

# Hepatitis B virus genotypes A and D in Uganda

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## Abstract

**Background:** The prevalence of hepatitis B virus (HBV) infection in Uganda is 10%. Hepatitis B virus genotypes impact on treatment response, rate of spontaneous recovery and progression of chronic HBV infection and hepatocellular carcinoma. There is little information on the HBV genotypic distribution in Uganda.

**Objectives:** To determine HBV genotypes in Uganda.

**Methods:** The MBN clinical laboratory performs HBV viral load and genotype testing in Uganda. It receives hepatitis B surface antigen (HBsAg)-positive samples from all over the country for additional HBV testing. Samples are stored for 6 months before being discarded. Our study used delinked stored samples. PCR-positive samples had DNA extracted and used as template for HBV genome amplification by nested PCR. Reverse hybridisation was performed and genotypes were determined by the line probe assay method (INNO-LiPA).

**Results:** One hundred stored HBsAg-positive plasma samples with detectable viral loads were analysed. Of these, 93 samples showed PCR amplification products and gave genotype-specific probe lines on the INNO-LiPA assay. Of the patients, where gender was recorded, 60.9% were female, and the overall median age (IQR) was 25 (2–60) years. There was a predominance of HBV genotype D (47 patients; 50.5%), followed by genotype A, (16 patients; 17.2%). One patient (1.1%) had genotype E. In 28% of the samples mixed infections were detected with genotypes A/E (9.7%) and A/D (6.5%) being most common. Genotypes B, C, E and H only occurred as part of mixed infections.

**Conclusion** Hepatitis B genotypes D and A were predominant in our study population.

## Introduction

Hepatitis B virus (HBV) is a common medical problem globally with 2 billion persons exposed, 350 million of whom are living with chronic infection. Sub-Saharan Africa has a high burden of infection with most infections acquired early in life, leading to chronicity and fatal complications of cirrhosis, liver cancer and liver failure [1].

In Uganda 10% of the 36 million population live with chronic HBV infection but the distribution varies from region to region. The highest prevalence is found in the northern part of the country where rates of up to 25% have been reported [2].

To date 10 genotypes of hepatitis B (A–J) have been reported with variations in the global distribution [3,4]. In Africa certain genotypes have been shown to be more prevalent in some parts of the continent. For example, while genotype D has been identified in Tunisia, Egypt and Sudan, West and Central Africa have mainly genotype E [5]. In Eastern Africa, genotypes A and D were reported in Kenya [6]. Two studies in Uganda showed a predominance of genotypes A and D [5,7]. However, these studies consisted of small patient numbers and were hospital-based, making it difficult to draw meaningful conclusions on the distribution of genotypes in the country.

Different hepatitis B genotypes may be associated with different rates of disease progression and treatment outcome. Progression to chronicity occurs more in genotype A than D, liver cancer in Taiwanese patients was seen more commonly in genotypes B than C, response to interferon- $\alpha$  is better in A than B and worse with genotype D [3,8,9].

Despite the high prevalence of hepatitis B in Uganda there is limited information about the hepatitis B genotypes in the country.

This information could be important in prognostication and treatment planning in patients with chronic hepatitis B infection. We conducted this cross-sectional study to determine hepatitis B genotypes in samples that were found to be hepatitis B surface antigen (HBsAg)-positive with a detectable viral load.

## Materials and methods

This was a laboratory-based study that used samples stored at a certified referral commercial laboratory in Kampala, Uganda. The MBN clinical laboratory is located in Kampala City and at the time we conducted the study it was the only one that was licensed to perform HBV viral load and genotype testing in the country. It received HBsAg-positive samples from health centres all over the country. Most samples are referred for determination of HBV viral load and HBeAg testing to characterise the phase of infection and to determine treatment candidacy. After testing is completed the samples are stored at  $-80^{\circ}\text{C}$  for about 6 months before discarding. This is in case the referral site requires further testing for patient care. Samples are usually submitted with a request that contains the name of the patient, age, gender and the health centre it comes from.

We used stored plasma samples with detectable HBV viral load of more than 20 IU/mL (Roche TaqMan). All patient identifiers were removed and thereafter samples were tested for hepatitis B genotype using the INNO-LiPA HBV Genotyping Assay (Innogenetics, Ghent, Belgium).

## Laboratory methods

### DNA extraction

In the DNA extraction laboratory, pure viral DNA was extracted from plasma using GenoType DNA isolation Kit (Hain Lifescience, Nehren, Germany) according to the manufacturer's manual. The extracted DNA was amplified with the LiPA procedures. DNA extracts if not used immediately, were stored at  $-20^{\circ}\text{C}$ .

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## PCR procedure

The extracted DNA was amplified by primary PCR and the PCR product was used in a nested PCR using PCR primers complementary to a conserved region in the PreS1 region (amplification of the HBsAg region) to provide a biotinylated product according to the manufacturer's manual. Genotype Amp 9700 Series Thermocycler (Applied Biosystems) was used. The amplified product was visualised on 3% agarose gel. For the samples that showed no DNA product on the gel after the primary amplification, a nested amplification was performed using the product from the primary amplification as DNA template. The primary amplicon (5 µL) was added to 40 µL of the PCR reagent mix. The thermocycler was set to carry out steps as for the primary amplification, except a repeat of 35 cycles was used.

## LiPA test procedure (hybridisation)

The biotinylated PCR products underwent chemical denaturation, and thereafter were incubated with a test strip (containing genotype-specific probes immobilised as parallel lines on each strip) in reverse hybridisation with the hybridisation solution at 49°C in a shaking incubator. Following hybridisation, the strips were washed with stringent wash buffers at 49°C in the incubator shaker and then incubated with a streptavidin conjugate to allow colour development from the biotinylated DNA bound to the strip according to the LiPA manufacturer's manual. Thereafter, the HBV genotype was read from the test strip. The INNO-LiPA HBV genotyping strip contains one red marker line, two control lines, and 14 parallel probe lines. The conjugate control line is a control for the colour development reaction and the amplification control line contains universal HBV probes to check for the presence of amplified HBV genomic material.

Using a data abstraction sheet, the age, gender, sample origin, HBV viral loads, HBeAg, and genotypes were recorded and entered in Microsoft Excel 2003. This was exported to STATA 13 (www.stata.com) for analysis.

The study was approved by the School of Medicine, Makerere University Research and Ethics Committee (SOMREC), and a waiver of consent was obtained for testing the delinked samples.

## Results

From July to August 2013, 100 HBsAg-positive stored plasma samples with detectable viral loads were retrieved and analysed. However, amplification products from only 93 samples were obtained. In the seven samples that did not undergo DNA amplification, the viral loads varied from 37 IU/mL to 19,200 IU/mL. On the request forms information about age and gender was missing in, respectively, 21 and one patients. Fifty-six (60.9%) of the 92 requests that had gender recorded were from patients of female gender; most patients were young, with median age 25 (range 2–60) years. In 68 (73%) samples the viral load was >2000 IU/mL (Table 1).

### Distribution of hepatitis B viral load in the 51 patients with known HBeAg status

In the 51 patients where HBeAg testing was performed, HBV DNA viral load was significantly higher in those who were HBeAg-positive (nine patients) with a median viral load of 6.88 (95% confidence interval [CI] 4.38–8.08) log<sub>10</sub> IU/mL, compared to those who were HBeAg-negative (42 patients) of 3.88 (95% CI 3.38–8.08) log<sub>10</sub> IU/mL,  $P=0.008$ .

**Table 1.** Demographic and laboratory characteristics of the enrolled 93 patients whose stored plasma samples were successfully genotyped

Variable	Study distribution	
	Number	Percentage
<b>Median age* [Range]</b> 25 years [2–60]		
<b>Gender*</b>		
Female	56	60.87
Male	36	39.13
<b>Viral load groups [IU/mL]</b>		
<2000	25	26.88
≥2000	68	73.12
<b>HBeAg*</b>		
Negative	42	82.4
Positive	9	17.6

\* 21 were missing age, 1 was missing gender and 42 were missing HBeAg

**Table 2.** Distribution of hepatitis B genotypes in HBV-infected samples in Kampala, Uganda (n=93)

Genotype	Number	%
A	16	17.2
D	47	50.5
E	1	1.1
A/D	6	6.5
A/D/E	3	3.2
A/D/E/G	1	1.1
A/E	9	9.7
B/C/D	1	1.1
D/E/G	1	1.1
D/E	2	2.2
D/G	3	3.2
Indeterminate	3	3.2
<b>TOTAL</b>	<b>93</b>	<b>100</b>

## Hepatitis B genotypes

Genotypes A, B, C, D, E and G were identified in the 93 samples. The most common genotypes were genotype D, found in 47 (50.5%) followed by genotype A in 16 (17.2%) (Table 2). Mixed infection was seen in 26 (28%) of the samples with genotypes A/E being the most common (9.7%). In three samples the genotypes could not be determined.

## Discussion

Our study has shown that HBV genotypes A and D were the most prevalent in our population. We also demonstrated a wide range of mixed infections in the population studied. Two previous small studies of 27 samples each in Uganda also found predominance of genotype A [5,7]. Genotypes A and D have also been reported in Kenya and genotypes D, E and A in Sudan [6,10–12]. This indicates that the east African region predominantly has HBV genotypes A and D and this has also earlier been reported [3,4]. This could potentially explain the high incidence of hepatocellular carcinoma in the region as well as the high prevalence of

HBeAg-negative chronic HBV infection that has been reported by many studies in sub-Saharan Africa [13,14]. The high frequency of HBeAg-negative chronic HBV in predominant HBV genotype D could also be a result of mutations [15].

In our study, despite the high prevalence of HBeAg-negative chronic HBV infection, viral loads were generally higher than 2000 IU/mL. This means that, from a virological point of view, many patients would eventually require some form of treatment for their chronic infection. This is also compounded by the fact that genotype D tends to lead to more rapid progression of disease, consequently requiring more regular monitoring to determine indication of treatment. An Indian study showed a correlation between advanced liver disease and mutation in genotype D HBeAg-negative chronic HBV infection [15].

As would be expected, the viral loads were significantly higher in those who were HBeAg-positive than those HBeAg-negative.

Our study has several limitations. Seven of the samples did not undergo amplification on both primary and nested PCR, and this may have been related to low viral load for some of the samples. It may also have been related to inherent Taq polymerase hepatitis B virus DNA inhibitors or bloodborne inhibitors that may include haemoglobin and immunoglobulin G, which are thought to inactivate DNA polymerase, and/or capturing or degradation of the target DNA and primers [16]. Also, the probe used was not able to describe all the genotypes such as the genotypes I and J. Since this was a laboratory-based study depending on information provided on request forms that were not uniform, some information was missing. Parameters such as age and gender were not available for all patients and also, HBeAg assay was not performed on all the samples. However, within the limitations, the main outcome for the study, which was the genotype, was performed for all the samples. The patients were generally young and could have been either in the immune tolerant phase or immune reactivation phase of infection. This could not be differentiated as liver enzyme assays were not performed. In addition, HIV status was not determined as mixed infections have been reported in HIV-infected patients [17].

In conclusion, hepatitis B genotypes A and D were predominant in a sample of Ugandan patients infected with HBV, with about one-half of these patients having genotype D.

## Acknowledgements

### Conflict of interest

None declared by the authors.

### Funding

This study was financed using funding from European and Developing Countries Clinical Trials Partnership (EDCTP) Senior Fellowship Scheme, grant award TA. 2011.40200.004.

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