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GENOTYPES OF HBV AND HCV AMONG HIV-1 CO-INFECTED INDIVIDUALS IN NGONG SUB-COUNTY, KENYA.
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GENOTYPES OF HBV AND HCV AMONG HIV-1 CO-INFECTED INDIVIDUALS IN NGONG SUB-COUNTY, KENYA.

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ABSTRACT

Background: **Hepatitis B and Hepatitis C viruses are the major causes of liver disease worldwide. Co-infections with HBV and HCV have turned out to be increasingly very common among people living with HIV, leading to a major public health concern**
Objective: **To determine HBV and HCV diversity among HIV infected patients attending the Ngong sub-county hospital comprehensive care clinic.**
Design: **A cross-sectional study**
Setting: **HIV research laboratory in Centre for virus research at Kenya medical research institute, Nairobi**
Subjects: **HIV infected patients attending the comprehensive care clinic at Ngong sub-county hospital between May and August 2015.**
Results: **One hundred and ninety (190) HIV-1 positive patients participated in this study, consisting of 78.9% females and 21.1% males. Out of the 190 participants, 11(5.8%) were positive for Hepatitis B surface antigen and eight (4.2%) were positive for anti-HCV antibodies. 5/11 samples were positive for HBV DNA PCR and five belonged to HBV genotype A and E . However, none of the eight samples for HCV were positive for HCV RNA PCR.**
Conclusion: None of the patients was infected with HCV. HBV genotype A1 was the most dominant circulating genotype in Ngong sub-County followed by genotype E. Nevertheless, there could be other HBV genotypes circulating in Kenya especially among higher risk populations.

INTRODUCTION

An estimated 35 million people are currently infected with HIV globally, and of those, two thirds are co-infected with HBV and one third with HCV due to the similarity in the modes of transmission (1). The long-term consequences of chronic HBV and HCV infections include End stage liver disease, liver cirrhosis, and hepatocellular carcinoma (1, 2). Although the survival of HIV infected patients has markedly improved since the introduction of highly active anti-retroviral therapy and deaths from AIDS-related causes reduced, studies document that liver diseases caused by HBV and HCV co-infection as well as hepatocellular toxicity associated with anti-retroviral therapy has emerged as the leading cause of mortality (3). HIV infected Individuals who are co-infected with either HBV or HCV are at a higher risk of serious and life threatening complications such as hepatocellular carcinoma and end stage liver disease (1). Co-infection with hepatitis can also complicate the management of HIV infection, because clinical management of these chronic infections requires intensive treatment regimens, hence placing tremendous burden on resources limited countries such as Kenya and more importantly the infected patients (4). HBV have ten genotypes (A-J) based on nucleotide divergence while HCV have six genotype (1-6), each of which can be further subdivided into subtypes (5, 6, 7). In additional these genotypes have different geographic distribution (8). Determination of viral genotype is a key parameter for patients with chronic HCV infection because genotyping helps in predicting the duration of HCV treatment as well

as the response to therapy (9). However, the role of HBV genotyping in disease progression, response to therapy and disease severity is still unclear (10).

MATERIALS AND METHODS

Study design, setting and population: This was a cross sectional study that comprised of 190 HIV infected patients attending the comprehensive care clinic at the Ngong sub-County hospital. Patients were consecutively recruited and only those who met the inclusion criteria, that is, Patients of 18 years of age and above, volunteered and consented to participate in the study were recruited to participate in the study. A researcher-administered questionnaire was used to collect socio-demographic information and about 5 ml of blood was collected from all the participants and dispensed into EDTA vacutainer tubes, Blood was then centrifuged for five minutes at 3000 revolutions per minutes, and plasma was collected and dispensed in cryovials for storage at -20ºc until tested. Both Hepatitis B surface antigen (HBsAg) and Hepatitis C virus IgG (anti-HCV) were determined using Maglumi 1000, a fully automated chemiluminescence immunoassay according to the manufacturer's instructions (Shenzhen New Industries Biomedical Engineering Co., Ltd) (11). Ethical clearance was obtained from Kenyatta national hospital and University of Nairobi ethical review committee

Hepatitis C virus RNA extraction, RT-PCR and cDNA Amplification : Hepatitis C virus RNA was extracted from plasma samples that were positive for Hepatitis C virus IgG using QIaAmp RNA Mini kit (Qiagen, Hilden Germany) following the manufacturer's instructions (12). For each reverse transcription reaction, 5.0 µl of RNA wa added to the reaction mixture [2 µl of random primers,1 µl of dNPT mix, 5 µl of 5× QIAGEN one step RT-PCR buffer, 11 µl of RNase-free water, 1 µl Qiagen One-Step RT-PCR enzyme mix (Invitrogen, Carlsbad, CA, USA),]. Reverse transcription was performed using the GeneAmp PCR Systems 9700 (Applied Biosystems, Foster, CA, USA) using the following conditions: 50°C for 30 min, 95°C for 15 min, 35 cycles at 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and a final hold at 4°C(13). Primers KY80 (sense;5'-GCAGAAAGCGTCTAGCCATGGCGT-3') and KY78 (antisense; 5'-CTCGCAAGCACCCTATCAGGCAGT-3') were used for HCV RT-PCR. Each nested PCR reaction contained 12.5 µl of 2x dreamtaq PCR Master Mix (Invitrogen, Carlsbad, CA, USA) 1 µM of each primer and 5 µl of the cDNA to be tested in a final volume of 25 µl by adding DEPC-treated H_2O. PCR was be performed in the GeneAmp PCR System 9700 (AppliedBiosystems)using the following amplification conditions: 10 min at 95°C; 35 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min; 72°C for 10 min; and a final hold at 4°C. Primer Hep 21B (sense; 5'-GAGTGTYGTRCAGCCTCCAGG-3') and Hep 22 (antisense; 5'-GCRACCCAACRCTACTCTCGGCT-3') were used in the nested PCR. The PCR amplicons were then viewed on agarose gel .

Extraction and amplification of HBV DNA: Hepatitis B virus DNA was extracted from plasma samples that were positive for HbsAg using QIaAmp DNA Mini kit (Qiagen, Hilden Germany) following the manufacturer's instructions (14). HBV S gene was amplified by nested PCR using GeneAmp PCR System 9700 (AppliedBiosystems). Each PCR reaction contained 12.5 µl of 2x dreamtaq PCR Master Mix (Invitrogen, Carlsbad, CA, USA), 1 µM of each primer and 5 µl of the DNA to be tested in a final volume of 25 µl by adding DEPC-treated H_2O. The primer pairs S1A (sense; 5'-TCCTGCTGGTGGCTCCAG-3') and S1B (antisense;5'- CGTTGACATACTTTCCAATCAA-3') were used in the first round, and primers S2A (sense;5'-ACCCTGYRCCGAACATGGA-3') and S2B (antisense;5'-CAACTCCCAATTACATARCCCA-3') in the second round (14, 15). The amplification conditions for the first and second round PCR were: Initial denaturation for 5 min at 94°C; then 40 cycles of denaturation at 95°C for 30 s, annealling at 55°C for 1 min and extension at 72°C for 2 min followed by final extension of 72°C for 4 min then 4°C for infinity. The PCR amplicons were then viewed on agarose gel(15).

Nucleotide sequencing and geneotyping: Purified DNA products were directly sequenced using automated DNA sequencer ABI 377 (Applied Biosystems, Foster City, USA), using fluorescence-labelled dideoxynucleotide chain terminators (ABI Prism Big Dye™ Terminator Cycle Sequencing Reaction kit; Applied Biosystems) (16). About 1.25 µL of primers S2A (sense; 5'-ACCCTGYRCCGAACATGGA-3') and S2B (antisense;5'-CAACTCCCAATTACATARCCCA-3') were used for forward and reverse sequencing reactions, respectively. Pairwise contiguous sequences were generated using DNA Baser Sequence Assembler version 4.20.0 (Heracle Software, Germany). Consensus sequences were aligned with complete HBV genotypes A-J reference sequences from Genbank using ClustalW. A phylogenetic analysis was performed using MEGA version 6 (17).

Data analysis: Statistical analyses were performed using SPSS version 19.0 (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.). Descriptive analysis was done for the demographic variables using frequencies and proportions, and prevalence for HBV and HCV were

expressed as a percentage of the study population.

RESULTS

A total of 190 HIV-1 infected patients were included in the study. Out of these, 150 (78.9%) were females and 40 (21.1%) were males. Their mean age (SD) was 36.7 (10.3) years. Majority 72.6% were aged 30 years and above. Most (83.2%) had multiple sexual partners while 82.1% were on anti-retroviral drugs, and 95.3% were not vaccinated against HBV (Table 1)

Table 1
Distribution of socio-demographic characteristics of HIV infected patients attending the comprehensive care clinic at Ngong sub-County hospital.

Variable	Frequency (%)
Age, mean (SD)	36.7 (10.3)
Age	
<30 years	52 (27.4)
≥30 years	138 (72.6)
Gender	
Male	40 (21.1)
Female	150(78.9)
Education level	
No formal education	24 (12.6)
Primary	96 (50.5)
Secondary and above	70 (36.8)
Marital status	
Married	121 (63.7)
Single	69 (36.3)
Employment	
Informal	98 (51.6)
Formal	18 (9.5)
Not working	74 (38.9)
Income	
None	47 (24.7)
1-9999	107 (56.3)
10000-14999	18 (9.5)
≥ 15000	18 (9.5)
Sex partners	
Nil	32 (16.8)
≥1	158 (83.2)
Condom usage	
Yes	140 (73.6)
No	50 (26.4)
Frequency of condom usage (n=140)	
Always	108 (77.1)
Occasionally	32 (22.9)
Condom breakage	
Yes	31 (22.1)
No	159 (77.9)
Hepatitis immunisation	
Never	181 (95.3)
Completed dose	9 (4.7)
ART treatment	
Yes	156 (82.1)
No	34 (17.9)
CD4, median (IQR)	469 (317-582)
Viral load, , median (IQR)	150 (150-4509)

KEY:
ART: Anti-retroviral therapy
IQR: Interquartail range
CD4: Cluster of differential 4

Of the 190 samples, only eight were positive for anti-HCV antibodies and none of the eight were positive for HCV-RNA by PCR. Eleven samples were positive for HbsAg, out of which five were positive for HBV-DNA by PCR. Sequencing of HBV was successfully completed for the S gene of five HBV–DNA positive samples. Phylogenetic analysis of HBV sequences indicated the existence of HBV genotypes A1 and E as shown by table 2

Table 2

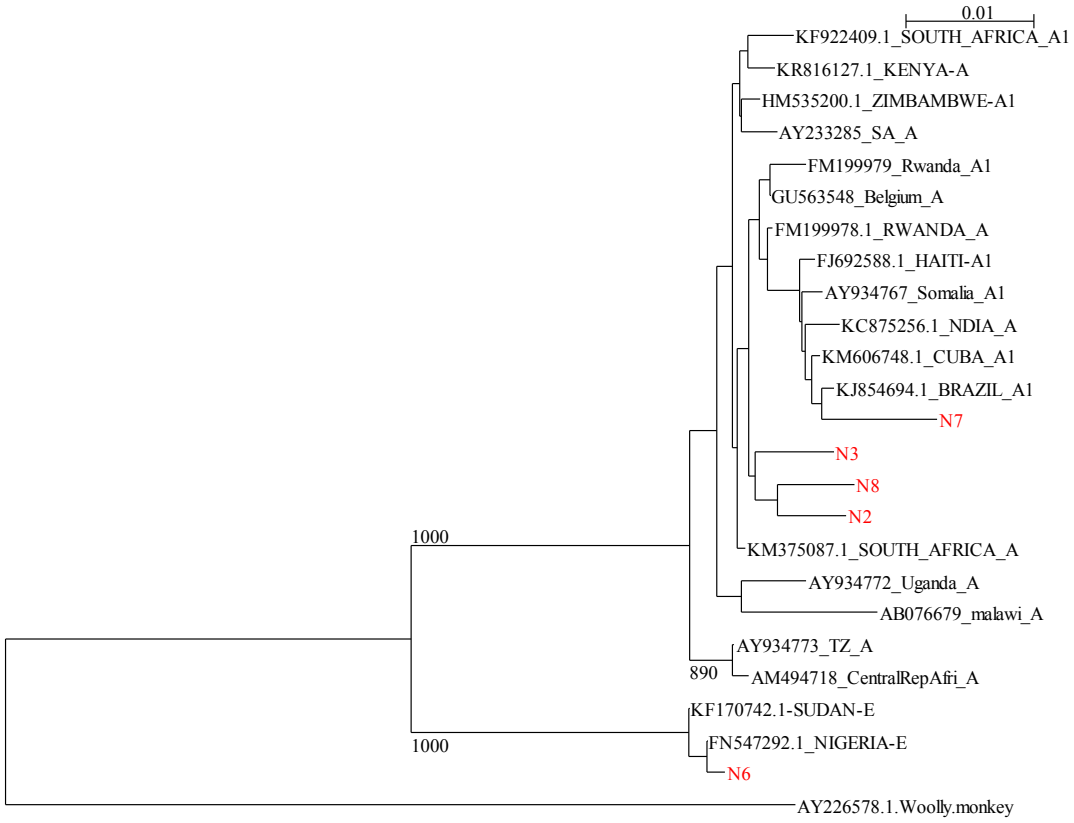
prevalence of HBV and HCV, and their genotypes among HIV infected patients attending the comprehensive care clinic at the Ngong sub-County hospital

characteristic	HbsAg positive (%)	HbsAg negative (%)	HBV PCR Positive (%)	HBV Geno-types (Sub-geno-type)	Anti-HCV Positive (%)	Anti-HCV Negative (%)	HCV PCR Positive (%)
Males	4	36	2	A (A1)	3	37	0
females	7	143	3	A (A1), E	5	145	0
Total	11 (5.8)	179(94.2)	6 (3.2)	-	8 (4.2)	182(95.7)	0

KEY:
HbsAg: *Hepatitis B surface antigen*
HCV: *Hepatitis C virus*
PCR: *Polymerase chain reaction*

Figure 1:

Phylogenetic tree of HBV isolates. Neighbour-Joining method based on 1000 bootstrap replicates and p-distances were used for generating the phylogenetic tree [23]. References from Genbank together with their country of origin are presented as accession numbers. Woolly monkey HBV (AY226578-WMHBV) was used as the out-group. HBV isolates from study participants are in red. Bootstrap values above 70% are indicated.



DISCUSSION

In this study, we determined the genotypes of HBV and HCV among HIV-1 infected patients attending the comprehensive care clinic at Ngong sub-County hospital in Kajiado County. Hepatitis B virus genotype A1 was detected in four samples while one sample belonged to genotype E. The findings of this study are in agreement with the findings from previous study carried out in Kenya among blood donors where HBV genotypes A1 and E were detected (22). However, a study conducted among HIV infected in Nairobi detected only HBV genotype A1 (18). Another study conducted among commercial sex workers in Mombasa reported HBV genotype A1 as the circulating genotype (19, 20). In addition, a study carried out among IDUs and non-IDUs that were either HIV positive or negative in the Kenya coastal region, reported HBV genotype A1 to be the most dominant circulating genotype in Kenya (21).

However, previous studies carried out in Kenya among Blood donors were able to detect HBV genotype D in addition to genotypes A and E (22). In a cohort of hepatitis patients in Egypt HBV genotype D and F were reported to be the most prevalent genotypes (14). A number of studies conducted in West African countries have reported the presence of HBV genotype D and E in additional to genotype A which the most dominant genotype in Africa (23-24) The possible reason why five samples did not turn positive for HBV-DNA PCR despite being positive for HbsAg could be due to mutation. Further, the use of HbsAg marker as the only marker for HBV diagnosis is insufficient for categorising patients according to the various stages of HBV infection. This drawback may lead to either undiagnosis or Misdiagnosis, thus posing a challenge in disease detection as well as monitoring (26-28).

None of the eight samples that tested positive for Anti-HCV IgG turned positive for HCV PCR. However, a study conducted among drug user's in Kenya reported HCV genotypes 1 and 4 as circulating HCV genotypes in Kenya (29). In another study conducted among HIV infected patients in Nigeria HCV genotype 1a was reported as the circulating genotype (30). In Gabon HCV genotypes 4a and 4b were reported among pregnant women (12).

Although no HCV genotype is reported in this study, there exist HCV genotypes in Kenya especially among the higher risk population. Therefore, there is need for more research in order to determine regional prevalence and genotypes of HCV in Kenya. Variant HCV because of mutations, false-positive serology results, or improper serum storage are possible explanations for these HCV PCR results (31). In this study specimens were collected and transported following the standardised procedures according to the study protocol. Plasma samples were frozen under proper conditions in the repository with minimal manipulation. Therefore, it is unlikely that storage conditions might have affected our results.

There was predominance of women in this study, which is a reflective of higher female HIV burden in Kenya as well as the observation that men find it harder to reveal their HIV status, leading to poorer health-seeking behaviour in seeking HIV care services. However, the role of gender in the disparity of HBV and HCV burden is not fully known.

This study had limitations. First, the study did not determine the HBV and HCV viral loads. Secondly, this study determined HBV and HCV PCR for samples that tested positive for HbsAg and anti-HCV antibodies, therefore missing out occult infection. In addition, other HBV serological markers were not included in this study.

In conclusion, HBV genotype A1 is the most dominant circulating genotype in Ngong sub-Country followed by genotype E. In addition, the prevalence of HCV remains very low in Kenya and the serological assays alone are not sufficient in diagnosis of HCV infection due to low sensitivity, specificity and cross reactivity leading to false positive results.

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