study 4 isolates were of genotype E as HBV genotype E strains are found exclusively in West and South Africa [5]. This genotype is restricted to Africa and little information is available on its clinical significance. However, our findings suggest that this genotype is also circulating in the eastern parts of Africa. From the results of this study, it appears that the HBV subtypes circulating in Kenya reflect the distribution of the genotypes in Africa.

It is important to note that all our isolates were from self-selected ‘healthy’ individuals in blood donor settings. It would be interesting to find out if the situation is different within the clinical settings and further elucidate the impact of diversity in HBV infections and disease management in Kenya and in this region. Cloned isolates gave different genotypes while the direct sequencing only grouped isolates into one genotype, thus cloning could be required for future work to further understand the complexity of genotypes in Kenya.

In conclusion, we have established the HBV genotypes and the existence of their variants in Kenya for the first

time. These findings suggest the existence of different genotypes and subgenotypes in Kenya. There is need to further monitor the diversity of HBV in the region since the cross-border effect, presence of refugees and selective presence of certain genotypes could have an impact on viral evolution, transmission and disease management. Because genotype may impact disease progression and response to treatment, additional studies are needed to add to these findings and improve treatment and prevention in our region.

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