

East African Medical Journal Vol. 98 No. 2 February 2021

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HEPATITIS B AND C VIRUS CO-INFECTIONS AND GENETIC DIVERSITY AMONG HIV-1 INFECTED INDIVIDUALS IN SIAYA COUNTY, KENYA

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ABSTRACT

Objectives: The present study determined the prevalence of HBV/HCV co-infections; genetic diversity and drug resistance of HBV among HIV infected patients visiting Siaya County Referral Hospital, Kenya.

Design: This was a hospital based cross-sectional study.

Setting: This study was conducted at Siaya County Referral Hospital Laboratory and KEMRI HBV laboratory, Nairobi, Kenya.

Subjects: A total of two hundred and twenty-five (225) HIV patients randomized from HIV comprehensive clinic of Siaya County Referral Hospital between August and December, 2018.

Results: From the 225 samples that were analyzed, 6.2% (14/225) were HBV/HIV co-infected while that of HCV/HIV was 4.0% (9/225). However, no participant was co-infected with three viruses. Of the 11 samples that were successfully sequenced, the phylogenetic analysis revealed the sequences belonged to HBV genotype A1. Mutation rt169F was detected in one of the patient.

Conclusion: From this study, HBV/HCV and HIV co-infections could be higher than reported here. HBV genotype A1 is the most predominant circulating genotype in Siaya County. All the detected HBV were susceptible viral strains with only one harboring HBV strain with rt169F mutation. There is therefore a need for a continuous surveillance of HBV/HCV/HIV co-infections, circulating HBV genotypes and drug resistant variants in this region in order to guide vaccine and optimization of treatment.

INTRODUCTION

Human Immunodeficiency Virus (HIV) remains a major public health concern globally having claimed more than 32 million lives so far (1). It is estimated that about 37.9 million of world's population are living with HIV as of 2018 with 1.7 million new infections yearly (1). Majority of these cases are found in sub-Saharan Africa where about 25.6 million are infected and about 1 million new infection in 2018 (1). In Kenya HIV prevalence has remained relatively stable at 5.6% by end of 2019 with prevalence of HIV infections in Siaya County remaining high (15.3%) (2). Viral hepatitis B and C (HBV and HCV) co-infections with HIV is becoming common due to their shared routes of transmission. These infections are critical to the global public health with infection levels of about two billion people and 130 million HBV and HCV infected population respectively (3). In Kenya, HBV prevalence in general population is 8% with HCV being >2%. However, in high-risk population, the prevalence is higher with highest levels of 9.6% and 32% being reported for HBV and HCV respectively (4, 5). These co-infections predispose patients to a variety of opportunistic infections following immune system suppression by the HIV virus (6). Genetic diversity of HBV in HIV infection is of great clinical significance with ten HBV genotypes (A-J) having been reported distributed worldwide (5, 7). HBV genotypes A-E have been widely identified in Africa with genotypes A, D and E having been detected in Kenya with genotype A (A1) as the most Predominant (5). Studies on circulating HBV genotypes in Kenya have been conducted mostly in urban centres of Nairobi and Mombasa cities. Analysis of circulating genotypes in Siaya County is still limited. The

primary antiviral agents for treatment of chronic HBV infection are nucleotide/nucleoside analogues (NAs) such as lamivudine (3TC), tenofovir (TDF), telbivudine (LdT), entecavir (ETV), efavirenz (EFV) and adenofovir dipivoxil (ADV) due to their high viral suppress by inhibiting HBV reverse transcriptase (RT) (8). However, the main draw-back is the appearance of drug resistance mutations following long-term treatment with NAs. This study was therefore conducted to evaluate the prevalence of HBV, HCV infections, circulating HBV genotypes and drug resistance in Siaya County but among HIV infected patients. It is expected that generated information could be critical in management of viral hepatitis B and C infections and surveillance of circulating genotypes in this region.

MATERIALS AND METHODS

A cross-sectional study was conducted on HIV infected individuals visiting Siaya County Referral Hospital (SCRH). Approximately 5ml of venous puncture blood was collected into 10ml vacutainer tubes (Becton Dickson, San Jose, California) from each consenting study participant and used for analysis. The blood samples were screened for HBsAg and anti-HCV using Onsite HBsAg Rapid Test Cassette and Onsite Rapid Test Kit for qualitative detection of anti-HCV IgM (CTK Biotech, Inc, San Diego, USA) following the manufacturer's instruction (Table 1).

Viral DNA extraction and amplifications of HBV-pol gene: Viral DNA was extracted from HBsAg positive plasma samples using QiaAmp™DNA Mini kit (Qiagen Inc, Valencia, USA) according to manufacturer's guidelines (5). Partial HBV *pol* gene was

amplified in nested PCR using specific sense and antisense primers for both first and second rounds PCR. Primers HBPr1 (position: 2850-2868, 5'-GGGTCACCATATTCTTGGG-3') and HBr135 (position: 803-822, 5'CAAAGACAAAAGAAAATTGG-3') in first round and HBPr2 (position: 2867-2888, 5'-GAACAAGAGCTACAGCATGGG-3') and HBPr3 (position: 3226-3246, 5'-CCACTGCATGGCCTGAGGATG-3') in the second round of the PCR (5).

Amplification was performed using master mix Hot start PCR in reaction of 25 μ L consisting of 12.5 μ L of 2 X phusion high-fidelity master mixes, 5.0 μ L of DNA, 0.625 μ L of each sense and antisense primers and 6.25 μ L of distilled water. PCR conditions were: one cycle at 94°C for 10 minutes, 40 cycles at 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 1 minute. The final extension was performed at 72°C for 10 minutes in both PCRs (5). The PCR products were visualized using agarose gel electrophoresis by staining amplicons with ethidium bromide (0.05%) and loaded for visualization using trans illuminator (UVP, San Gabriel, A, USA) ((5, 9). The amplicons were then directly sequenced using automated DNA sequencer ABI 377 (Applied Biosystems, Foster City, USA) using BigDye Terminator kit (Applied Biosystems). Pair wise contiguous sequences were generated using DNA Baser sequence assembler version 4.20.0 (Heracle Software, Germany).

The HBV genotype was determined by phylogenetic analysis of the HBV *pol* gene sequence. The generated sequences were aligned with reference sequences of previously described HBV genotypes retrieved from Gene bank database to construct phylogenetic tree based on partial HBV *pol* gene by CLUSTAL W (version 1.81) and neighbor-Joining method, following Kimura's two- parameter distances

using Molecular Evolutionary Genetic Analysis (MEGA X software). Bootstrap resampling was performed at 1000 replicates (5) (Figure 1).

Statistical analysis: Data analysis was done using scientific programme for social sciences (SPSS) version 24.0 and prevalence of HBV/HIV and HCV/HIV co-infections are expressed in percentages. The association between the presence of HBV/HIV, HCV/HIV and gender, age, marital status, academic level and area of residence was determined using Pearson's chi-square test with the significant p-value of $p \leq 0.05$.

Phylogenetic analysis: Pair wise contiguous sequences were generated using DNA Baser sequence assembler version 4.20.0 (Heracle Software, Germany). The generated sequences were aligned with viral hepatitis B strains A-J reference sequences from Gene bank to construct phylogenetic tree based on partial

HBV *pol* gene by CLUSTAL W (version 1.81) and neighbor-Joining method, following Kimura's two- parameter distances based on 1000 bootstrap replicates using Molecular Evolutionary Genetic Analysis (MEGA X version 10.0.4) (5). HBV-*pol* sequences were analysed for drug resistance using *insilico* tool platform;

<https://hbvdb.ibcp.fr/HBVdb/HBVdbResistance> and (<http://hivdb.stanford.edu/HBV/HBVseq/development/HBVseq.html>). (10).

Ethical Consideration: This study was ethical approved by Kenyatta University Research and Ethical Review Committee (KU-ERC) (Ref: KU/ERC/APPROVAL/VOL.I (149) and permitted by Siaya County Referral Hospital Institutional Review Committee (IRC) (Ref: SYA/MED/VOL.I (99) before its commencement.

RESULTS

Socio-demographic variables of studied population:

Among the 225 participants in this study, males were 68 (30.2%) while the females formed the majority, 157 (69.8%). The ages of the participants ranged between 3 and 76 years old. The average age of the patients was 38.26 ± 15.46 years (mean \pm SD) whereas the mean ages of female and male patients were 36.29 years (SD \pm 15.21) and 42.82 years (SD \pm 15.18) respectively. Most of the patients were married adults (80.9%) (Table 1). Majority of the participants had at least post primary education and were residence of Siaya Township. However, on the treatment line, only 11(4.9%) of the participants were on second-line treatment of HIV infection whereas majority 214 (95.1%) were on first-line treatment of HIV infection.

Prevalence of HIV/HBV and/or HCV co-infections:

From 225 plasma samples that were screened, 14 (6.2%) were infected with HBV/HIV while HCV/HIV were 9 (4.0%). None of the participants had trio-infections in this study.

Co-infection was higher ($p = 0.059$) among the male patients (16.2%) as compared to the females (7.6%), though this was not statistically significant. The prevalence of HBV/HIV co-infection among male and female patients showed 7.4% and 5.7% respectively. However, this was not significant ($p = 0.764$) as both gender were infected. Age was not a predisposing risk factor ($p = 0.964$). On the other hand, residential place was found to be a risk factor with most co-infected patients from North Alego $p = 0.023$ (Table 1). Marital status and level of education were also found to be insignificantly associated with the risk of being HIV/HBV co-infected. The detected prevalence of HCV-HIV co-infection among male and female patients was 8.8% and 1.9% respectively. This was statistically significant ($p = 0.024$). The highest HCV/HIV co-infected age group was children in the ages of 5–15 years (12.5%). This was, however, not scientifically significant ($p = 0.344$). The prevalence of HCV/HIV co-infection among the single study participants was significantly high ($p = 0.013$) (Table 1).

Table 1*Demographic characteristics of the study participants attending Siaya county referral hospital, Siaya*

Categories	N = 225	HIV-Mono	HIV-Co	P-Value	HIV/HBV	P-Value	HIV/HCV	P-value
Gender								
Males	68 (30.2)	57 (83.8)	11(16.2)		5 (7.4)		6 (8.8)	
Females	157 (69.8)	145 (92.4)	12(7.6)	0.059	9 (5.7)	0.764	3 (1.9)	<0.024*
Age group (yrs)								
< 5	2 (0.9)	2 (100)	0 (0.0)		0 (0.0)		0 (0.0)	
5 – 15	16 (7.1)	13 (81.2)	3 (18.8)	0.629	1 (6.3)	0.964	2 (12.5)	0.344
15 - 24	25 (11.1)	22 (88.0)	3 (12.0)		2(8.0)		1 (4.0)	
>25	182 (80.9)	165 (90.7)	17 (9.3)		11 (6.0)		6 (3.3)	
Marital Status								
Married	146(65)	133 (91.1)	13 (8.9)		10 (6.8)		3 (2.1)	
Single	38(17)	34 (89.5)	4 (10.5)	0.312	1 (5.3)	0.961	3 (15.8)	<0.013*
Divorced	19(8)	15 (78.9)	4 (21.1)		2 (5.3)		3 (7.9)	
Widowed	22(10)	20 (90.9)	2 (9.1)		1 (4.5)		0 (0.0)	
Academic Level								
Primary	56(25)	51 (91.1)	5 (8.9)		3 (5.4)		3 (5.4)	
Secondary	116(52)	105 (90.5)	11 (9.5)		5 (4.3)		6 (5.2)	
Post-secondary	48(21)	42 (87.5)	6 (12.5)	0.801	6 (12.5)	0.224	0 (0.0)	0.409
None	5(2)	4 (80)	1 (20)		0 (0.0)		0 (0.0)	
Residence								
Township	99(44)	93 (93.9)	6 (6.1)		3 (3)		4 (4)	
North Alego	24(11)	18 (75)	6 (25)		5 (20.8)		1 (4.2)	
Central Alego	23(10)	20 (87)	3 (13)		3 (13)		0 (0.0)	
West Alego	24(11)	23(95.8)	1 (4.2)	0.053	0 (0.0)	<0.023*	1 (4.2)	0.497
S.E Alego	28(12)	23 (82.1)	5 (17.9)		2 (7.1)		3 (10.7)	
Usonga	17(8)	16 (94.1)	1 (5.9)		1 (5.9)		0 (0.0)	
Others	10(4)	9 (90)	1 (10)		0 (0.0)		0 (0.0)	
Regimen								
Line-One	214(95.1)	192 (89.7)	22 (10.3)	1				
Line-Two	11(4.9)	10 (90.9)	1 (9.1)					

* $P \leq 0.05$

HBV genetic diversity: The phylogenetic analysis of the 11 samples that were successfully analysed revealed that all the sequences belonged to HBV genotype A1 (Figure 1).

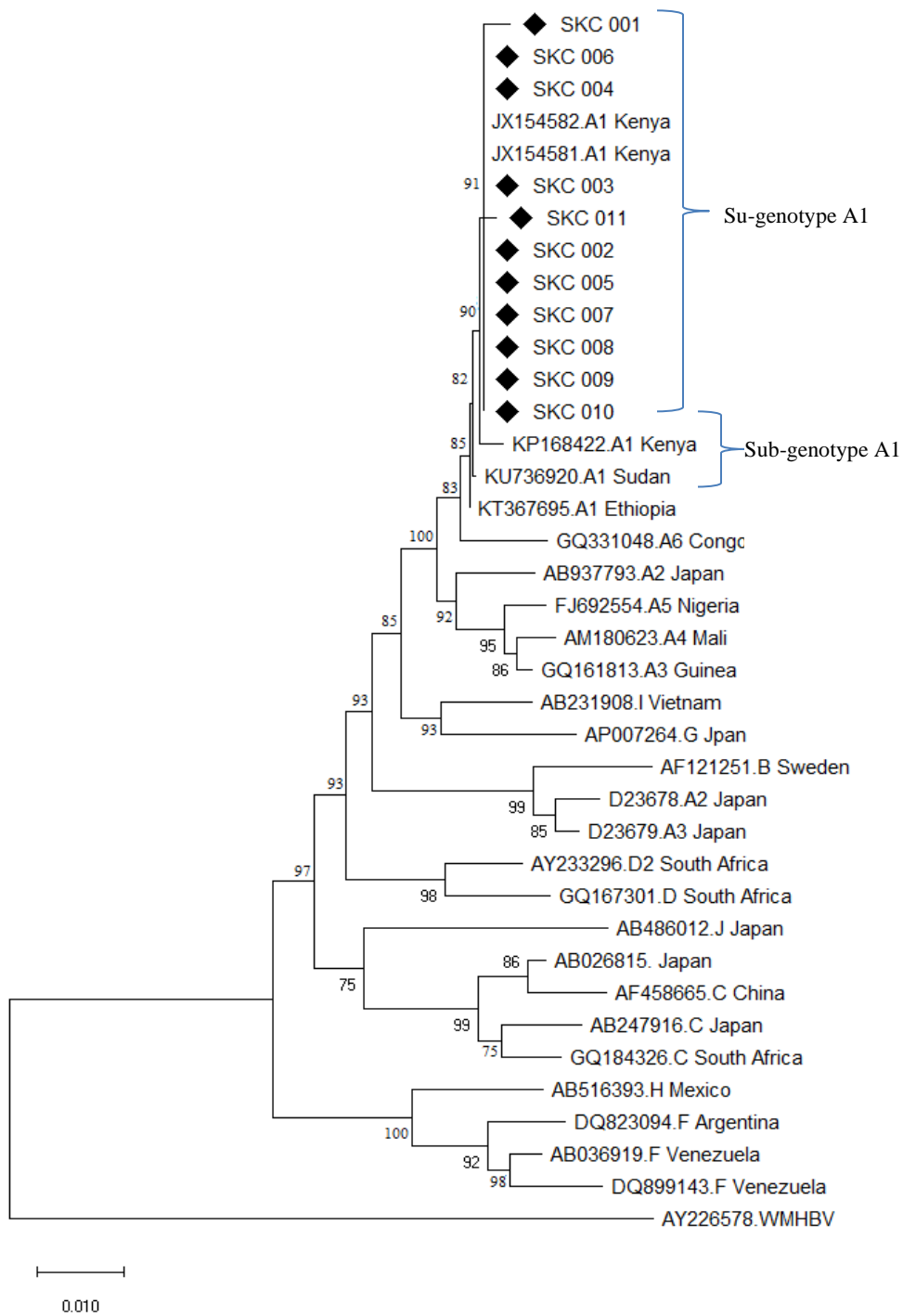


Figure 1: Phylogenetic tree of HBV-pol gene sequences. Neighbor-joining method was used to generate the phylogenetic tree based 1000 bootstrap replicates. Woolly Monkey HBV (AY226578-WMHBV) was used as an out group. Relative bootstrap values > 70% are indicated. HBV isolates from study participants are indicated with diamond sign.

Hepatitis B virus Drug resistance: From the two hundred and twenty-five (225) patients that were recruited to participate in this study, 214 (95.1%) of the patients were on first line of HIV antiretroviral therapy, a combination that contains lamivudine (TDF+3TC+EFV). HBV

mutation rt169F was detected in one participant. However, the rest of the 10 Individuals were infected with HBV drug susceptible strains. The mutation rt169F is a mutation that is not yet associated with any drug resistance.

Table 2

Patterns of hepatitis B virus mutations among study participants

Mutation patterns	n(11)	Frequency (%)	Drug associated
rt169F	1	9	No drug associated
No major mutations	10	91	Susceptible

DISCUSSION

In this study we determined the prevalence of HBV, HCV among HIV infected patients seeking health services in Siaya County referral hospital, Siaya, Kenya. From the study findings, the detected prevalence of HBV/HIV co-infections (6.2%) was found to be higher compared to previously (0.83-2.88%) obtained in Nyanza region (10). The findings are, however, consistent with those previously obtained in Kenya Nairobi 7.2% (12), 6.0% (13), and 5.7% Eldoret (14) and elsewhere in Nigeria (5.7%) (15), Tanzania (7.3%) (16), Mozambique (9.1%) (17) and Zambia (11.3%) (18). These levels of infections of HBV could be associated with target study population of HIV infected patients seeking medical care in the sampled regions following improved access to antiretroviral therapy that has led to their prolonged life span hence increased risk to HBV infection. However, the findings from this study were also in contrast with those previously conducted in Nairobi, (0.7%-1.1%) (19) and elsewhere in Ethiopia and Tanzania (2.0%-2.3%) (20-21). Nevertheless, our findings were also lower compared to those previously obtained in Kenya 50.6%, however, this study involved clinical jaundiced patients and

already infected cases explaining the probability of high infected cases (22). In this study, most of the HBV/HIV co-infected individuals were residents of North Alego region (Table 1). This region is largely rural; probably explain possible association with significantly infections due to lack of knowledge on risk was associated with HBV infections. In addition, Siaya region like any Luo region, residents are known with some cultural and behaviour practices that could be risk to these infections like widow inheritance, polygamy, multiple sexual partners and permissiveness in boy sexuality (23).

For HCV/HIV co-infections, the overall prevalence of HCV/HIV (4.0%) in Siaya was found to be higher than the national prevalence of 2% (11). Though high risk could be in existence among studied population, the levels of HCV infections needs further surveillance. Siaya County is among the counties in Nyanza that have high prevalence of HIV. Its transmission is shared with HCV hence factors associated with high prevalence rates in this region; injecting drug use (IDUs), men who have sex with men (MSM) or prostitution or sex for fish and sex-based socio-cultural practices could similarly predispose them to these infections (23). Similar levels of

HCV infections in Siaya County have been obtained elsewhere in Ethiopia (5.5%) (20) and Cameroon (7.2%) (24). On the other hand, our findings were in contrast with the previous study findings in some parts of Kenya (0.4%-1.6%) (19) and elsewhere in Gambia (0.6%) (25), Libya (0.9%) (26) and Nigeria (1.7%) (27).

Nevertheless, the findings were found to be lower compared to those previously reported in Kenya (10.3%) (13) and those in Gabon (9.2%) and Burundi (11.3%) (28). High prevalence levels observed in these studies are associated with high-risk populations. Gender and marital status were significantly associated with HCV/HIV co-infections. This was associated with sexual behaviours of these category of population as well as males tend to engage in high-risk activity like IDUs (23). However, age of the patients was not associated with infection, although, co-infection was insignificantly common among adults with cases of infections increasing with increase in age similar to a study among pregnant women in Rwanda (29).

From the HBV phylogenetic analysis of the 11 sequences obtained from the study, all sequences were of HBV genotype A1. These findings confirm the consistent predominance of Hepatitis B virus genotype A1 in the country (9). In this study all the 11 sequences clustered together with references sequences from Kenya, Sudan and Ethiopia. This suggests origins transmission with Kenya and her neighbouring countries. The drivers of this transmission could probably be associated with population migration within these countries (9, 22). The detection of only existing HBV genotype A1 in this region confirms its sustainability in the region by inbreeding of this infection within the region (12). Nevertheless, Siaya being one of the counties with highest HIV prevalence, by virtue of the shared transmission mechanism, the

population behavior of the residents could be a driving force in facilitating this infection among residents. These finding confirms previous studies that have been conducted in most urban centres that drives the transmission networks of this virus across the country (5, 9, &12). Detection of hepatitis B genotype A and sub-genotype A1 reaffirm the predominance of HBV genotype A in Kenya. The successive detection of HBV genotype A in the country is an indicator of transmission within Kenyan border (12). Sub-genotype A1 was first detected in southern Africa and subsequently in other African countries a suggestion of possible transmission dynamics or much emphasis on monitoring HBV genotypes surveillance across countries especially with this era of HIV infections (22). In spite of HBV genotype, A being predominant in the country, genotype D and E have also been reported in Kenya from previous studies which could be existing even though none was detected in this study (22). Their detection suggests that there could be other HBV genotypes circulating in Siaya County than reported in the present study.

In this study, we also analysed HBV drug resistance from the 11 HBV isolate sequences. From the drug resistance evaluation, no drug associated mutations were detected. This confirms good response to treatment of HBV infection hence implying possible recovery to infection. However, one patient had mutation at position 169 (rt169F), a mutation that is not yet associated with any drug resistance. The occurrence of this mutation could be due to HAART pressure on rt gene on HBV. Compared to similar studies conducted in the country, this finding is contrary, since a low level of lamivudine-resistant HBV were detected among patients on a 12 month follow up (30) comparable to a study in Gambia (14.3%) (31). The differences could mean a

possible suggestion that most of these HBV/HIV co-infected patients could be due to previous treatment strategy for HIV failing to capture HBV from onset hence possible upsurge of drug resistance due to drug non adherence.

Nevertheless, this finding was also contrary to previous studies in Kenya and china that reported high levels of lamivudine-resistant HBV at 46% and 89.4% respectively (32). The high levels of lamivudine resistance observed were associated with long treatment period and possible non adherence to treatment.

Although this study could be limited by small sample size that may not represent the entire Siaya County, we conclude that the prevalence of HIV/HBV and HIV/HCV co-infections could be high with HBV genotype A1 being the most predominant. This study indicates a likelihood of high proportion of HIV co-infections in this region. The recorded level of HIV Co-infections therefore calls for a routine surveillances for HIV, HBV and HCV infections in Siaya County. Despite low lamivudine-resistant HBV observed in this study, the sustained use of 3TC drug as the primary HBV active drug for highly active antiretroviral therapy (HAART) could possibly result into an increased HBV resistant strains which may impact negatively on HBV treatment in the region.

Sequence data

The HBV pol sequences were deposited at the GeneBank under the following accession numbers: MK903343- MK903353.

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