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Genetic Diversity and Prevalence of Hepatitis B among Rural HIV Infected Populations Receiving Antiretroviral Therapy in Kenya

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Abstract
Hepatitis B virus infection can result to an aggressive liver disease. It is estimated that two billion people worldwide have been exposed to HBV infection, with 150 million chronically infected. Of those chronically infected, an estimated 1% are also infected with Human Immunodeficiency Virus (HIV). Infection with HIV accelerates progression of HBV to both chronic and development of liver complications. This study aimed at determining the genetic diversity and prevalence of hepatitis B infection in HIV infected persons receiving antiretroviral care in a level 4 hospital, in rural Kenya. A cross-sectional study was conducted among 413 study participants. Structured questionnaires were used to capture social demographic data whereas plasma samples were tested for hepatitis B using HBsAg. Positive samples were then genotyped to establish the circulating HBV genotypes and finally sequenced to screen for mutations and establish potential resistance to the recommended HBV treatment regimen. An overall prevalence of HBV-HIV co infection was established at 3.9%. Genotype A was the most common (92.3%), while the remaining 7.7% all belonged to genotype D. Mutations at S207N (associated with immune escape and hepatic carcinoma and liver cirrhosis), N122H (associated with Occult Hepatitis B) as well as M129L and V163I (both associated with resistance to Lamivudine and Telbivudine) were identified in high frequencies. These results demonstrate the negative impact of HIV/HBV co-infection in the progression to liver complications as well as raises questions in the continued use of Lamivudine and Telbivudine based drugs in the management of HBV in the HIV infected populations, especially in the low and middle income countries, where out of pocket treatments remain beyond reach of the general population.
Keywords: Hepatitis B Virus, Human immunodeficiency virus, Lamivudine, Telbivudine, Hepatocellular carcinoma, Cirrhosis, LMIC
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1. Introduction

Hepatitis B virus (HBV) is the causative agent of an aggressive liver disease which causes acute or chronic infection. Acute infection is characterized by a rapid onset of sickness with vomiting, yellowish skin, tiredness, dark urine and abdominal pain. Chronic infection with hepatitis B virus may be asymptomatic or may be associated with a chronic inflammation of the liver, leading to cirrhosis over a period of years. This type of infection dramatically increases the incidence of hepatocellular carcinoma (HCC) (Pollicino et al., 2011). The virus belongs to *Hepadnaviridae* family (Liang, 2009); a double stranded DNA virus with a unique characteristic of possessing reverse transcriptase enzyme. it is estimated that 254 million people were living with chronic HBV by 2022, with an infection of about 1.2 million new people annually, and an estimated 1.1 million deaths (*Global Hepatitis Report 2024: Action for Access in Low- and Middle-Income Countries*, n.d.). It is estimated that up to 1% HBV infected patients are co-infected with HIV, and these people account for 7.4% globally (*Global Hepatitis Report 2024: Action for Access in Low- and Middle-Income Countries*, n.d.). Within the African Region, Hepatitis B is endemic and affects an estimated 5-8% of the population, mainly in west and Central African Republic (Corcorran & Kim, 2023). Hepatitis is additionally a growing explanation for mortality among people living with HIV. The prevalence of HBV/HIV co-infections in Kenya is estimated at 5.5% (Mabeya et al., 2016). HIV infection is significant in the explanation of chronic HBV infection. Co-infection with HIV leads to accelerated progression to chronic HBV condition and hepatocellular carcinoma (Singh et al., 2017) conversely, the presence of HBV in HIV infected patients, affects the course of the disease, increasing the progression to AIDS disease as well as affecting the efficiency of antiretroviral therapy (ART). Both HBV and HIV infections share transmission patterns and predisposing factors which cannot be overlooked when exploring how the 2 interrelate (Bedassa et al., 2022) hence the increasing rate of HIV/HBV co infections and chronic HBV infections among the HIV infected. Although the burden of HBV in the HIV infected patients has been documented (WHO 2022) especially in the African countries (Abesing et al., 2020; Ajuwon et al., 2021; Makokha et al., 2023). Testing for HBV in the HIV population in these low resource countries has been minimal, partly due to the assumption that sufficient HIV with the current regiment

would automatically clear HBV in the blood. It is therefore important to document not only the burden of the co-infection, but also the resistance to HIV/HBV coactive drugs.

2.0 Materials and Methods

2.1 Study site

The study was conducted at Masinga Sub County Hospital Comprehensive Care Center, a level 4 hospital located in the Eastern region of Kenya, Machakos County.

2.2 Study design

This was cross-sectional study.HIV infected plasma samples were collected from patients attending the comprehensive care clinic (CCC) and serological assay conducted for HBV infection, at the site.

2.3 Sample Size

The sample size was determined using the Naing (2003) with Postulated prevalence of 7.25% (sepha m *et al.,* 2016). A sample size of 103 was attained**.** However, the sample size was adjusted to 413 to increase statistical power

2.4 Sampling criteria

The study participants were sampled from the CCC consultation room through convenience sampling method where study participants were recruited based on availability and willingness to take part in the study.

2.5 Sample collection

Venous blood (5 mls) was drawn from the Median Cubital, Cephalic or the Basilic vein using the closed needle system into a plasma preparation tube (PPT), and properly mixed by gently inverting the tube 3 to 4 times. The tubes were centrifuged at 3000 rpm for 15 minutes to obtain plasma which was used to test for HBV using the HBsAg rapid dipstick. Samples that turned positive for HBsAg were stored at 2^0C for 2 days at the Masinga level 4-hospital laboratory before being transported, while maintaining cold chain to the Kenya Medical Research Institute Center for Virus Research (KEMRI-CVR) Nairobi and stored at -20^0C for molecular testing and further analysis. Collection of plasma was accompanied by administration of a questionnaire which captured the socio-demographic and clinical data including age, sex, marital status, residential location, vaccination status and knowledge on HBV, which were directly gathered from the patient. Clinical information was extracted from the hospital records.

2.6 Laboratory analysis

Serological tests were done using HBsAg rapid dipstick (Guangzhou Wondfo Biotech Co., Ltd). Samples that turned positive for HBsAg were used for molecular analysis.DNA extraction was done using the Qiagen DNA extraction kit following the manufactures' protocol. The product was amplified using ABI Thermo cycler 9700 system. First round of the PCR was performed using specific primer pair S1f (5'-TCCTGCTGGTGGCTCCAG-3'as sense primer and S1r (CGTTGACATACTTTCCAATCAA)-3' as anti-sense primer targeting position 55-995 bp of S gene (Osiowy et al., 2010). 40 cycles of PCR amplification were done using the following thermo cycling parameters, 94ºC for 5 min; followed by 40 cycles at 94ºC for 40 seconds, annealing at 55ºC for 1 min, extension at 72ºC for 2 min and final extension at 72ºC for 7 minutes. The Second round nested PCR was conducted using specific Inner primers pair S2f (5'-ACCCTGYRCCGAACATGGA-3'as sense primer and S2r (5'-CAACTCCCAATTACATARCCCA -3' as anti-sense primer targeting position 155-835 bp of S gene (Osiowy et al., 2010) under the same thermo cycling conditions as of the 1st PCR with the modification of the annealing temperature at 50ºC,25 µl of master mix and 5 µl of the first-round product as per the method (Osiowy et al., 2010). The purity of amplified PCR products were examined on a 2% agarose gels stained with cyber green and viewed using a UV trans-illuminator. The products with the expected band size were then purified, prepared for sequencing, using the same PCR primer pair and an automated sequencing using Sanger technique Applied Biosystems ABI3500xl sequencer (Macrogen, Netherlands) was done.

2.7 Sequence, Genotyping, and Mutations Analysis of *S Gene* Sequences

All Forward and reverse sequences obtained by the sanger technique were analyzed and edited using BioEdit sequence alignment editor software version 7.2.5 (*BioEdit 7.2 Download (Free) - BioEdit.Exe*, n.d.) and then subjected to NCBI nucleotide BLAST for quality check. Genotypes and sub-genotypes of the assembled HBV sequences obtained were determined using geno2pheno HBV online software (https://hbv.geno2pheno.org/index.php) version 2.0 (*Geno2pheno Hbv*, n.d.).

A phylogenetic analysis was performed using MEGA version 11 (Tamura et al., n.d.). The analysis was done using the Maximum-likelihood statistical method, Tamura-Nei model, and the bootstrap method of 1000 replicates

to determine the genotypes and ancestral relation.

These sequences were translated to the protein sequences using EMBOSSTranseq (*EMBOSS Needle < EMBL-EBI*, n.d.) and aligned with the referenced amino acid sequence accession No. FM199974.1,from HBV database in Bioedit software version 7.2.5 (*BioEdit 7.2 Download (Free) - BioEdit.Exe*, n.d.). The clinical significance of mutations was flagged out from the search of peer-reviewed journals using Pubmed search engine and later submitted to geno2pheno HBV online software (*Geno2pheno Hbv*, n.d.) version 2.0 for further confirmation of drug resistance and immune escape mutation identification

2.8 Ethical Approval

Ethical approval was sought from the JKUAT ethical review board (JKU/IERC/02316/0133) and permission to conduct the study was obtained from the hospital management.

2.9 Data analysis

Social demographic, clinical and risk factors data was entered and cleaned using Ms Excel and transferred to Epi Info7, which was used to generate the frequencies, means percentages and logistic regression analysis.MedCalc Software Ltd was used to determine factors associated with HBV infections with significance set at $P \leq 0.05$ and odds ratio with corresponding 95% confidence interval.

3.0 Results

3.1 Study participants characteristics

A total of 413 participants were successfully enrolled in the study where 139 (33.7%) were males and 274(66.3%) females. The overall mean age was 47 years, ranging from 18 – 77 years. 65% of the study participants were married and most of the participants had primary education (60%). Majority of the study participants were on 1st line ART intervention (92%) while 96% had not been vaccinated against HBV and 98% reported having no underlying condition.

3.2 Prevalence of HBV in HIV patients receiving antiretroviral therapy at Masinga level 4 hospital

The overall sero-prevalence of HIV–HBV co-infection was 3.9% (16/413) with a prevalence of 2.7 %(11) in females and1.2% (5) in males as shown in figure 1

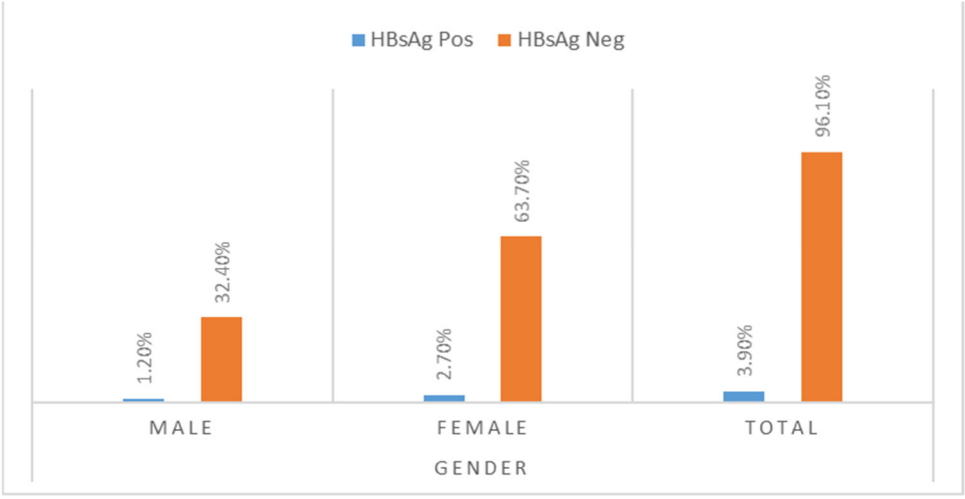

Figure 1: Sero-prevalence in gender and overall of HBV among HIV positive patients from Masinga sub-county hospital

3.2.1 HBV and HIV co infection prevalence and associations of socio demographic factors among the study participants

The prevalence of HBV- HIV co-infection was 4.0 % among the females and 3.6% among the males. Those in the age group 40-50 had a prevalence of 5.9% with 8.3 % prevalence in the widowed category. A prevalence of 4% was found among the HBV unvaccinated individuals and 8.3% in those who had low level high viremia (LLHVL (200-999).

The probability of getting infected with HBV among HIV infected individuals was determined to be higher among those who had primary level of education(OR 1.2556 95%CI 0.07-23.05 P value 0.8781),age group 30-40(OR 5.0870 95%CI 0.01-296.71 P value 0.4220),those that had underlying condition(OR 3.8667 95% CI 0.45-33.45 P value 0.2193) and ART use of between 0-5 years(OR 1.5580 95%CI 0.37-6.65 P value 0.5493).Within the marital status category, the risk of HBV infection was five time higher among singles(OR 6.3333 95% CI 0.74-

53.9 P value 0.0911) .However no significant associations of the demographic variables and HBV infection was found as shown in table 1

Table 1: HBV and HIV co infection prevalence and associations of socio demographic factors among the study participants

Variable	Variable group	N=413	No. positive(p)	%Prevalence (p/n)x100	Odds ratio(OR)	Confidence interval (95%)	Z statistic	P<0.05
	Female	274	11	4.0	0.8960	0.31-2.63	0.200	0.8416
Sex	Male	139	5	3.6	Referent			
	<20	11	0	0	1.0	0.02-54.83	0.000	1.0
	20-30	16	0	0	1.4348	0.03-77.7	0.177	0.8593
Age	30 40	58	0	0	5.0870	0.01-269.7	0.803	0.4220
	40 – 50	153	9	5.9	0.7025	0.04-12.8	0.238	0.8118
	50 – 60	112	4	3.6	1.08570	0.06-21.5	0.055	0.9563
	60 – 70	52	3	5.8	0.6522	0.03-13.51	0.276	0.7822
	70 – 80	11	0	0	Referent			
	Married	265	9	3.4	2.4537	0.85-7.12	1.651	0.0987
Marital status	Single	76	1	1.3	6.3333	0.74-53.9	1.689	0.0911
	Widowed	72	6	8.3	Referent			
	None	8	0	0	0.8095	0.01-45.2	0.103	0.9180
Level of Education	Primary	250	9	3.6	1.2556	0.07-23.05	0.153	0.8781
	Secondary	145	7	4.8	0.9238	0.05-17.03	0.053	0.9577
	Tertiary	10	0	0	Referent			
Vaccination status	NO	396	16	4.0	0.6866	0.04-11.92	0.258	0.7962
	YES	17	0	0	Referent			
Underlying condition	NO	406	15	3.7	3.8667	0.45-33.45	1.228	0.2193
	YES	7	1	0	Referent			
Treatment regimen	1st line	381	15	3.9	0.7938	0.10-6.20	0.220	0.8257
	2nd line	32	1	3.1	Referent			
	0-199	337	11	3.3	1.2760	0.07-23.00	0.165	0.8688
	200-999	60	5	8.3	0.4783	0.02-9.26	0.488	0.6256
Viral load	>1000	5	0	0	0.4783	0.01-27.4	0.357	0.7211
	N/A	11	0	0	Referent			
Knowledge of HBV	NO	354	14	4.0	0.8571	0.19-3.87	0.200	0.84
	YES	59	2	3.4	Referent			
	0	5	0	0	0.5789	0.02-16.15	0.322	0.7476
# of sexual partners	1	380	15	4.0	0.9048	0.12-7.10	0.095	0.9241
	>1	28	1	3.6	Referent			
	Employed	68	3	4.4	0.6296	0.16-2.5	0.658	0.5108
Employment status	Self employed	93	6	6.6	0.4306	0.14-1.31	1.480	0.1389
	Unemployed	252	7	2.8	Referent			
	0-5	129	3	2.3	1.5580	0.37-6.65	0.599	0.5493
Duration on ART(years)	6-10	146	8	5.5	0.6612	0.21-2.07	0.710	0.4775
	>10	138	5	3.6	Referent			

3.3 Logistic Regression of social demographic variables associated with HBV infection

Logistic regression analysis found no significance associations that would be related with any of the demographic variables and HBV infection. However a cross tabulation indicated that some variable groups were associated with a higher risk of HBV infection than others.

Age, Marital status, level of education, viral load and history of underlying medical condition were associated with a higher risk of HBV infection compared to other variables. (Table2).

Table 2: Logistic Regression Analysis of social demographic factors

Term	Odds Ratio	95%	C.I.	Coefficient	S.E.	Z-Statistic	P-Value
Age	1.0224	0.9699	1.0777	0.0221	0.0269	0.8230	0.4105
Duration of ART use	0.9754	0.8552	1.1125	-0.0249	0.0671	-0.3713	0.7104
Employment status	0.4121	0.1365	1.2440	-0.8864	0.5637	-1.5726	0.1158
Knowledge of HBV	0.6393	0.1283	3.1848	-0.4474	0.8193	-0.5460	0.5850
Level of Education	1.2684	0.4085	3.9389	0.2378	0.5781	0.4113	0.6809
Marital status	1.6271	0.5581	4.7437	0.4868	0.5459	0.8917	0.3726
# of sexual partners	0.9049	0.1094	7.4880	-0.0999	1.0782	-0.0927	0.9261
Treatment Regimen	0.6444	0.0674	6.1570	-0.4394	1.1516	-0.3816	0.7028
sex (M/F)	0.8354	0.2548	2.7393	-0.1798	0.6059	-0.2968	0.7666
Underlying Condition	2.7985	0.2589	30.2548	1.0291	1.2146	0.8473	0.3968
Vaccination Status	0.0000	0.0000	>1.0E12	-12.3553	414.8750	-0.0298	0.9762
Viral load	2.5644	0.8946	7.3512	0.9417	0.5373	1.7527	0.0797
CONSTANT	*	*	*	46.1187	135.4852	0.3404	0.7336

3.4 Phylogenetic Analysis of Sequenced Samples from Masinga level 4 Hospital Comprehensive care center in Kenya

Of the 16 HBV positive samples (HBsAg detection), only 13 were of good quality for genotyping and mutation analysis. A total of 12 (92.3%) of the 13 sequenced samples showed genotype A as the most prevalent genotype with the remaining sample belonging to genotype D. Of interest were 2 samples of non-African lineages mainly 1 genotype A and the genotype D which aligned to viruses circulating in Canada and Germany respectively (Figure 2).

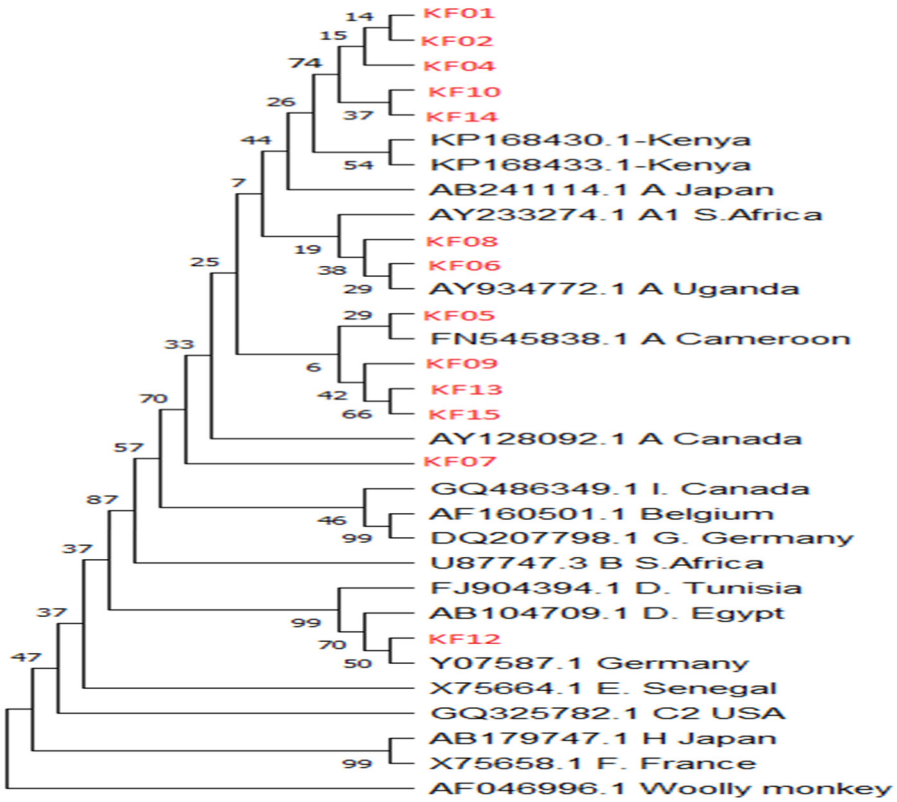

Figure 2: showing 13 samples labeled in red and 19 reference sequences selected from HBV database that were closely related with the samples

3.5 Mutation analysis

Mutations were identified from the RT and SHB gene of the Hepatitis B genome. Several mutations were found and occurred in high frequency in samples that belonged to genotype A. No mutations were recorded in the sample which belonged to genotype D as shown in table 3.

Table 3:Summary of genotypes and the mutation identified from the RT and SHB gene of the Hepatitis B genomes

Sample ID	Genotype	RT Domain Mutation	SHB Protein Mutation
KF01	A A1	N122H, Y126H, M129L, V163I,	S204N, S207N,
KF02	A	N76D,,N122H,Y126H,M129L,W153R, V163I,	A194V, S204N, S207N
KF04	A; A1	N122H, Y126H, M129L, V163I	I68T, A194V, S204N, S207N
KF05	A; A1	N122H, M129L, W153R, V163I	S207N
KF06	A;A1	N76D,N122H, M129L, V163I,	A194V, S204N, S207N
KF07	A; A1	N122H,M129L,W153R, V163I,	,S207N
KF08	A; A1	N122H, M129L, V163I,	A194V, S207N,
KF09	A; A1	N122H, M129L,W153R, V163I,	Q101R,K122R, A194V, S207N
KF10	A; A1	N122H, Y126H, M129L, V163I,	M198I, S207N
KF12	D;D1		
KF13	A; A1	N122H, M129L, W153R, V163I,	Y161F M198I, S207N,
KF14	A; A1	N122H, Y126H,M129L,V163I,A181S	I68T, S204C,S207N
KF15	A;A1	N122H, M129L, W153R, V163I	Q101R,K122R, S207N

3.5.1 Mutations and their clinical significance

The most predominant mutations of clinical importance in the analysis were S207N (associated with immune escape, hepatic carcinoma and liver cirrhosis), N122H (associated with Occult Hepatitis B), M129L, and V163I (both associated with resistance to Lamivudine and Telbivudine) where identified in high frequencies. Others included, A181S found in one sample which confers resistance to Lamivudine, Adefovir, and Telbivudine and I68T which has been associated with Hepatocellular carcinoma (HCC).Table 4

Table 4: Identified predominant mutations from the samples and their clinical significance

Mutation	Frequency	Clinical implication	Resistance conferred
S207N	12	Immune escape, HbsAg detection failure, Increased HBsAg reactivity in immunological diagnostic assays, causes hepatocellular carcinoma/cirrhosis and is an asymptomatic carrier	
A194V	5	Immune escape	Tenofovir
K122R	2	Immune escape and is an occult infection related mutation	
Y161F	1	Immune escape	
A181S	1		Lamivudine, Adefovir, and Telbivudine
M129L	12	Occult hepatitis B virus infection (OBI)	Lamivudine
N122H	12	Occult hepatitis B virus infection (OBI)	
V163I	12		LMV & LdT
W153R	6		
Y126H	5	Immune escape, Occult hepatitis B virus infection (OBI)	
S204N	3	vaccination escape diagnostic failure	
N76D	2		
I68T	2	Immune escape, Hepatocellular carcinoma (HCC)	
M198I	2	Immune escape	
Q101R	2	Occult hepatitis B virus infection (OBI)	

4.0 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. 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 3.9% (16/413) among HIV positive participants based on laboratory analysis of HBsAg through rapid

chromatographic immunoassay for qualitative HBsAg testing. Although Africa is classified as high HBV endemic area, the results of this study indicate HBV intermediate endemicity (2-7%) according to WHO HBV classification. This could be explained by the fact that the study was carried out among HIV infected individuals who were currently on ART intervention and majority being on the 1st line ART regimen (TDF/3TC/DTG) which has co active drug against HBV. These co active drugs can effectively suppress HBV replication and reduce the risk of both new infections and HBV reactivation in those already co infected (Wlazłowska et al., 2022). ART also improves overall health and immune function making the individuals less susceptible to opportunistic infections such as HBV (Sabir et al., 2024).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 due to lack of prevention or control measures such as limited access to HBV vaccination and insufficient healthcare infrastructure. Social economic factors such as poverty lack of education and limited access to health care have also played a role in these areas. (Muriuki et al., 2013 &Tadewos et al., 2024).A recent systematic review and meta-analysis on the burden of Hepatitis B virus infection in Kenya showed an overall pooled prevalence estimate of 7.8% and 8.2% in general population and HIV-infected individuals respectively attributed to the large sample size used(Makokha et al., 2023).

A recent study on Hepatitis B virus prevalence and its genotypic association with drug resistance in HBV/HIV co-infected patients in Western Kenya reported a prevalence of 5.4% (Onyango et al., 2024). This result was also in agreement with other studies carried out in Kenya including; 4.9% (Gitau et al., 2017), 5.8% (Maina N et al., 2017), 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 4.3% in Rwanda (Umutesi et al., 2017) which was attributed to HBV vaccination uptake.

The prevalence of 3.9% in this study were found to be lower than those of other studies carried out including jaundiced patients in Kenya which reported 53 % (Otedo, 2004) and 50.6 % (Ochwoto et al., 2016), 9.7% among HIV infected persons in western Kenya (SB Okoth et al., 2017) 9.6 % among HIV IDUs in Mombasa, Kenya (Webale et al., 2015) and 8.8% in Western Kenya among high risk populations(Koroney et al.,2020),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), and 8% among people with HIV in Botswana (Phinius BB et al., 2023). The higher HBV prevalence rates in this studies compared to the current study could have been as a result of using subpopulations 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 of the samples being collected from a HBV prevalence intermediate (2- 7%) area as classified by WHO (Zenebe et al., 2014).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 behavioral characteristics of each population.

The current study looked for associations between the social demographic, clinical history and risk/behavioral factors with HBV among HIV positive. Although, no significant statistical associations were found;age(OR 1.234 95% 0.95735 C.I. 1.0758),marital status (OR 1.5496 95% 0.5379 C.I 4.4646),level of education(OR 1.2084 95% 0.3965 C.I. 3.6835) and history of underlying condition(OR 3.3741 95% 0.3309 C.I 34.4013) were found to increase the risk of HBV infection among the HIV infected. Those in age group 30-40 had a high risk of HBV infection compared to other age groups. This may be attributed to this group having more exposure to risk factors such as sexual activity, needle sharing among drug users or occupational exposure. In the marital status category, singles had a high risk of infection probably due to not having a long term sexual relationship, having multiple sexual partners and engaging in unprotected sex (Kolou M et al., 2017). Increased HBV infection risk was also seen in those who had primary education. This is likely due to a combination of factors such as lower knowledge about HBV prevention and transmission, limited access to healthcare and vaccinations and potentially higher risk behaviors (Zhao et al., 2021).Participants who had underlying medical condition also had a high risk of HBV infection probably due to weakened immune system, making it harder for the body to fight off the virus or suppress it after initial infection, leading to increased risk of chronic HBV infection or reactivation. These results were in agreement with other studies curried out in Kenya (James Gitau et al., 2017)

Phylogenetic analysis of the HBV genotypes in the current study showed that genotype A was found to be the prevalent genotype (92.3%) with Genotype D being found in one sample (7.7%).This could be attributed to the geographical distribution of HBV genotypes where genotype A is mainly found in Africa (Sunbul M et al.,

2014).Genotype D on the other hand is prevalent in middle East where interactions among this populations are common due to trade and employment. This observation was in agreement with a study by Down done in Kenya that reported a prevalence of 92%,6% and 1% of genotype A,D and D/E recombinants respectively(Downs LO et al., 2023), 90.3% genotype A and 9.7% genotype D among HBV DNA positive specimens (Ochwoto et al.,2016), 100 % (Webale et al.,2015) of genotype A1 and 88% (Mwangi et al., 2008). Detection of Genotypes A also reported in Uganda and Cameroun could signify a potential importation of the genotypes through road travels along the trans-African highway, whereas the linkages to genotypes from Canada and Germany demonstrate how easily HBV is transmitted on a global scale. Therefore, any mutations occurring and the negative implications may not be restricted only to a specific geographical location (Sant'Anna TB et al., 2023).

The most predominant mutations of clinical importance in the analysis were associated with immune escape (S207N, A194V, K122R &Y161F), drug resistance (A194V, A181S, V163I&M129L and hepatocellular carcinoma (168T). similar findings were reported in a study in kenya by king (king et al .,2023) A181S found in one sample confers resistance to three HBV active drugs (Lamivudine,Adefovir and Telbivudine).This could be an indicator of the detrimental effect HBV-HIV co infection has on the progression to liver complications as well as the challenge in continued use of the HBV-HIV co active drugs in the management of HBV in this population. This was consistent with other studies carried out in Kenya such as (Aluora PO et al., 2020) and (Langat BK et al., 2023) which reported mutations conferring resistance against lamivudine and immune escape were predominant. Similar studies on mutations to the aforementioned drugs have been documented ((Bernard Wandera Onyango et al., 2024) indicating a slow decline in drug affectivity within the HBV/HIV co-infected populations.

Conclusion

The HBV-HIV prevalence of 3.9 % among patients attending Masinga Sub-county hospital Comprehensive Care Centre is most likely driven by lack of HBV testing and surveillance of the circulating genotype as well as possible mutation that can pose a challenge in the management of HBV in the population at large. Genotype A was the most prevalent (92.3%) while (7.7%) belonged to genotype D an observation that relates well with the distribution of HBV genotypes among the Kenyan general population. Mutations that confer drug resistance to antiretroviral activity for both HIV and HBV therapy were also identified as well as those that lead to immune escape. The realization of key mutations in these samples especially on drug resistance is of concern especially with regards to HIV treatment with HIV/HBV coactive drugs. Currently existing HIV/HBV coactive drugs for use among the rural populations include Tenofovir (TDF) and Lamivudine(3TC) and therefore, resistance to this drugs within this pool will present a treatment burden not only to the patients, but also to public health, especially, if the mutant viruses were to be transmitted. There is therefore the need to routinely diagnose HBV among new comprehensive care centers recruits to ensure proper therapeutic management using antiretroviral drugs as well as vaccination of those that turn negative for HBV. Knowledge on hepatitis B prevalence and circulating genotypes are essential to care givers and health planners in providing personalized patient care as each genotype has specific clinical association with course of infection, severity of the disease, prognosis and response to antiviral treatment

Recommendations

This study recommends sensation of the community on HBV infection, as well as scaling up HBV testing among the HIV infected as well as the general population and subsequent vaccination of those eligible. Due to the number of mutation and frequency at which they are occurring, the study also recommends surveillance of HBV genotypes and mutations. Further studies in this area are recommended to fully understand the extent of HBV burden

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