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PREVALENCE AND ASSOCIATED FACTORS OF HEPATITIS B VIRUS AMONG PATIENTS ATTENDING AN STI HEALTH FACILITY IN NAIROBI, KENYA
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ABSTRACT

Objectives: **The study aimed at determining the distribution of HBV infection, immunity status and associated factors among STI clinic attendees in Kenya.**
Study design: **The study was a cross sectional descriptive study that followed a quantitative approach. It was conducted from April to June 2014. Questionnaires were used to collect data and from the consenting participants blood was drawn and screened for HBsAg, anti- HBV and anti-HBc serology**
Setting: **The study was carried out at the STI clinic -specialized treatment Centre in Nairobi. Subjects: Systematic random sampling was used to enroll the subjects.200 consenting STI attendees aging between the ages of 18 to 60 years were recruited.**
Results: **The mean age of the study subjects was 32.77 years (SD=8.638) and a median of 31 years. Among the 200 samples analyzed, the prevalence rates of HBsAg and anti- HBV were found to be 19/200 (9.5%) and 53/200 (26.5%) respectively. The major associated factors of hepatitis B transmission that were found to statistically significant among the study population were unprotected sex (p -value 0.004*<0.05), number of sexual partners (p-value 0.003<0.05) and those with genital ulcers (p-value <0.001). Majority of the study subjects had immunity from natural infection 53/200 (26.5%).**
Conclusion: **Sexual mode of transmission of hepatitis B was found to be associated with HBV infection among the STI attendees in Kenya. HBV is a vaccine preventable infection and the results highlight the importance of HBV vaccination among STI clinic visiting patients and their clients/partners.**

NTRODUCTION

Hepatitis B is one of the major and common infectious diseases of the liver which is caused by small enveloped DNA virus called the hepatitis B virus (HBV) (Tong *et al.,*2005). It is estimated that over 2 billion people are infected by HBV worldwide with 350 million people being chronically infection (Uneke *et al*2005). The chronically infected individuals have higher risks of dying from liver cancer that kills about 1 million people every year (WHO, 2000). In endemic zones like Kenya, where carrier rates are >5%, most persons are infected during early childhood through vertical transmission (wright TL, 2006) since HBV is sexually transmitted diseases (STDs).The disease poses a serious public health problem with a large number of cases leading to disease progression, social and

economic impacts on more severely affected countries, (Tessema *et al.,* 2010).STDs are usually common among the core groups, such as female sex workers (FSWs), who mainly have multiple sex partners and receive poor health care (Znazenet al., 2010). Sex workers (SW) are considered a high-risk group for acquisition of STIs (Hvan etal., 2010; Wang et al., 2009) because of their social vulnerability and other factors like history of multiple sex partners and inconsistence usage (Fernandes et al., 2014; Brussa et al., 2009).

The fact that HBV is sexually transmitted, sex workers remain a high-risk group due to their numerous clients. Therefore, there is great potential for some of these clients to act as a bridging population for further spread of this important STD into the general population (Ghys PD et al., 2001) Safe and effective vaccines against HBV infection have been available since 1982 in Kenya (Kenya National Hepatitis guidelines 2014).

The implementation of the mass immunization programmes in most countries which have been recommended by the WHO since 1991, have dramatically decreased the incidence of HBV infection among infants, children, and adolescence (WHO, 2000). However, not all countries have adopted these recommendations by WHO and there remain a large number of persons that could have been with HBV prior to the implementation of immunization programs.

In Kenya, infant vaccination was adopted in 2002, though the same has not been rolled out to other populations such as Sex workers thus, leaving them at risk of contracting HBV infection. Despite this, a high increase in liver cancer cases is being noted in the country that could partly be caused by HBV. The extent of this silent killer disease has been studied and documented in most risk groups thus little is known of this viral infection among the STD patients who are recognized as a high risk group also. Studies in Kenya have shown a prevalence rate of HBsAg of 8.8% among the general population but values in urban areas range from between 8-30% (Mutuma et al., 2011;). In one of the studies, 34% of those positive for HBsAg were children aged between 5 and 10 years (Bagshawe et al., 1973). As a result of the complications related to this infection and the fact that the HBV and Human Immunodeficiency Virus (HIV) have a shared mode of transmission, there is need to find the magnitude of HBV infection among this risk group which could be in the increase as the HIV epidemic.

MATERIALS AND METHODS

This study was conducted at specialized STI clinic in Nairobi, Kenya. Ethical clearance was obtained from the National Ethical Review Board, (Kenya Medical Research institute Committee). The center has been specially established to offer subsidized health services to individuals with sexually transmitted infections (STIs). Its clients are mostly low income earners and commercial sex workers. About 400 both new and old patients are seen and treated on a daily basis. The facility is meant to serve residents of Nairobi and its environs**.**

Study population:

STI patient attendees between the ages of 18 and 60 years, who consented to participate and be tested for HBV markers serologically, were recruited. A sample size of 200 was calculated as representative of the participants. Individuals were approached at the clinic's waiting bay after registering and informed about the study. Using the registered individuals for the day, a systematic sampling method was employed to recruit subjects into the study. Those willing to participate were invited and explained further about the study and informed consent obtained. HBsAg, HBs and HBc were screened using ELISA test Methods. Semi structured questionnaires were administered to the participants by the researchers to obtain demographic characteristics and knowledge about the disease under study. Then 5ml venous blood was collected from the consenting patients. Specimens were immediately

processed by separating serum from blood products using a centrifuge at 1,500 revolutions per minute (rpm) for 10 minutes (KUBOTA KS-5000) for samples that had not separated at room temperature. The serum was carefully separated into cry vials, properly labeled and then transported to KEMRI Hepatitis laboratory using cold chain and only non-hemolysis blood samples were used for the screening process.

The samples were screened for HBV using Kemri Hepcell, a reverse passive haemaglutination based kit. Briefly, 25 µl each of Hepcell diluent was added to three wells of a micro-titter plate. Then 25 µl of the sample was the added to the first well and serially diluted to the third well. Twenty-five microliters of the Hepcell sensitized cells was added to the third wells. Then it was incubated at room temperature for two hours. Where there was haemagglutination in the third well the sample was regarded as positive and subjected to confirmation.

During confirmation, two rows each of six wells were used. In each well of the first row 25 µlof Hepcell diluent was dispensed. Corresponding rows of the second row were dispensed with equal volumes of inhibition buffer. Twenty-five microlitres of sample will then be added into each of the first wells of the two rows and serially diluted to the sixth wells. Then sensitized cells were added to the last four wells of the rows and the micro plate incubated at room temperature for two hours.

The number of rows with haemagglutination in the second row were counted and subtracted from that of the first row. Where the difference was equal to two or more, the sample was termed as positive. Where the difference was less than two the sample was reported as negative.

HEPATITIS B SURFACE ANTIBODY TESTING:

The strip holder was fitted with the required number of Monolisa strips, a volume of 25 uls of specimen diluent was added into the assigned wells, then 100mls of undiluted sample or control; then the mixer was incubated at 37oc for 60 minutes, a volume of 50uls of the conjugate was added into each well and then incubated the strips at 37oc for 60 minutes.

The microplate was then washed six times with phosphate buffer solution; a volume of 100uls TMB substrate was added into each well and the strips incubated at 15oc for 30 minutes in the dark. The reaction was stopped by adding 100ul 1mol/1 sulphuric acid to each well. Then the solution absorbance was read at 450 nm within 15 minutes. Results were then interpreted according to the manufacturer's instruction.

ANTI-HBC ASSAY PROCEDURE:

A volume of 200 uls of Specimen diluent was pipetted into all wells of the micro titer plate except well 1A. Ten microliter of controls or serum samples was pipe pipetted into the appropriate wells. Cover seal was then applied and the plate incubated at 37oC ± 1oC for 1-hour. The plate was washed five times with Wash Buffer. 200ul of Antibody Conjugate was then added to all wells except 1A.

Cover seal was used to cover the plate and incubate at 37oC ± 1oC for 1 hour. Test wells and controls were then filled substrate and then all the wells were washed five times with Wash Buffer. Add 200 ul of Substrate Solution to all the wells including 1A. The plate was incubated at room temperature for 30 minutes in the dark. 50 ul of 4N sulfuric acid (H2SO4) was then added to all wells including 1A and their action was read at 492 nm in the ELISA reader. Data analysis: The proportion and the mean value were computed in appropriate situations. To find out any association between categorical data, Chi square test was employed using the SPSS (Version 16). The study finding data were presented as frequency ± SD and 95% Confidence Interval. The determinants for HBV infection is determined by calculation of Odd Ratio. Chi square used to determine the significance of differences between the risk factors.

RESULTS

Demographic Characteristics of Respondents

Summary of socio-demographic of patients attending a sexually transmitted infections clinic are shown in table 4.1.

Table 4.1

Distribution of participant's response according to social demographic variables

Variable	Frequency (n=Samples)	Percent %
Age in years		
18-23	27	13.5
24-29	52	26
30-35	51	25.5
36-41	35	17.5
42-47	20	10
48-53	11	5.5
54-60	4	2
Gender		
Male	80	40
Female	120	60
Marital status		
Married	139	69.5
Single	44	22
Cohabiting	4	2
Widowed	6	3
Divorced	7	3.5
Religion		
Christians	194	97
Muslims	6	3
Level of education		
None	2	1
Primary	59	29.5
Secondary	98	49
Tertiary	41	20.5
Occupation		
Unemployed	57	28.5
Formal employee	40	20
Self employed	97	48.5
Student	6	3
Housing		
Temporary	6	3
Semi-permanent	46	23
Permanent	148	74

A total of 200 patients aged 18-60 years with a mean age±SD of 32.8±SD=8.6 and a median of 31 years participated. Majority, 26% (52/200) were aged 24-29 years. More than half, 60% (120/200) were females while 40% (80/200) were males. Most were married 69.5% (139/200) while 22% were single. Ninety-seven percent (194/200) were Christians while only 3% were Muslims. Majority, 49% (98/200) had attained secondary education. In terms of occupation status, 48.5% (97/200) of those who were self-employed formed the highest proportion. Majority lived in permanent houses at 74% (148/200).

Prevalence of HBsAg, anti HBc and HBsAb

The prevalence of HBsAg and HBsAb are shown in table 4.2.

Table 4.2

Proportion of HBsAg and HBsAb among the respondents

Variable	n=Samples	%	95% CI
HBsAg status			
Positive	19	9.5	5.81-14.43
Negative	181	90.5	85.56-94.18
HBsAb status			
Positive	53	26.5	20.52-33.18
Negative	147	73.5	66.81-79.47

The crude prevalence of HBsAg and HBsAb was found to be 9.5% (19/200) and 26.5% (53/147) respectively among patients attending a sexually transmitted clinic (STC) in Nairobi County.

Socio-demographic characteristics associated with HBsAg positivity

The socio-demographic characteristics of patients are summarized in table 4.3 below.

Table 4.3

Social Demographic factors

Variable	HBsAg status							
	Positive		**Negative**	**%**	**OR**	**p-value**	**Total**	**95% CI**
Gender	n=Samples	%	n=Samples			0.844		
Male	8	42.1	72	38.8	1.101		80	20.25-66.50
Female	11	57.9	109	57.9			120	33.49-79.74
Anti-HBc Results								
Negative	2	1.5%	129	98.5%	0.148	<0.0001*	131	0.04-0.550
Positive	17	24.6%	52	75.4%			69	2.36-4.106
Age in years						0.535		
18-23	4	21.1	23	12.7	1.00		27	6.05-45.56
24-29	4	21.1	48	26.5	2.087		52	6.05-45.56
30-35	3	15.8	48	26.5	2.783		51	3.38-39.57
36-41	5	26.3	30	16.6	1.043		35	9.14-51.20
42-47	2	10.5	18	9.9	1.565		20	1.30-33.13
48-53	0	0	11	6.1	1.04		11	0-17.64
54-60	1	5.3	3	1.7	0.522		4	0.13-26.02
Marital status						0.616		
Married	13	68.4	126	69.6	1.00		139	43.44-87.42
Single	5	26.3	39	21.5	0.20		44	9.14-51.20
Cohabiting	1	5.3	3	1.7	1.21		4	0.13-26.02
Widowed	0	0	6	6.3	0.31		6	0-17.64
Divorced	0	0	7	3.9	1.01		7	0-17.64
Religion						0.543		
Christians	18	94.7	176	97.2	0.511		194	73.97-99.86
Muslims	1	5.3	5	2.8			6	0.13-26.02
Education						0.66		
None	0(0%)	0	2	1.1	1.00		2	0-17.64
Primary	6(31.6%)	31.6	53	29.3	0.50		59	12.57-56.55
Secondary	11(57.9%)	57.9	87	48.1	0.54		98	33.49-79.74
Tertiary	2(10.5%)	10.5	39	21.5	0.59		41	1.30-33.13
Occupation						0.721		
Unemployed	7	36.8	50	27.6	1.00		57	16.28-61.64
Formal employee	3	15.8	37	20.4	1.73		40	3.38-39.57
Self employed	9	47.4	88	48.6	1.33		97	24.44-71.13
Student	0	0	6	3.3	0.80		6	0-17.64
Housing						0.693		
Temporary	0	0	6	3.3	1.00		6	0-17.64
Semi-permanent	4	21.1	42	23.2	1.80		46	6.05-45.56
Permanent	15	78.9	133	73.5	1.12		148	54.43-93.94

**Significant factors p-value<0.05*

In regard to age, majority of patients who tested positive for HBsAg were aged 36-41 years, 26.3% (5/19). In terms of gender, females formed the highest proportion at 57.9% (11/19). Sixty eight point four percent (13/19) were married whereas 94.7% (18/19) were Christians representing the majority. In regard to the level of education, majority 57.9% (11/19) had attained secondary level. In terms of occupation, majority were self-employed at 47.4% (9/19) followed closely by those who were unemployed at 36.8% (7/19). Lastly, in regard to housing standards, majority, 78.9% (15/19) lived in permanent houses. None of the socio-demographic factor was found to be significant in explaining the outcome of HBsAg status.

Factors associated with HBsAg sero-prevalence among patients attending STC

The factors associated with HBsAg sero-prevalence are shown in table 4.4.

Table 4.4

Distribution of hepatitis B markersamong the respondents

Results for Test				
Elisa HBsAg results	HBsAb	anti-HBc	n=Samples	%
Negative	Negative	Negative	131	65.5
Negative	Positive	Positive	53	26.5
Positive	Negative	Positive	16	8

The number of sexual partners was found to be statistically significant associated with HBsAg sero-prevalence ($\chi 2=15.831$; $p<0.0001$*). However, those who had more than two sex partners formed the highest proportion at 94.7% (18/19).

Ever bought or sold sex was also found to be related to HBsAg sero-prevalence ($\chi 2=4.945$; $p=0.026$). However, 11/19(57.9%) of those who tested positive for HBsAg had never bought or sold sex were the majority. Have you injected yourself with drugs was significantly associated with HBsAg positivity ($\chi 2=3.854$; $p=0.05$). However, majority, 18/19 (94.7%) of those who tested positive for HBsAg reported to haven't injected themselves with drugs. Lastly, history of genital ulcers was also found to be statistically significant in explaining theHBsAg positivity ($\chi 2=114.696$; $p<0.0001$*). However, majority of those who tested positive for HBsAg reported history of genital ulcers.

DISCUSSION

HBV infection is a public health problem given that a third of the world's population has been infected with HBV at sometimes in their life, and of these approximately 400 million remain infected chronically (Uneke et al, 2005).

Kenya has been considered among high endemic areas for HBV infection with upward trends of sero-prevalence These results are inconsistent with the present study especially the prevalence of HBsAg as it was found to be high (9.5%) as compared to the former study. However, the prevalence of anti-HBs in the former study was in agreement with present study as both found a prevalence of 26.5% despite different populations studied.

Moreover, uni-variate analysis for risk factors associated with HBV infections revealed that history of tuberculosis (p value 0.0091*) which was significantly associated with HBV incidence. History of injections in past 6 months (p-value 0.0079*) did not increase the risk of acquiring HBV infection. Additionally, a history of inguinal swelling also (p-value0.0571) revealed borderline significance. However, in the present study, the number of sexual partners (p value 0.0047*), history of injecting with drugs (p value 0.003*) and history of genital ulcers (p value 0.001*) were found to be statistically significant at multivariate level which involved fitting of binary logistic model.

Forbi et al., 2008, evaluated seven hundred and twenty (n = 720) Female Sex Workers (FSWs) in Nigeria and revealed that HBsAg was present in one hundred and twenty-three (n = 123) giving overall prevalence of 17.1%. This prevalence was however high as compared to the present study that recorded HBV incidence of 9.5% (n=19). The difference might be due to different study populations studied. In a survey aimed at determining the seroprevalence of viral hepatitis B and C in females who engage in illegal sex behavior in Isfahan, Iran showed that HBsAg was detected in only 1 participant (1.1%), anti-HBc in 4 (4.4%), anti-HBs in 60 (65.9%), and HCV-Ab in 9 (9.9%). Evidence of vaccination was seen in 54 subjects (59.3%) (Kassaian et al., 2011). among blood donors being observed (Kwena, 2014).

This study was significantly different from the present study because the prevalence of HBsAg was extremely low as compared to the present study that recorded 9.5% incidence. However, the prevalence of anti-HBs was found to be high 65.9% as compared to the present study that revealed 26.5% (53/200).

A Brazilian study revealed that anti-HBc marker, HBsAg and anti-HBs were 13% (57/440), 3.4% (15/440) and 8.5% (37/440) respectively. In addition, the previous study revealed that; homo/bisexual behavior, past homosexual, anal intercourse, HIV infection, syphilis infection and blood transfusion were independently associated with HBV infection. However, the present study showed that the number of sexual partners, injection with drugs and history of genital ulcers were the only factors that were significantly associated with HBV exposure (Oliveira et al., 2001). This discrepancy may be due to different study populations in different geographical settings studied.

CONCLUSIONS AND RECOMMENDATIONS

This study showed an intermediate prevalence of HBV (9.5%) and anti-HBsAg (26.5%) among STI clinic attendees. This high prevalence of anti- HBV serologic marker suggesting previous exposure to or natural infection was found to be high among this study population. The Multiple sexual practices, drug use and having genital ulcers were found to be statistically significant predictors of prevalence of HBV.Based on findings of this work the following recommendations were made, the significant burden of hepatitis B infection among STI attendees highlights the need for educational programmes such as health education to explain how the disease spreads and its control measures to this risk group. Regular screening of HBV be done among all patients visiting STI clinics is highly recommendable to reduce the prevalence of HBV. It is also important that safe and effective HBV vaccines to be administered to sex workers and their clients. Future studies to determine the stage of infection and circulating genotypes are highly recommended.

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