



(RESEARCH ARTICLE)



DISTRIBUTION OF FIVE HEPATITIS B VIRUS MARKERS IN HIV INFECTED AND UNINFECTED INJECTING DRUG USERS IN COASTAL KENYA

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ABSTRACT

Aims: This study determined HBV sero-markers profile in HIV-1 infected (n=157) and uninfected (n=214) IDUs from Mombasa, Kenya HIV-1 diagnosis was performed using Determine™ and Unigold™ rapid tests.

Methodology: The CD4+ T cells were enumerated using the FacsCalibur flow cytometer and categorized according to the Centre for Disease Control and Prevention (CDC) immunological staging criteria. HBV seroprofile was determined using HBV-5 panel rapid test cassettes. Clinical staging was based on serological profile of the five HBV markers.

Results: HBsAg ($P=0.004$) and HBcAb-IgM ($P=0.008$) positivities were higher in the HIV-1 infected compared to the uninfected individuals while the opposite was observed for HBsAb positivity ($P=0.019$). Rates of acute ($P=0.033$) and chronic ($P=0.021$) clinical stages were higher in the HIV-1 infected compared to the uninfected individuals but the inverse was noticed for vaccine type response rate ($P=0.008$). Only genotype A, sub-genotype A1 was detected with higher frequencies noted in the HIV-1 infected (n=12) compared to the uninfected (n=4) individuals.

Conclusion: HBsAg and HBcAb-IgM sero-markers and acute and chronic clinical categories are higher while HBsAb sero-marker and vaccine type response stage are lower in HIV-1 infected compared to uninfected IDUs at Mombasa, Kenya. Taken together, HIV infected injection drug users are at risk of acquiring HBV and progressing to chronic infection.

Keywords: HBV sero-markers; HIV-1; IDUs

1 INTRODUCTION

Globally, it is estimated that 12.7 million people are injection drug users of whom 1.02 million are living in Africa [1]. In Kenya, the population of injection drug users is estimated at 49,169 and is concentrated in specific geographic areas mainly Nairobi and Mombasa [2]. Injection drug use compromises immunological status of a person as well as

increasing the chances of acquiring blood borne infections [3, 4]. Thus, identification of HBV sero-markers, clinical stages and genotypes is weighty in improvement of injection drug users' health status.

An exponential increase in HBV and HIV prevalence occurs with the arrival of injection drug use in a community [3]. It has been reported that about 1.2 million and 1.7 million IDUs worldwide are living with HBV and HIV, respectively [1, 5]. Africa accounts for at least 8.9% and 7.2%, respectively, of global HBV and HIV infection rates among injection drug users [1, 5]. Due to shared intravenous infection route and HIV-related immune suppression, between 7% to 10% of HIV infected IDUs are co-infected with HBV [6]. It has been reported that about 4.4% of injection drug users from Mombasa, Nairobi and Kisumu are HBV-infected [7]. These data corroborate previous studies showing HBV prevalence rates of 4.5% [8] and 3% [9] among injection drug users residing at Nairobi. However, these estimates are inaccurate since the five hepatitis B virus sero-markers namely hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B pre-core antigen (HBeAg), hepatitis B pre-core antibody (HBeAb) and hepatitis B core antibody (HBcAb) were not determined simultaneously in these studies.

The five panel HBV sero-markers provide accurate diagnosis and clinical staging of hepatitis B disease into acute, occult, chronic, window, convalescent, immunized and susceptible [10-12]. Hepatitis B clinical stages guide genotyping, clinical management as well as predicting disease reactivation and exacerbation [13, 14]. Meanwhile, it is estimated that 6.9% of HIV-1 infected individuals on antiretroviral therapy at Nairobi [15] and HIV-1 infected female commercial sex workers at Mombasa are living with chronic hepatitis B virus [16]. However, hepatitis B clinical stages among Kenyan injection drug users, especially those resident in Mombasa, are unknown. This study, therefore, examined hepatitis B virus sero-markers, clinical stages and genotype distribution in HIV-1 infected and uninfected IDUs residing at Mombasa, Kenya.

2 METHODOLOGY

2.1 Study site

The present study was conducted at Bomu Medical Hospital in Mombasa County, Kenya (Appendix 2). Mombasa County lies between latitudes 3°56' and 4°10' South of the equator and longitudes 39°34' and 39°46' East, and has a population of about 523,183 people [17]. There are about 27,000 (range: 2,000 to 60,000) people who inject drugs in this coastal town [2]. Drug use debut usually starts at an early age of between 10-15 years and heroin is the most common injected drug in Mombasa [18]. Apart from Mombasa town being an important tourist town, Mombasa port is frequently used as a transit route for illicit drug dealers [19]. Nearly 17% of annual HIV incidence in Kenya's former Coast Province is due to intravenous drug injection [20].

2.2 Study design and study population

This was a cross-sectional study targeting injection drug users resident in Mombasa, Kenya. Injection drug users were defined as those who had injected drugs during the past one month. The specific criteria for ascertaining injection drug users' status was track marks checking. The injection drug users were stratified into either HIV-1 infected or uninfected group based on their HIV-1 statuses'. Injection drug users who were 18 years or older; resident in Mombasa, Kenya; signed an informed consent; and able to disclose any information that were required during the study period.

2.3 Human immunodeficiency virus-1 testing

Each participant underwent pre- and post-HIV test counselling after completion of the interview questionnaire. Approximately, 3 mL of venipuncture blood was collected in BD™ vacutainer anticoagulant tubes (Becton, Dickinson and Company, Franklin Lakes, USA) from each study participant. A drop of the blood was used for HIV-1 serological testing. HIV-1 sero-positivity was determined using Determine™ (Abbott Laboratories, Tokyo, Japan) and Unigold™ (Trinity Biotech Plc, Bray, Ireland) according to the Kenya national guidelines for HIV testing and counselling [21].

2.3.1 Determination of Hepatitis B virus sero-markers

The five hepatitis B virus sero-markers (HBsAg, HBsAb, HBeAg, HBeAb and HBcAb-IgM) were determined by testing of plasma samples using HBV-5 panel rapid test cassette (Healthaw Medical limited, Hangzhou, China) (Appendix 5) according to the manufacturer's instructions. Briefly, 5 µl of plasma was added to the five sample wells (HBsAg, HBsAb, HBeAg, HBeAb and HBcAb-IgM sample wells) followed by two drops of buffer. The assay was let to stand for 20 minutes after which the sero-reactivity was recorded. A coloured band at both test and control zones indicated positive results for HBsAg, HBsAb and HBeAg. Coloured band at either control or test zone indicated negative or invalid results, respectively, for HBsAg, HBsAb and HBeAg. On the other hand, a coloured band at both test and control zone indicated negative results for HBeAb and HBcAb. Coloured band at either control or test zone indicated positive or invalid results, respectively, for HBeAb and HBcAb.

2.4 Determination of Hepatitis B virus clinical stages

Hepatitis B clinical staging was based on interpretation of the five HBV sero-markers (HBsAg, HBsAb, HBeAg, HBeAb and IgM HBcAb) reactivities (Table 1) [10-12].

Table 1: Hepatitis B clinical staging criteria

Hepatitis B Clinical stage	HBsAg	HBsAb	HBcAb-IgM	HBeAg	HBeAb
Acute infection	+/-	-	+/-	-	-
Chronic infection	+	-	+	-	+
Vaccine type response	-	+	-	-	-
Resolved infection	-	+/-	+/-	-	+/-
Uninfected	-	-	-	-	-

The clinical classification of the hepatitis B infection status was adopted from [10-12]. +, positive sero-marker; -, negative sero-marker.

2.5 Data analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Age and CD4+ T cell counts were compared between the study groups using Mann Whitney U tests. The CD4+ T cell immunological stages, gender, and hepatitis B virus sero-markers and clinical categories were compared between the HIV-1 infected and uninfected injection drug users using Chi-square test and Fisher's exact test whenever appropriate. All tests were two-tailed and statistical significance was determined using *P*-value of less than 0.05. Phylogenetic analysis was performed using Neighbor-Joining method based on 1000 bootstrap replicates in MEGA version 6.0 [22].

2.6 Ethical considerations

Initial approval to carry out this study was provided by School of Graduate Studies (SGS) of Maseno University. This study was ethically approved by Kenyatta University Ethical Review Committee number PKU019/116 of 2012 (Appendix 7). This study was conducted according to Helsinki Declaration [23]. Written informed consent (Appendix 8) was obtained from each participant before enrolment. All the study participants were provided with free health education on sexually transmitted infections (STIs) including HIV, hepatitis B and C, tuberculosis, hygiene and nutrition. Participants testing positive for HIV were referred to the comprehensive care centers at Bomu Hospital for treatment, care and support.

3 RESULTS

3.1 Demographic and laboratory characteristics of study participants

The demographic and laboratory measures of the study participants are presented in Table 2. A total of 371 individuals were enrolled in to this study comprising of 157 (42.3%) HIV-1 infected, and 214 (57.7%) HIV-1 uninfected injection drug users. Gender distribution differed significantly between the study groups, such that the HIV-1 infected injection drug users had a higher frequency of females compared to the HIV-1 uninfected injection drug users (54.8% vs. 6.5%; $P < 0.0001$). However, age distribution was similar between the study groups (median, 30.60; IQR, 6.50 vs. 31.71; 9.13 years; $P = 0.271$). Most importantly, the HIV-1 infected injection drug users presented with significantly lower CD4+ T cell counts relative to the HIV-1 uninfected injection drug users (median, 456; IQR, 449 vs. 905; 641 cells/ μ l; $P < 0.0001$). Consistent with the reduced CD4+ T cell counts, the frequency of CD4+ T cell counts < 200 cells/ μ l (38.2% vs. 13.6%) and CD4+ T cell counts $\geq 200 < 500$ cells/ μ l (15.9% vs. 8.4%) were significantly higher in the HIV-1 infected compared to the HIV-1 uninfected injection drug users ($P < 0.0001$).

Table 2: Demographic and laboratory characteristics of HIV-1 infected and Uninfected injection drug users from Mombasa, Kenya

Characteristic	HIV-1 uninfected, n=214	HIV-1 infected, n=157	P-value
Gender, n (%)			
Females	14 (6.5)	86 (54.8)	<0.0001 ^a
Male	200 (93.5)	71 (45.2)	
Age (IQR), yrs.	31.71 (9.13)	30.60 (6.50)	0.271 ^b
CD4 T cells $\times 10^3/\mu\text{L}$ (IQR)	905 (641)	456 (449)	<0.0001 ^b
<200 ^c	29 (13.6)	60 (38.2)	
$\geq 200 < 500^d$	18 (8.4)	25 (15.9)	<0.0001 ^a
499 ^e			
$\geq 500^e$	167 (78.0)	72 (35.9)	

CD4+ T cells were categorized based on CDC categorization (^cCategory 3; ^dCategory 2; ; ^eCategory 1) [24]. ^aChi-Square test. ^b Mann Whitney U test.

3.2 HBV sero-markers in HIV-1 infected and uninfected injection drug users

The HBV sero-marker reactivities of the study participants are presented in Table 3. Of the 371 injection drug users recruited, 20 (5.4%), 49 (13.2%), 0 (0%), 47 (12.7%), and 23 (6.2%), respectively, were sero-positive for HBsAg, HBsAb, HBeAg, HBeAb and HBcAb-IgM sero-markers. Analysis of the HBV sero-marker reactivities revealed that HBsAg positivity was significantly higher in the HIV-1 infected injection drug users compared to HIV-1 uninfected injection drug users (9.6% vs. 2.3%; $P=0.004$). In contrast, HBsAb positivity was significantly lower in the HIV-1 infected injection drug users relative to the HIV-1 uninfected injection drug users (8.3% vs. 16.8%; $P=0.019$). Although none of the study participants in HIV-1 infected and uninfected injection drug users were reactive to the HBeAg sero-marker, HBeAb positivity was similar between the groups (13.4% vs. 12.1%; $P=0.754$). Consistent with the pattern observed for HBsAg reactivity, a significantly higher HBcAb positivity was observed in the HIV-1 infected injection drug users compared to the HIV-1 uninfected injection drug users (10.2% vs. 3.3%; $P=0.008$).

Table 3: Hepatitis B sero-markers in HIV-1 infected and uninfected injection drug users from Mombasa, Kenya

Hepatitis B sero-marker	HIV-1 uninfected, n=214	HIV-1 infected, n=157	P-value
HBsAg	5 (2.3)	15 (9.6)	0.004 ^a
HBsAb	36 (16.8)	13 (8.3)	0.019 ^a
HBeAg	0 (0.0)	0 (0.0)	-
HBeAb	26 (12.1)	21 (13.4)	0.754 ^a
HBcAb-IgM	7 (3.3)	16 (10.2)	0.008 ^a

Data shown are number (n) and proportions (%). HBsAg, hepatitis B surface antigen; HBsAb, hepatitis B surface antibody; HBeAg, hepatitis B pre-core antigen; HBeAb, hepatitis pre-core antibody; HBcAb-IgM, Immunoglobulin M hepatitis B core antibody. ; ^a Chi-square test.

3.3 Hepatitis B clinical stages in HIV-1 infected and uninfected injection drug users

The frequencies of HBV clinical stages are presented in Table 4. In this study, 12 (3.2%), 10 (2.7%), 43 (11.6%), 37 (10.0%) and 269 (72.5%) study participants were diagnosed with acute, chronic, vaccine type response, past/resolved infection and uninfected statuses, respectively. Stratification based on HBV infection status illustrated a significantly higher frequency of acute infection in the HIV-1 infected injection drug users compared to the HIV-1 uninfected injection drug users (5.7% vs. 1.4%; $P=0.033$). Likewise, a significantly higher frequency of chronic HBV infection was observed in the HIV-1 infected injection drug users compared to the HIV-1 uninfected injection drug users (5.1% vs. 0.9%; $P=0.021$). A significantly lower frequency of vaccine type response was observed in the HIV-1 infected injection drug users relative to the HIV-1 uninfected injection drug users (6.4% vs. 15.4%; $P=0.008$). The frequencies of past/resolved (8.3% vs. 11.3%; $P=0.385$) and HBV uninfected statuses (74.5% vs. 71.0%; $P=0.482$) were similar between the HIV-1 infected and uninfected injection drug users.

Table 4: Hepatitis B clinical stages in HIV-1 infected and uninfected injection drug users from Mombasa, Kenya

Hepatitis B Clinical stage	HIV-1 uninfected, n=214 (%)	HIV-1 infected, n=157 (%)	P-value
1	3 (1.4)	9 (5.7)	0.033 ^a
2	2 (0.9)	8 (5.1)	0.021 ^a
3	33 (15.4)	10 (6.4)	0.008 ^b
4	24 (11.3)	13 (8.3)	0.385 ^b
5	152 (71.0)	117 (74.5)	0.482 ^b

1=Acute (HBsAg+/-, HBcAb-IgM+/-); 2=Chronic (HBsAg+, HBeAb+, HBcAb-IgM+/-); 3=Vaccine type response (isolated HBsAb+); 4=Resolved infection (HBsAb+/-, HBeAb+/-, HBcAb-IgM+/-); 5=Susceptible (HBsAg-, HBsAb-, HBeAg-, HBeAb-, HBcAb-IgM-) [10-12]. ; ^a Fisher's exact test.

^b Chi-square test.

4 DISCUSSION

The higher rates of HBsAg (9.6%) with concomitant lower rates of HBsAb (8.3%) positivity detected in the HIV-1 infected injection drug users indicate increased susceptibility to hepatitis B virus infection. These findings, are in part, similar to previous studies in Tanzanian HIV-1 infected injection drug users showing 9.0% HBsAg positivity [25], and previous studies in HIV-1 infected Burmese injection drug users showing 11.3% HBsAg positivity [26]. Most importantly, these findings are partly consistent with previous studies in HIV-1 infected female sex workers from Mombasa (the same region as the current study area) showing HBsAg sero-positivity rates of 6.9% [16]. The HBsAb positivity of 8.3% in the HIV-1 infected injection drug users is comparable to Iranian studies showing a 6.9% HBsAb rates in injection drug users [27]. Likewise, these findings are comparable to studies in India showing HBsAb positive rates of 10.3% in HIV-1 infected adults [28]. In this study, HIV-1 infected injection drug users presented with significantly lower CD4+ T cell counts. Reduced HBsAb production by B cells due to diminished CD4+ T cells counts occur in HIV-1 infected individuals [29]. In addition, impaired HBsAb response has been reported in HIV-1 infected individuals [30]. It therefore, appears that the lower rates of HBsAb positivity in the HIV-1 infected injection drug users reflect reduced HBsAg sero-conversion resulting from immune suppression.

The lack of HBeAg sero-positivity and the comparable rates of HBeAb positivity in this study population are related to the rarity of the co-occurrence of HBeAg and HBeAb positivity in HBV infections [10]. Additionally, the findings of this study showing HBeAb rates of 13.4% in HIV-1 infected injection drug users mirrors a prevalence of 12.5% previously reported in HIV-1 infected Nigerians [31]. Taken together, these findings suggest reduced viral replication in the acute and chronic injection drug users at this coastal population.

The finding of this study showing HBcAb-IgM positivity of 10.2% is comparable to previous studies showing rates of 10.7% HBcAb-IgM positivity among HIV-1 infected Indians [32]. Also, this finding parallels rates of 7.8% HBcAb-IgM positivity in healthy Egyptians [33]. The higher rates of HBcAb-IgM positivity identified in the HIV-1 infected injection drug users, suggest increased exposure rates to the hepatitis B virus.

Although complete serological testing for the five hepatitis B virus sero-markers is vital in accurate diagnosis and clinical staging of hepatitis B virus infection [10, 11], this is the first study to examine the utility of the five hepatitis B virus sero-markers in identifying the clinical stages of hepatitis B virus infection in HIV-1 infected and uninfected injection drug users from Kenya. The findings of this study showing acute hepatitis B infection rates of 5.7% in the HIV-1 infected injection drug users corroborates previous studies showing acute hepatitis B virus infection rates of 5.1% among injection drug users from a high HIV-1 risk region of Scotland [34]. Thus, HIV-1 infected injection drug users are at increased risk of acute hepatitis B virus infection. The higher rates of chronic hepatitis B virus infection of 5.1% identified in the HIV-1 infected injection drug users suggest that HIV-1 infection directly increase the risk of acquiring and developing hepatitis B in injection drug users. This finding is in part consistent with rates of 7.1% observed in HIV-1 infected injection drug users in the United States of America [35], and the 6.9% prevalence of chronic hepatitis B virus infection detected in HIV-1 infected female sex workers from Mombasa (same study area as the current study) [16].

The lower rates (6.4%) of vaccine type responses observed in the HIV-1 infected injection drug users are related to the immunosuppression state. This observation is similar to 7.0% prevalence of vaccine-type response previously detected among HIV-1 infected illicit drug and non-drug using women in the United States [36]. Although not determined in the current study, the reductions in the hepatitis B virus vaccine response during HIV-1 infection is possibly due to the decline in CD4+ T cells [37] responsible for priming specific memory B cells to produce HBsAb after HBV vaccination [38] and diminished HBsAg-specific memory B cells [39]. In addition, HIV-1 infected individuals experience a more

accelerated decline in serum HBsAb following HBsAg vaccination [38]. The implication of these findings is that incorporating immune modulators and vaccine booster dose regimens greatly enhances immunity in the co-infected individuals.

Consistent with the pattern observed for vaccine-induced immunity, the rates of resolved infection were lower in the HIV-1 infected injection drug users indicating immune suppression. Findings of this study showing resolved infection rates of 8.3% in HIV-1 infected injection drug users is comparable to 6.7% previously reported among drug users in Luxembourg [40]. This lower rate of resolved infection in HIV-1 infected injection drug users also relate to the reduced CD4+ T cell levels leading to failure of HIV-1 infected individuals to resolve hepatitis B virus infection [41]. Taken together, the lower rates of the resolved infection in the HIV-1 infected injection drug users imply weakened immune-mediated hepatitis B infection resolution.

The sero-exposure rates of 25.5% and 29.0% identified, respectively, in the HIV-1 infected and uninfected injection drug users, suggest high exposure to HBV infection in this population. These results are partly consistent with previous studies among drug addicts in Bangladesh showing exposure rates of 27.8% [42] and among injection drug users from San Francisco showing exposure rates of 21.0% [43]. Similarly, these exposure rates are, in part, consistent with previous studies showing exposure rates of 26.5% among antiretroviral treatment naive HIV-1 infected South Africans [44], suggesting that injection drug users from this coastal Kenya region experience intermediate hepatitis B virus endemicity (rates of 2-8% based on the HBsAg positivity) [45]. Although no studies from Kenya have applied the five HBV sero-markers in evaluating HBV sero-reactivity, previous studies in eastern Kenya utilizing three HBV sero-markers (HBcAb, HBsAb and HBsAg) illustrated total sero-reactivity rates of 56.2% among outpatients attending three distinct hospitals in the towns of Mombasa, Kilifi, and Malindi [46]. This contrasting results are attributable to the detection of HBcAb-IgG (indicating a past exposure) in the previous studies [46], the rapid disappearance of HBcAb-IgM and the persistence of HBcAb-IgG [12]. Therefore, the five sero-marker testing can be applied in accurately detecting recent exposure rates to hepatitis B virus infection in individuals most-at-risk including injection drug users.

5 CONCLUSION

Higher HBsAg and HBcAb-IgM sero-positivities and Acute and chronic clinical stages are higher in HIV-1 infected injecting drug users indicate that the HIV infected injection drug users are at higher risk of acquiring HBV and progressing to chronic infection.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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