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Prevalence and Associated Risk Factors of Hepatitis B Virus Infections Among HIV-1 Infected Patients Attending the Comprehensive Care Clinic in Malindi Sub-County Hospital in Kenya

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Abstract
Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV) co-infections are common all over the world. Infection with HIV increases rates of HBV chronicity, prolong the time the HBV stays in circulation and increase liver-related morbidity. Factors such as intravenous drug use, multiple blood transfusions, presence of tattoos, unsafe sexual practices and being health workers have been implicated as drivers of infection & transmission of HBV & HIV. This study aimed to determine the prevalence and genotypes of HBV associated risk factors among HIV infected patients in a descriptive cross-sectional study. Malindi was chosen as a suitable study site because of the high numbers of residents involved in sex tourism as well as intravenous drug use. A structured questionnaire was used to capture social demographic data such as age, gender, employment status, occupation, the level of education and marital status, clinical history information such as duration since diagnosis with HIV, ART drug history, duration taking ARVs and baseline CD4 count and risk factors associated with HBV infections such as intravenous drug use, history of blood transfusion, tattooing/scarification, and the sexual history from 446 consenting randomly selected HIV infected participants. Five millilitres of whole blood was obtained from each participant, 50µl of which was used for CD4 cell counts using a flow cytometer. HBsAg serology was done using Diaspot® rapid diagnostic test and confirmed by Hepanostika® HBsAg Ultra ELISA kit (BioMérieux SA) and HBV DNA was extracted from all HBsAg positive samples. Nested polymerise chain (PCR) reaction and sequencing of the Pre S1 region was done. Sample sequences were compared with published HBV genotypes sequences from GenBank and Phylogenetic trees were constructed using the NJ Plot software using a PHB file created through DNA Database of Japan (DDBJ) to determine the HBV genotypes. Out of the 446 HIV positive participants, 126 (28.3%) were males and 320 (71.7%) females. Only 19/446 (4.26%) participants were positive for HBV based on rapid strip test while 22/446 (4.93%) participants had HBV based on ELISA. Twelve of the 22 ELISA positive samples were successfully amplified by PCR. Out of the 12 PCR positive samples 10 were successfully sequenced. Phylogenetic analysis revealed that 9/10 (90%) samples belonged to genotype A while 1/10 (10%) belonged to genotype E. Males (p=0.028) and intravenous drug use (p= 0.08) were significantly associated HBV infections. The high prevalence (4.9%) of HBV among HIV patients attending Malindi Sub-county hospital is most likely highly driven by intravenous drug use and multiple sexual partners among the male gender and is predominantly genotypes A and E which is similar to the general population.
Keywords: Hepatitis B virus, HIV, Co-infection, HBsAg, genotypes, intravenous drug use

Introduction

Hepatitis B virus (HBV) and Human Immunodeficiency Virus (HIV) are major public health problems and are among the 10 highest causes of infectious deaths in the world (Alter, 2006). The two viruses share similar routes of transmission (Alter, 2006, Otedo, 2004) with HBV having a hundred fold efficiency in transmission compared to HIV (Otedo, 2004). Individuals with HIV-HBV co-infection have higher HBV DNA levels (Dore *et al.,* 2010) which is associated with the immune deficiency due to HIV which in turn translates to increased HBV disease progression (Diwe *et al.,* 2013). On top of this, increased mortalities and morbidity in HIV patients as a result of liver disease are partly due to co-infection with HBV and HCV since these viruses increase intra-hepatic apoptosis which is a consequence of liver fibrosis (Diwe *et al.,* 2013).

In the world, it's approximated that 33 million people are infected with HIV and among these about 3 million (10%) are co-infected with HBV (Kourtis *et al.,* 2012). In Kenya the prevalence of HBV-HIV co-infection has been reported to be as high as 6% and significantly higher in males 19.2% compared to 14.2% in females in a study done among several Nairobi based health centres. (Muriuki *et al.,* 2013).

HBV has a wide genetic variability which has resulted into the emergence of 10 genotypes with several sub-genotypes which are labelled alphabetically from A-J (Tangkijvanich *et al.,* 2013). There is remarkable evidence suggesting that HBV genetic variability is an important factor in determining disease progression and also response to antiviral treatment (Poukarim *et al.,* 2011). In East Africa where Kenya resides, genotype A is the most

prevalent (88%) followed by genotype E (8%) and then Genotype D (4%) (Mwangi *et al.,* 2008). Webale et.al (2015) reported 100% genotype A1 among HIV-1 infected and non-infected injecting and non-injecting drug users in coastal Kenya. Genotype A1 had also been reported exclusively among HIV infected IDUs from Mombasa, a region along the Kenyan coast. (Kibaya et al., 2015)

HBV is more prevalent among HIV–infected individuals than in the general population which can be attributed to the shared risk factors for transmission and acquisition of these two life threatening viruses (Chen *et al.,* 2013). Some of these risk factors associated with the transmission of HIV and HBV include: involvement in intravenous drug use, scarification/tattooing, having several heterosexual partners/involvement in commercial sex, male having sex with men and occupational hazards for health workers (Alter, 2006, Chen *et al.,* 2013, Ranjbar *et al.,* 2013).

The primary aim of this study was to determine the prevalence of HBV and circulating genotypes among HIV positive participants attending the comprehensive care clinic at Malindi Sub-county hospital. The diversity of countries of origin of the tourists to this area and their interaction with the local people would arouse the epidemiological curiosity about the introduction of new strains, their genetic diversity and the implications on the clinical presentation of disease in cases where patients are co-infected. Characterization of HBV among the HIV patients will help in understanding the distribution of specific genotypes in the study setting. The genetic pattern of the virus will in turn help policy makers and clinicians in making sound decisions during prescription of drugs to these patients since different HBV genotypes have different responses to the antiretroviral drugs.

Methods

Study site and patients recruitment

This was a descriptive hospital based cross sectional study carried out between May 2015 and April 2017 at the Comprehensive Care Centre (CCC) at the Malindi sub-county hospital. Malindi was selected as a suitable study site because it is located along the coastal strip of Kenya, a region that is popular with tourists both local and foreign. Foreign tourist in Malindi come from all over the world with the Italians, Germans and those from the United Kingdom topping the list. This region is on record for having a fairly large population of persons who engage in sex tourism as well as recreational intravenous substance abuse and in some cases they may share needles to inject themselves with the narcotics and/or engage in unprotected sexual contact. Simple random sampling was used to recruit patients aged 18 years (due to ethical issues) and only those from the CCC. Any vulnerable persons such prisoners and the mentally challenged were not recruited for the study. Participation was upon providing a written informed consent. Those who were randomly selected and did not consent were not recruited and hence replaced.

Data on socio-demographic, clinical and associated risk factors

A study questionnaire was designed to obtain information from patients and also from the patient's hospital records. The information captured in the questionnaire included: social demographic characteristics including Age, gender, employment status, the level of education and marital status, Clinical history of patients including Duration since diagnosis with HIV, ART drug history, duration taking ARVs and baseline CD4 count. The questionnaire also sought to obtain information on risk factors associated with transmission of HIV and HBV including Intravenous drug use, history of blood transfusion, tattooing/scarification, and the sexual history. The questionnaire was administered in a confidential counselling room by a professional counsellor who was recruited for this study.

Sample collection, processing, shipping and storage

Five millilitres of blood was collected aseptically into EDTA vacutainer bottles (BD New Jersey, USA) using venepuncture. Fifty microliters (50µl) of the sample were analysed for CD4 counts within 24 hours of collection through flow cytometry using the BD Facs Caliber machine. The samples were then separated using centrifugation at 3000 RPM for 10 minutes and the plasma stored at -20 C. The samples were then packaged using the basic triple packaging system according to the International Air Transport Association (IATA) regulations. This included primary receptacles (screw capped containers) which were leak proof covered with a layer of absorbent material, a secondary receptacle (zip lock bags) which was also leak proof enclosing the primary receptacle and finally a leak-proof outer packaging i.e. cool box labelled with an easily noticeable biohazard symbol. The samples were then transported to KEMRI under a cold chain through a paid for courier services. After un-packaging they were stored at -80 C until serological and molecular tests were done.

HBV serology.

The samples were screened for HBsAg using the DiaSpot® HBsAg test kit which is a rapid one step test for the qualitative detection of HBsAg and Hepanostika® HBsAg Ultra ELISA kit (BioMérieux SA) which is based on a "sandwich" principle. The tests were done according to the manufacturer's instructions.

DNA extraction and PCR amplification of HBV PRE S1 region

DNA from the 22 samples that tested positive for HBsAg was extracted from 50µl of plasma using Qiagen® DNA extraction kit (Qiagen) using the manufactures instructions. Extracted DNA was used as template in a PCR to amplify the Pre S1 (681bp) region by nested PCR using a set of primers (table1). To describe this briefly, the master mix for the first amplification included 2.5µl of 10x PCR buffer, 2.0µl of 10mM dNTPs, 0.3µl of first set of primer S1F and S1R (Table 1), 1µl Taq polymerase and 5µl of extracted DNA.

Table 1: Primers used for HBV PCR amplification and sequencing

Primers	Nucleotide position	Sequences	Expected fragment size region (bp)
PS1 forward PS1 Reverse	55-72 995-974	TCCTGCTGGTGGCTCCAG CGTTGACATACTTTCCAATCAA	940
PS2 Forward PS2 Reverse	155-173 835-814	ACCCTGYRCCGAACATGGA CAACTCCCAATTACATARCCCA	680

The PCR conditions started at 94 ^{0}C for 7 minutes, followed by 38 cycles of 94 ^{0}C for 40 seconds, 60 ^{0} C for 1 minute and 72 ^{0} C for 2 minutes and final extension for 72 ^{0} C for 15 minutes. The second master mix will include 5µl of 10x PCR buffer, 1.5µl magnesium chloride, and 4.0µl of dNTPs, 0.25µl of second set of primer S2F and S2R (table 1) and 2µl Taq polymerase. Visualization of amplified DNA was done on 1.2% agarose gel in 1x TAE buffer stained with ethidium bromide.

Sequencing and viral genotyping

Purified PCR products were directly sequenced using BigDye terminator v3.0 in a Sequencing Ready Reaction Kit (Macrogen Inc, Korea) using the 2nd PCR primers S2F and S2R (table 1). The sequencing reactions were performed using ABI3730 automated sequencer (Applied Bio systems). The resulting forward and reverse sequences from each sample were assembled using the GENETYX® Ver.9 software. The sequences were aligned with references sequences from gene bank and trimmed with MEGA 7.0.25 software. Phylogenetic relationship were inferred by creating a PHB file through the DNA Data bank of Japan (DDBJ) and opening the file through NJ plot software.

Ethical consideration

The study was ethically approved by the KEMRI scientific steering committee (SSC) and the Ethical review committee (ERC) (SSC Protocol number 2913) before implementation. The study was also approved by the Kilifi County Research committee (Ref:DOH/KLF/RESCH/VOL.I/21) and the management of Malindi Sub-county Hospital.

Data analysis

Social demographic, clinical and risk factors data was entered and cleaned using Ms Excel and transferred to Statistical package for social sciences (SPSS) version 21. SPSS was used to analyse the frequencies, means and percentages for the social demographic data, clinical data and the risk factors against the HBV sero-positivity through cross-tabulation. Independent t test was used for comparison of means for the CD4 counts between the HIV mono-infected and HIV-HBV mono-infected. STATA was used to perform bivariate and multivariate analysis to determine factors associated with HBV infections using Poisson regression with significance set at $P \leq 0.05$ and odds ratio with corresponding 95% confidence interval.

Results

Baseline characteristics of study participants

A total of 446 HIV positive participants including 126 (28.3%) males and 321(71.8%) females were enrolled for the study. Their ages ranged from 18-76 years with a mean age of 42.98 +/-10.750 years. The mean age of males was 44.93 years (SD ±10.16) while that of females was 42.22 years (SD ±10.88). Other baseline characteristics of the study participants have been summarised in table 2

At the time of sample collection 69 (15.5%) individuals had not started ARVS. Those who were taking AZT, 3TC, NVP regimen were the majority (33.4%) while 26.9% were on 3TC, TDF, EFV. The rest (24.2%) were on TDF, 3TC, NVP

Table 2- Baseline characteristics among HIV positive patients attending Malindi sub-county Hospital

Variable	Sample size (N = 446)	
	No	%
Age (Years)		
Mean (± SD)	42.98	(± 10.75)
Median (IQR)	43	(34.75 - 50)
Range	58	(18 - 76)
≤30	62	13.9
31-40	125	28.1
41-50	158	35.4
≥50	101	22.6
Gender		
Males	126	28.3
Females	320	71.7
Level of Education		
Primary	240	53.8
Secondary	77	17.3
Tertiary	21	4.7
none	108	24.2
Employment status		
employed/self employed	246	55.2
unemployed	200	44.8
Marital status		
Single	48	11
Married	236	53
widow/divorced	162	36
ARV drug currently on use		
3TC, TDF, EFV	120.0	26.9
AZT, 3TC, NVP	149	33.4
TDF,3TC, NVP	108	24.2
Not started	69	15.5
CD4 count		
Mean (± SD)	417.49.	(±239.643)
Median (IQR)	403.5	(247.5-562)
Range	1331	0-1331
CD4 less 500	292	65.5
CD4 ≥500	154	34.5
Duration Since HIV diagnosis		
0-5 yrs	227	50.9
6-10 yrs	204	45.7
>10yrs	13	3.4
Duration since ART initiation		
0-5 yrs	264	59.2
6-10 yrs	175	39.2
>10yrs	7	1.6
Intravenous Drug Use		
Yes	13	2.7
no	433	97.3
History of blood transfusion		
Yes	36	8.1
No	410	91.9
Scarification/Tatooing		
Yes	9	2
No	437	98
Involvement in CSW		
Yes	23	5.2
No	423	94.8
Sexuality		
Heterosexual	441	98.9
Homosexual/Bisexual	5	1.1
Number of partners in the past 6 months		
None	86	19.3
One	329	73.8
2 to 3	24	5.4
≥4	7	1.6

Laboratory results

A total of 19 (4.26%) participants were HBsAg positive based on rapid strip test while 22 (4.94%) participants were positive for HBsAg based on ELISA. All the 19 samples that were positive via the rapid test were confirmed positive via ELISA. Males had the highest prevalence of the HIV-HBV co-infection 11 out of 126 (8.7%) compared to 11 out of 320 females (3.4%). HBV DNA was successfully amplified in 12 out of 22 (54.5%) samples positive for HBsAg ELISA. 10 out of the 12 HBV-DNA positive samples were successfully sequenced. This has been summarised in table 3

Table 3: A summary of the laboratory tests done and their results

	HBsAg serology (n=446)		**HBV DNA PCR positive (n=22)**	**HBV Sequencing (n=12)**
	Rapid	**ELISA**		
Positive	19 (4.26%)	22 (4.94%)	12 (54.5%)	10 (83.3%)
Negative	427 (95.74%)	424 (95.06%)	10 (45.5%)	2 (16.7%)

The mean CD4 count for the study population was 417.5 (SD = ±240.3 cell/µl). Although not statistically significant (p = 0.429) those who were HIV-HBV co-infected had a lower CD4 count (378 cell/µl) compared to those who were HIV mono-infected (419.5 cell/µl)..

Phylogenetic analysis and genotypes

Phylogenetic analysis revealed that 9/10 (90%) belonged to genotype A which were all categorised into sub-genotype A1 while 1/10 (10%) belonged to genotype E. A phylogenetic tree was constructed by NJ plot software using a phb file generated from DNA Data Bank of Japan (DDBJ) as shown in fig 1.

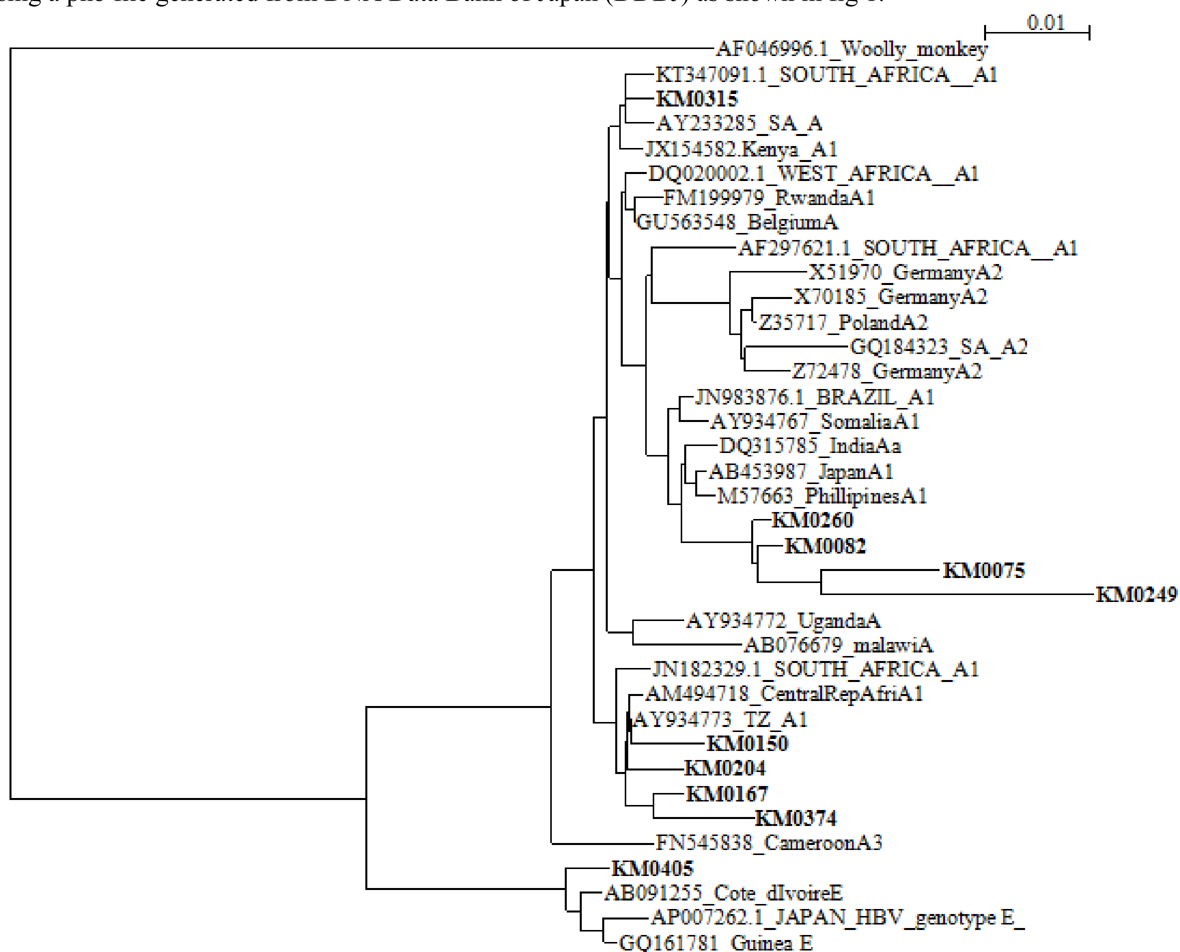

Fig 1: Phylogenetic analysis based on the Pre-S region from 10 HBV strains from HBV-HIV co-infected patients attending the Malindi Sub-County Hospital and selected reference sequences representing some of the known HBV genotypes/Sub-genotypes from the Genebank. The 10 HBV characterised in this study are indicated in bold and 9/10 fall under genotype A1 while 1/10 fall under Genotype E. The references are labelled with their accession numbers, country of origin and the genotype they belong to. The tree is rooted from the Woolley Monkey virus genome sequence.

Factors associated with HBV infections

Univariate analysis showed that gender (p = 0.029) and Intravenous drug use (p = 0.008) were significantly associated with HBV infection whereas all other factors were not statistically significant. In Multivariate analysis gender (p = 0.013) and Intravenous drug use (p = 0.05) were also statistically significant (Table 4)

Table 4: Univariate and Multivariate analysis of factors associated with HBV infection among HIV patients attending CCC at Malindi Sub-County Hospital. (N=446)

Variable	Sample size (N = 446)				Bivariate		Multivariate
	No.	HBV No. (n=22)	HBV %	P - value	OR (95% CI)	P - value	OR (95% CI)
Gender							
Male	**126**	**11**	**8.7**	**0.029**	**2.2(1.1-5.9)**	**0.013**	**3.2(1.3-8.1)**
Female	320	11	3.4	Referent	Referent	Referent	Referent
Age (Years)							
≤30	62	2	3.2	0.34	0.5(0.1-2.2)	0.542	0.6(0.1-3.0)
31-40	125	4	3.2	0.218	0.5(0.1-1.5)	0.384	0.6(0.2-2.0)
41-50	158	9	5.7	0.697	0.8(0.3-2.2)	0.961	1.0(0.4-2.7)
>50	101	7	6.9	Referent	Referent	Referent	Referent
Employment status							
employed/self employed	246	14	5.7	0.426	1.4(0.6-3.4)	0.378	1.5(0.6-3.8)
unemployed	200	8	4	Referent	Referent	Referent	Referent
Level of Education							
Primary	240	14	5.6	0.92	1.1(0.4-2.7)	0.606	0.8(0.3-2.2)
Secondary	77	1	1.3	0.178	0.2(0-1.9)	0.056	0.1(0-1)
Tertiary	21	1	4.8	0.887	0.9(0.1-7.1)	0.511	0.5(0.1-4.3)
Non formal	108	6	5.6	Referent	Referent	Referent	Referent
Marital status							
Single	48	1	2.1	0.352	0.4(0.0-3.0)	0.532	0.5(0.1-4.2)
Married	236	12	5.1	0.841	0.9(0.4-2.1)	0.514	0.7(0.3-1.8)
Widow/Widower/Divorced	162	9	5.6	Referent	Referent	Referent	Referent
ARV drug currently on use							
3TC, TDF, EFV	120.0	8	6.7	0.15	4.6(0.6-36.8)	0.995	0
AZT, 3TC, NVP	149	10	6.7	0.144	4.6(0.6-36.2)	0.995	0
TDF,3TC, NVP	108	3	2.8	0.573	1.9(0.2-18.4)	0.995	0
Not started	69	1	1.4	Referent	Referent	Referent	Referent
CD4 count							
Less than 500	292	16	5.5	0.476	1.4(0.6-3.6)	0.433	1.5(0.6-3.9)
Greater than 500	154	6	3.9	Referent	Referent	Referent	Referent
Duration since HIV diagnosis							
0-5yrs	227	9	4	0.995	0	1	0
6-10yrs	204	13	6.4	0.965	0	1	0
>10yrs	15	0	0	Referent	Referent	Referent	Referent
Duration since ARV initiation							
0-5yrs	264	11	4.2	0.996	0	0.999	0
6-10yrs	175	11	6.3	0.996	0	0.999	0
>10yrs	7	0	0	Referent	Referent	Referent	Referent
Intraveneous drug use							
Yes	**13**	**3**	**23.1**	**0.008**	**5.3(1.6-17.8)**	**0.05**	**4.1(1.0-16.6**
no	433	19	4.4	Referent	Referent	Referent	Referent
History of blood transfusion							
Yes	36	0	0	1	0	0.995	0
No	410	22	5.4	Referent	Referent	Referent	Referent
Scarification/Tatooing							
Yes	9	0	0	0.999	0	0.997	0
No	437	22	5	Referent	Referent	Referent	Referent
CSW							
Yes	23	1	4.3	0.897	0.9(0.1-6.5)	0.813	0.8(0.1-6.0)
No	423	21	5	Referent	Referent	Referent	Referent
Sexuality							
Homosexual	1	0	0	1	0	0.999	0
Bisexual	4	0	0	1	0	0.998	0
Heterosexual	441	22	5	Referent	Referent	Referent	Referent
No. of patners in past six months							
One	329	14	4.3	0.161	0.5(0.2-1.3)	0.398	0.7(0.2-1.7)
2 to 3	24	1	4.2	0.531	0.5(0.1-4.2)	0.846	0.8(0.1-8.8)
≥4	7	0	0	0.994	0	0.998	0
None	86	7	8.1	Referent	Referent	Referent	Referent

Discussion

Several studies have been carried out in Sub-Saharan Africa including Kenya to access the burden of hepatitis B virus among HIV infected individuals. However majority of this studies focus on populations that form risk sub-

populations for HIV and HBV transmission or newly diagnosed HIV patients. The present study focused prevalence, risk factors and genotypes of HBV in a generalized HIV infected patients population receiving medical care in one of government hospital set up in Kilifi County.

Generally, the prevalence of HBV is higher among HIV–infected individuals than in the general population which can be attributed to the shared risk factors for transmission and acquisition of these two life threatening viruses (Chun *et al.,* 2012). The present study reported a HBV prevalence of 4.9% (22/446) among HIV positive participants based on laboratory analysis of HBsAg through ELISA. The rates of HIV-HBV co-infections have previously been reported to be as high as 10–20% in countries where HBV infection is either endemic or intermediate to high HBV cases (Muriuki *et al.,* 2013). In Kenya, a study by Muriuki *et al.,* 2013 on the prevalence of HBV and HIV co-infection showed a prevalence of 6% with co-infection rates significantly higher among males (19.2%) compared to females (14.3%). This result was also in agreement with other studies carried out in Kenya including; 5.7% (Wambani *et al.,* 2015), 4.26 % (Kerubo *et al.,* 2015) and 3.6 % as reported by Webale *et al.,* (2015) among HIV positive non-IDUs in Mombasa, Coastal Kenya. The results were also similar to those reported in other parts of the world including 5.2 % in Morocco (Rebbani *et al.,* 2013) and 3.8 % in Brazil (Brandaoa *et al.,* 2015). In South Africa between 5% and 17% of HIV-infected patients in the country were found to be co-infected with Hepatitis B virus (Chun *et al.,* 2012).

The results of this study were found to be lower than those of other studies carried out in other studies including jaundiced patients in Kenya which reported 53 % (Otedo, 2004) and 50.6 % (Ochwoto *et al.,* 2016), 25.5 % among HIV patients in Morocco (Magoro *et al.,* 2016), 19.2 % among HAART naïve patients in China (Chen *et al.,* 2013), 12 % among HIV patients in Columbia (Bautista *et al.,* 2014), 10.5 % among HIV patients in Lesotho (Mugomeri *et al.,* 2015), 9.6 % among HIV IDUs in Mombasa, Kenya (Webale *et al.,* 2015). The higher HBV prevalence rates in this studies compare to the current study could have been as a result of using sub-populations that have evident signs associated with HBV such as the jaundiced patients who were attending clinics due to liver related complication, being selected from HBV high risk sub-populations such as the intravenous drug users or newly diagnosed patients who had not started ART.

On the other hand, the results reported in this study were contrarily higher compared to reports from Nigeria as reported by Dore *et al.,* (2010) and Diwe *et al.,* (2013) indicating HBV/HIV prevalence rates of 2.4% and 2.2% respectively, 2.2 % in Malawi (Varo *et al.,* 2016) and 2.5 % in Brazil (Freitas *et al.,* 2014). The higher prevalence rate in the present study could be as a result the samples being collected from a HBV prevalence intermediate (2-7%) area as classified by WHO (Zenebe *et al.,* 2014). As reported by Mukami *et al*., (2013) other factors that might have resulted in disparities in the prevalence rates between this studies result and those conducted in other places could include but not limited to sample size used, specificity and sensitivity of kits used and diversity of behavioural characteristics of each population.

Phylogenetic analysis of the HBV genotypes in the current study revealed the presence of genotype A (90%) and E (10%) as would be expected of the Kenyan population. This observation is in agreement with other studies done in Kenya that reported a prevalence of 88% (Mwangi *et al.,* 2008), 90.3 % (Ochwoto *et al.,* 2016), 100 % (Webale *et al.,*2015) of genotype A1. Mwangi et al., (2008) had also reported genotype E although at a much lower prevalence than genotype A. All the genotype A sequences belonged to the HBV genotype A1 sub-genotypes. Five of the genotype A and the genotype E sequences clustered with sequences from countries in sub Saharan Africa including Tanzania, central Africa republic, South Africa Ivory coast and Guinea. This could be attributed to trade relationships between these African countries. The rest four sub-genotype A1 samples clustered with samples from Somalia and the Asian clade (Phillipines and Japan). This could be as a result of immigrants from Somalia who access the Kenyan coast and tourists from the Asian countries.

The current study looked for association between the social demographic, clinical history and risk/behavioral factors with HBV among HIV positive participants. Male gender was found to be significantly associated with HBV infection among the HIV positive participants. This finding agrees to other studies carried out in Lesotho, Brazil and Nigeria (Mugomeri *et al.,* 2015, Freitas *et al.,* 2014, Balogun *et al.,* 2012). Contrary to the findings of this study, Kerubo *et al.,* (2014) in a study involving participants from two informal settlements in Nairobi, Kenya did not find any association between HIV/HBV co-infection with gender. The fact that male gender is associated with HBV in the current study can be attributed to risky behaviours related to HBV transmission such as multiple sexual partners without use of protective measures and involvement in intravenous drug use more than their female counterparts.

Involvement in intravenous drug use was also found to be significantly associated with HBV infection OR, 5.3 (95% CI 1.6-17.8) among HIV participants of this study. Zenebe *et al.,* (2014) reported that individuals who used injectable drugs were 5 times more likely to have HBV infections which is in tandem with the current study. This is a clear evidence of the efficiency of intravenous drug use in transmission of blood borne viral infections.
Other studies have documented that factors such as increasing age (Freitas *et al.,* 2014), blood transfusion (Zenebe *et al.,* 2014, Alhuraiji et al., 2014) tattooing/scarification (Zenebe *et al.,* 2014) homosexuality (Freitas *et al.,* 2014, Brandaoa *et al.,* 2015) to be associated with HBV infections among HIV positive patients. This did not however

agree with the present study findings probably due to low numbers of participants who had the above characteristics.

Some of the limitations of this study included the use of a single serological marker. Not all serological markers of HBV were used to for diagnosis of HBV therefore chances of leaving out possible occult cases positive for HBV DNA extraction. In the risk factors analysis, majority of participants who are at higher risk of HBV acquisition such as the known men who have sex with men and intravenous drug users could not participate in the study as they have their special clinics where they receive similar services as those offered in the comprehensive care centre where this study was done. Finally the current study was not able to analyse liver function enzymes (Aspartate transferase and Alanine transaminase) and viral load which would have shown relationship between HBV infection and clinical variables among those who are HIV-HBV co-infected.

Conclusion

The high HBV-HIV prevalence of 4.9 % among patients attending Malindi Sub-county hospital Comprehensive Care Centre is most likely driven by intravenous drug use and multiple sexual partners among the male gender. Genotype A was the most prevalent 9/10 (90%) while 1/10 (10%) belonged to genotype E an observation that relates well with the distribution of HBV genotypes among the Kenyan general population. There is therefore need to routinely diagnose HBV among new comprehensive care centres recruits to ensure proper therapeutic management using antiretroviral drugs.

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