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Enzyme link Immunosorbent Assay-based Detection of Hepatitis B Virus Infection in Correctional Facilities in Sokoto Metropolis, Sokoto State Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors BRA designed the study. Authors BRA, ABS and BIA performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BRA and MFU managed the analyses of the study. Authors MB, AF and AJO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

ELISA-Based detection of HBV infection in correctional facilities in Sokoto metropolis, Sokoto state Nigeria was carried out using a questionnaire survey to determine the associated risk factors of the disease and serological method using [Enzyme link immunosorbent Assay (ELISA)] to determine hepatitis B surface antigen (HB_SAg) among inmates. Of the 180 participants from the two correctional facilities in Sokoto state (Sokoto central prison and Remand home) overall prevalence rate of (42.2%) was obtained. The distribution of HBV infection according to age was statistically significant, and the age groups of 21-25 and 36-40 years had the highest prevalence rates (61.3%).

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However higher prevalence was recorded among males (42.6%) than females (36.4%). Analysis of spatial distribution of prevalence of HBV infection showed that Remand home (juvenile detention centre) had the highest prevalence rate of (75%). It was concluded that hepatitis B virus infection was found to be prevalent in correctional facilities in Sokoto state in which duration of stay and multiple sexual partners were the main risk factors. Furthermore there is need for Nigerian government to establish a policy that would make general screening of all the inmates in correctional facilities for HBV infection and vaccinate them against the disease.

Keywords: Hepatits B virus; ELISA; Juvenlie detention center.

1. INTRODUCTION

Hepatitis B virus (HBV) is an envelope, double-stranded DNA virus belongingto Hepadnaviridae family and genus Orthohepadnavirus. It is among group VII viruses and possesses double-stranded DNA genomes asnd replicate using reverse transcriptase according to Baltimore classification of viruses [1].

Hepatitis B surface antigen or HBsAg, previously described as Australia antigen, is the most important protein of the envelope of Hepatitis B Virus [2].

Hepatitis B virus infection is a potentially life-threatening liver infection caused by the hepatitis B virus, in some people, the hepatitis B virus can cause chronic liver infection and puts people at high risk of death from cirrhosis and liver cancer [3]. However, some people have acute illness with symptoms that last several weeks, including yellowing of the skin and eyes (jaundice), dark urine, extreme fatigue, nausea, vomiting and abdominal pain [3].

Therefore the complications from hepatitis B include progression to hepatocellular carcinoma (HCC) and, rarely, cirrhosis [3].

The development of chronic infection is very common in infants infected from their mothers or before the age of 5 years.Infection in adulthood leads to chronic hepatitis in less than 5% of cases [4].

A small subset of persons with acute hepatitis can develop acute liver failure which can lead to death [3].

Hepatitis B prevalence is highest in sub-Saharan Africa and East Asia, where between 5–10% of the adult population is chronically infected [5]. High rates of chronic infections are also found in the Amazon and the southern parts of eastern and central Europe [4].

A vaccine against hepatitis B has been available since 1982. The vaccine is 95% effective in preventing infection and the development of chronic disease and liver cancer due to hepatitis B [3].

A correctional facility, detention centre, penitentiary, prison, or remand centre is a facility in which inmates are forcibly confined and denied a variety of freedoms under the authority of the state as a form of punishment.Prisons for juveniles are known by a variety of names, including "youth detention facilities", "juvenile detention centres", "Remand centres" and "reformatories" [6]. Like adult facilities, youth detention centres in some cases are experiencing overcrowding due to large increases in incarceration rates of young offenders, which can create extremely dangerous environments in correctional facilities. Overcrowding may also lead to the decrease in availability to provide the youth with much needed and promised programs and services, therefore the facilities can become unstable and create instability in simple logistics [7].

Correctional facilities is therefore a place where people of unknown health status, could spread infectious diseases.

Intravenous drug users (IDUs), and those sharing of nail cutter, clipper and or those engaged in homosexual activities, are at high risk for infection with several blood-borne pathogens such as HBV, hepatitis C1 virus (HCV), and human immunodeficiency virus (HIV) [4].

HBV infection and other blood-borne diseases among prison inmates are higher than the general population and maybe it is because of the over representation of IDUs and high-risk addiction-related behaviours, having multiple sexual partners and homosexuality, prison life style and limited educational opportunities of this Population group [4].

Currently, there is no periodic national survey for HBV in either the Nigerian population or in correctional facilities; however, prisoners worldwide are at high risk of contracting HBV, especially those who engage in high-risk behaviours and therefore could serve as potential reservoirs of infection to the uninfected incarcerated people or non-incarcerated population upon regaining freedom. In this study a cross-sectional survey was carried out in selected correctional facilities in Sokoto metropolis, Sokoto state Nigeria, to determine the prevalence of hepatitis B surface antigen and associated risk factors with this silent and deadly infection.

2. MATERIALS AND METHODS

2.1 Study Design

The study was descriptive cross sectional study.

2.2 Study Area

The study was conducted in Sokoto central prison, and Remand home (juvenile detention centre) in Sokoto metropolis, Sokoto state Nigeria. Sokoto is the capital city of Sokoto State, The metropolitan city of Sokoto lies between latitude 13°3' 490N, longitude 5°14' 890E and at an altitude of 272 m the sea level above [8].

2.3 Samples Collection and Transportation

Five milliliters (5 ml) ofVenous blood samples were collected from each subject using a sterile 5ml Syringe and needle. The collected blood samples were transferred into clean plain sample bottles and transported to the Medical Microbiology Laboratory of UsmanuDandodiyo University Sokoto (UDUS) where serum was harvested and stored at -20C .The samples were later transported to UsmanuDanfodio University Research Center Sokoto where ELISA test was conducted. During sample collection a total of 180 structured questionnaires on the general awareness of HBV infection were administered to the inmates study area. The questionnaire was initially tested for validity and reliability before the final copy was printed and used for the study.

2.4 Study Population and Sample Size Determination

One hundred and eighty (180) subjects were involved aged (≥15) comprising 169 males and 11 females in correctional facilities in Sokoto state. The sample size was calculated using the formula for Sample Size, $n=z^2pq/d^2$ [9].

2.5 Ethical Clearance

The study was approved by the State Ministry of Health and prison authority (Ministry of interior)

2.6 Inclusion Criteria

Inclusion criteria include inmates, and willingness to give an informed consent to partake in the study.

2.7 Assay of Serum Samples

The serum samples were analysed for the presence of HBsAg using the sandwich Enzyme Linked Immunosorbent Assay (ELISA) AccuDiag™ HBsAg ELISA and the procedure of the sandwich ELISA was carried out according to manufacturer's instruction.

- The reagents and samples were allowed to reach room temperature (18-30℃) for 30 minutes.
- Wells were numbered including one Blank (e.g. A1), three for the Negative control (e.g. B1, C1, D1), and two for the Positive control (e.g. E1, F1).
- 50 µl of Positive control, Negative control, and test sera were added into their respective wells.
- 50 µl HRP-Conjugate (Horseradish peroxidase-conjugated anti-HB_S) to each well except the Blank was added, and mixed by tapping the plate gently.
- The plate was covered with plate cover and incubated for 60 minutes at 37℃.
- Plate cover was removed and discarded.
- The plate was emptied by aspiration and washed each well 5 times with diluted Wash buffer.
- After the final washing cycle, the plate was dried by turning them upside down onto blotting paper.
- 50 µl of Chromogen A (Urea peroxide solution) and 50 µl Chromogen B (tetramethylbenzidine solution) was dispensed into each well including the Blank, and mixed by tapping the plate gently.
- The plate was incubated at 37° for 15 minutes avoiding light.

- Multichannel pipette was used to stop the reaction by adding 50 µl Stop Solution (diluted sulfuric acid solution 0.5 M H_2SO_4) into each well and was mixed gently.
- The cut-off value was calculated using, cut-off value (C.O.) = Nc x 2.1. The A (absorbance) value of the blank must be < 0.080, the A (absorbance) value of the positive control must be > 0.800, the A (absorbance) value of the negative control must be < 0.100 and the result was interpreted as: Negative results (A/ C.O. < 1), Positive results (A/ C.O. >1) and Borderline (A/ C.O. = 0.9-1.1).

2.8 Statistical Analysis

Data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS) software version 22. Chi-square and fisher's exact test were used to perform categorical comparison and level of significance at 99% confidence interval. Regression analysis was also used to investigates the relationship between variables (risk factor and HBV infection) P-value less than or equal to 0.01 was considered statistically significant.

3. RESULTS AND DISCUSSION

Of the 180 participants from the correctional facility in Sokoto state (Sokoto central prison), showed an overall prevalence rate of (38.1%), which was regarded as high prevalence rate. World Health Organisation (WHO) defines low prevalence to be <2%, moderate prevalence as 2-8% and high prevalence as > 8% HB_SAg positivity [10].This result is higher than the prevalence rate of 23% found among prison inmates in Nasarawa state [11], 6.7% among inmates in Lagos [12], 25.5% among Ghanian prisons [13] and (19.9%) obtained among blood donors in Osogbo, Osun state Nigeria [14]. The reasons for these variations may be related to the fact that infection tend to vary from one locality to another and from one country, depending on the level of associated risk factors [15], also the higher prevalence obtained in this study could be due to the fact that study population was among the most high risk group. The 38.1% prevalence observed in this study is also higher than the 20.0% observed in Greece prisons [16], 0.9% reported for prison inmates in USA [17]. The possible reasons for this difference may be due to difference in geographical locations and institutionalized infection control measures taking by some countries against HBV infections. Nigeria a tropical country, has been documented as highly endemic for HBV infection and about 75% of its population is likely to have been exposed to the virus at one time or the other in their lives [18]. The prevalence rate in Nigeria is high and 19 million Nigerians are considered to be infected with HBV [19] and have reached hyper-endemic levels with the prevalence of HB_SAg estimated to range from 10-40% [20].

There was no observed statistical difference in sex distribution of HBV infection in correctional facilities, however higher prevalence was recorded among males (38.3%) than females (36.4%) this could be attributed to the number of male to females in the study and males engaged themselves in practicing of illegal behaviours. This finding agrees with that of Pennap et al. [21] and Isah et al. [15] which showed higher prevalence of HBV infection among males than females. The result however contrast the report of Mustapha and Jibrin [22] in which prevalence of HBV infection was higher in female than male.

The distribution of HBV infection according to age was statistically significant, and the age groups of 21-25 and 36-40 years had the highest prevalence rates 48.8% and 52.4% respectively, this could be due to the fact that they are within the reproductive age group and their over representation among the age groups in the study. And this agreed with the earlier reports among the general population in Nigeria, Brazil and Ghana which spans the generally accepted sexually active age group of 15-45 years [13]. This finding is contrary to report by Isah et al. [15] and Okonko et al. [23] which showed higher prevalence rate in those above 40 years. Apart from HIV, many people are not aware with other sexually transmitted diseases and continue to have unprotected sex with other HIV negative partners who might be chronic carriers of HBV [15].

There was no significant difference in the prevalence of HBV infection in correctional facilities based on their ethnicity, however, higher prevalence rate was observed among Hausa's (42.2%) followed in decreasing order by Igbo, Yoruba, Fulani and [others (Gobirawa, Dakarkariand Zabarmawa)]. The high prevalence among Hausa`s could be due to their large number among the ethnic groups. Also no significance difference was observedin the

prevalence of HBV infection in correctional facilities based on their Nationality. High prevalence was observed among Nigerians (37.8%) this could be due to their over representation in the study.

Prevalence of HBV infection in correctional facilities based on their educational background was significant. Highest prevalence was observed among those with primary education followed by those that attended non formal education followed by those with secondary education and the least prevalence was observed among those with tertiary education. The high prevalence among these groups could be due to their low level of education and lack of knowledge on HBV infection (unawareness), while the lower prevalence among those in tertiary institution is because they are careful enough and usually take necessary precautions when negotiating new sexual partners.

The prevalence of HBV infection based on marital status was statistically significant. Higher prevalence rate was observed among those that were married followed by those that were divorcees, separated and singles, while none was found to be positive among widows. The high prevalence among married ones could be attributed to their indiscriminate sexual activities.

Significant difference in prevalence of HBV infection in correctional facilities was also observed based on their duration of stay in correctional facilities, inmates that spends 2-3 years had the highest prevalence followed by those with >3 years and the least 0-1 years. This shows that high chance increases with long period of incarceration.

There was no significant difference in the prevalence of HBV infection in correctional facilities based on their occupation. Those in public service and farming were most infected (60%), followed in decreasing order of prevalence by Businessmen, Students and those in civil service.

Significant difference in prevalence of HBV infection in correctional facilities was also observed based on their locations. Remand home (juvenile detention centre) had the highest prevalence rate of (75%), while Sokoto central prison had prevalence of (38.1%). The high prevalence among those in remand home might be associated to their dirty habit because they are still young and their untidy environment as compared to that of Sokoto central prison.

Among the associated risk factors result showed that multiple sexual partners are associated with higher prevalence rate of HBV infection in correctional. This could be as a result of indiscriminate sexual activities and lack of taking measures / unprotected sex which will increase the chance of infection.

No association was observed between blood transfusion and prevalence of HBV infection in correctional facilities. This tally with the reports of Adoga et al. [11] and Adekanle et al. [24] who reported insignificant association of HBV with blood transfusion. The result of the present study however contradicts the report of Agbede et al. [25] who observed significant association between the HBV and blood transfusion. Also, intravenous drug use (IDU) was not significantly associated with the prevalence of HBV infection in correctional facilities in Sokoto state. This agreed with the report of Abdul et al. [26]. This could be as a result of banning of IDU practice among the prison inmate in the study area. In addition, the result of this study did not reveal significant association between sharing of sharp objects with prevalence of HBV infection in correctional facilities in Sokoto state. It may be due to the restriction on the use of the sharp objects imposed on the inmates. This is in contrast with the reports of Samuel et al. [27], Ray and Hunter [28], who reported significant association between the sharp objects and infection with the HBV. Similarly, the result of this study did not reveal significant association between knowledge of the HBV infection and its modes of transmission in the study areas. This agreed with the study of Butler et al. [29] who reported that in Australia there was poor knowledge of hepatitis risk factors among their study subjects. Regression analysis revealed that multiple sexual partners and duration of stay were significantly associated with HBV infection ($p<0.01$). Therefore, multiple sexual partners and duration of stay were the only significant risk factors in the study area.

There is need for Nigerian government to establish a policy that would make general screening of all the inmates in correctional facilities for HBV infection, and those prior to incarceration, and vaccinate them against the disease. Also proper treatment should be made to the affected ones.

Table 1. Gender distribution of HBV infection among study populations (correctional facilities) in Sokoto state (using fisher's exact test)

Gender	No. tested	No. positive (%)	No. negative (%)
Male	169	72(42.6)	97(57.4)
Female	11	4(36.4)	7(63.6)
Total	180	76(42.2)	104(57.8)

P-value =0.7622 p> 0.01

Table 2. Age Distribution of HBV infection among study population (correctional facilities) in Sokoto state (using chi square test)

Age (Years)	No. tested	No. positive (%)	No. negative (%)
15-20	40	16(40.0)	24(60)
21-25	31	19(61.3)	12(38.7)
26-30	29	9(31.0)	20(69.9)
31-35	23	8(34.8)	15(65.2)
36-40	21	11(52.4)	10(47.6)
41-45	19	9(47.4)	10(52.6)
> 46	17	4(23.5)	13(76.5)
Total	180	76(42.2)	104(57.8)

P-value = 0.003 $\chi 2$= 19.75 p<0.01

Table 3. Distribution of HBV infection among study population (correctional facilities) in Sokoto state based on Ethnicity of the subjects (using chi square test)

Ethnicity	No. tested	No. positive (%)	No. Negative (%)
Hausa	102	49(48.0)	53(52.0)
Fulani	38	18(47.4)	20(52.6)
Igbo	4	0(0)	4(100)
Yoruba	4	2(50)	2(50)
Others	32	7(21.87)	25(78.9)
Total	180	76(42.2)	104(57.8)

***Dakarawa, Gobirawa, And Zabarmawa*
P-value = 0.0346 p>0.01

Our findings in this study stress the need for further research on molecular detection and molecular characterization to help understand the specific pattern of distribution of the virus in the society.

Other HBV infection serological markers such as anti-HBs,anti-HBc, HBeAg, and anti-HBe should be detected in future studies, this will help in knowing people that are susceptible, immune due to natural infection, immune due to vaccination, acutely infected and chronically infected.

Table 4. Distribution of HBV infection among study population (correctional facilities) in Sokoto state based on Educational background of subjects (using chi square test)

Level	No. tested	No. positive (%)	No. negative (%)
Primary	30	22(73.3)	8(26.7)
Secondary	74	22(29.7)	52(70.3)
Tertiary	26	8(30.8)	18(69.2)
Others*	50	24(48)	26(52)
Total	180	76(42.2)	104(57.8)

P-value =0.0001 $\chi 2$=25.07 p< 0.01
**non formal education*

Table 5. Distribution of HBV infection among study population (correctional facilities) in Sokoto state based on Marital status of subjects (using fisher's exact test)

Status	No. tested	No. positive (%)	No. negative (%)
Single	114	37(32.5)	77(67.5)
Married	31	22(70.9)	9(29.0)
Separated	12	6(50)	6(50.0)
Widow/Widower	6	0(0)	6(100)
Divorcee	17	11(64.7)	6(35.3)
Total	180	76(42.2)	104(57.8)

P-value =0.0001 p< 0.01

Table 6. Distribution of prevalence of HBV infection among prison inmates based on their Duration of stay (using chi square test)

Duration (Years)	No. tested	No. positive (%)	No. negative (%)
0-1	82	27(32.9)	55(67.1)
2-3	64	28(43.8)	36(56.4)
>3	14	6(42.9)	8(57.1)
Total	160	61(38.1)	99(61.9)

P<0.01 p-value=0.0031 X^2 =1.545

Table 7. Distribution of HBV infection among study population (correctional facilities) in Sokoto state based on Occupation of subjects (using chi square test)

Occupation	No. tested	No. positive (%)	No. negative (%)
Civil service	30	9(30)	21(70)
Public service	5	3(60)	2(40)
Farming	10	6(60)	4(40)
Business	55	28(50)	27(49)
Others	80	30(37.5)	50(62.5)
Total	180	76(42.2)	104(57.8)

P-value =0.1769; p> 0.01

Table 8. Distribution of HBV infection among study population by locations of correctional facilities in Sokoto state (using chi square test)

Location	No. tested	No. positive (%)	No. negative (%)
Central prison	160	61(38.1)	99(61.9)
Remand home	20	15(75)	5(25)
Total	180	76 (42.2)	104(57.8)

P-value =0.00036 $\chi 2=8.46$ P< 0.01

Table 9. Prevalence of HBV infection among prison inmates in Sokoto state in relation to some associated risk factors

Risk factor	No of samples tested	No. of positive samples	%positive	X^2	P-value
knowledge on HBV					
Yes	12	6	50	0.07	0.7932
No	168	70	41.7		
Knowledge on mode of transmission					
Yes	22	10	45.5	0.01	0.9225
No	158	66	41.8		
Sharing of sharp object					
Yes	163	65	39.8	2.94	0.0865
No	17	11	64.7		
Blood transfusion					
Yes	4	2	50		0.1344
No	176	74	42.0		
Intravenous drug use					
Yes	35	20	57.1	2.74	0.0981
No	145	56	38.2		
Homosexuality					
Yes	10	5	50	0.03	0.8548
No	170	71	41.8		
Multiple sexual partners					
Yes	85	17	20	30.9	0.0001
No	95	59	62.1		

Table 10. Regression analysis of HBV infection with some associated risk factors

Risk factor	Standardized regression coefficients	Sig
Knowledge on HBV	-0.01	0.992
Knowledge on mode of transmission	-0.133	0.069
Sharp objects	-0.15	0.836
Blood transfusion	-0.144	0.040
IDU	-0.125	0.074
Homosexuality	-0.035	0.623
Multiple sexual partners	0.260	0.000
Duration of stay	-0.267	0.000

4. CONCLUSION

Prevalence of 42.2% of HBV infection was obtained and shown that HBV infection is prevalent with males and age group of (21-25 years). Duration of stay and multiple sexual partners were the main risk factors in the transmission of HBV infection among prison inmates in Sokoto metropolis, Sokoto state Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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