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## Seroprevalence, Risk Factors and Immunological Response against Hepatitis B Virus Among Students of Usmanu Danfodiyo University Sokoto, Nigeria

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### Abstract

*Hepatitis B Virus is of major public health interest as it accounts for significant mortality globally. The study aimed to determine the seroprevalence of HBV infection, the possible potential risk factors, and the immunological response among students of Usmanu Danfodiyo University, Sokoto, from December 2022 to April 2023. Structured questionnaires were used to collect sociodemographic information as well as possible risk factors related to the infection. Out of 139 study participants (10 females and 129 males screened randomly for HBsAg using a rapid HBsAg test kit, 17 (12.2%) were seropositive, and 122 (87.8%) were seronegative. The sera of the positive participants were further screened for HBeAg, anti-(HBs, HBe, and HBc) using one-step cassette diagnostic kits. Of the 17 positive samples screened, none were reactive for anti-HBs antibodies and HBeAg.; while 15 were positive anti-HBc and anti-HBe, indicating a response to natural infection. Sexual activity, unprotected sexual practices, use of already used sharp objects, and awareness of HBV infection were significantly associated ( $P < 0.05$ ) with the viral infection. The study indicates high endemicity in the study area. It is recommended that Nigerian Universities mandate the Hepatitis B virus vaccine for newly intake students. More awareness should be created about the dangers of unprotected sexual practice use or sharing of used sharp objects, among other possible factors.*

**Keywords:** HBsAg, Hepatitis B Virus, Risk Factors, Seroprevalence, Usmanu Danfodiyo University Sokoto.

### INTRODUCTION

The Hepatitis B virus belongs to the Hepadnaviridae Family. It is a partly double-stranded circular DNA virus that causes hepatitis B virus infection. It is composed of an envelope-bearing surface antigen (HBsAg) and a core capsid containing viral DNA (Isa *et al.*, 2015). The morphology of the HBsAg particles varies widely. The virions are found in high numbers during the early acute phase of the infection and are released continuously during the chronic phase of the disease (Campos-Valdez *et al.*, 2021). As one of the biggest global risks to public health, hepatitis B virus infection has killed countless people in sub-Saharan Africa (Dagnew *et al.*, 2020). According to the Nigerian HIV-AIDS indicator and impact survey, 2018, Nigeria has a high endemicity with HBsAg prevalence of 8.1% among adults aged 15-64

years (WHO, 2023). Because of the high burden of morbidity and mortality caused by HBV infection, it has received the greatest concern from the World Health Organisation (WHO) with the ultimate aim of its eradication (Ansumana *et al.*, 2018). Undetected Hepatitis B virus infection can progress to liver cirrhosis and or hepatocellular carcinoma.

Moreover, HBV is considered the most common cause of hepatocellular carcinoma (Rabaan *et al.*, 2023). More than one-fifth of HBsAg carriers tend to develop liver cirrhosis, and 5% to 6% progress to hepatocellular carcinoma after many years. Despite the disease burden and vaccine availability, hepatitis B virus vaccination has not been integrated or mandated among students in most Nigerian Universities (Adenlewo *et al.*, 2017). This research aims to establish the

seroprevalence of possible potential risk factors and to determine the immunological response to HBV infection among students of Usmanu Danfodiyo University, Sokoto.

## MATERIALS AND METHOD

### Ethical Approval

Ethical approval was obtained from the State Ministry of Health, Sokoto State (Reference Number: SMH/1580/V.IV)

### Study Population

The study population is students of Usmanu Danfodiyo University, Sokoto (UDUS). Usmanu Danfodiyo University is one of the second-generation universities established in 1975 in the city of Sokoto, Nigeria. Sokoto State is located on Longitude 11'30" to 13'50" East and Latitude 40' to 60". Inclusion criteria are that all undergraduate students of Usmanu Danfodiyo University, Sokoto. While excluding criteria were all non consenting undergraduate students and postgraduate.

### Sample Size Determination

Sample size was obtained using Cochran's formula as follows;

$$n = \frac{pq}{d^2}$$

Where;

n= minimum sample desired

z= standard normal deviate 95%; confidence interval=1.96

p= hepatitis detection=9.2% (0.09) (Isa et al., 2015)

q=complementary probability of p=1-p= 1-0.09= 0.91

d=tolerable alpha error or level of precision=5%=0.05

Therefore,  $n = (1.96)^2 \times 0.09 \times 0.91 / (0.05)^2$

N=125.

An attrition rate of 10% was added, which makes the final sample size as;

$$n = 125 + 125 = 139$$

Therefore, one hundred and thirty-nine students were enrolled in the study.

### Informed consent

The objectives of the study were made clear to the participants and consent was obtained verbally. All the information provided was kept confidential and anonymised.

### Questionnaire administration

The questionnaire was administered to the study participants to assess relevant potential risk factors and their demographic data following their verbal consent.

### Hepatitis B Sero Markers Detection using RDT kit

Three millilitres (3ml) of venous blood samples were collected randomly from the study participants into a clean, dry tube. The test was conducted using the lateral flow chromatographic immunoassay-based Labtrus rapid HBsAg diagnostic test kit. The test strip comprises a nitrocellulose membrane with a test band and a control band, as well as a colored conjugate pad containing mouse anti-HBsAg antibody conjugated with colloid gold (HBsAg conjugate). The test band is precoated with non-conjugated anti-HBsAg antibody, whereas the control band contains goat anti-mouse IgG antibody. When an adequate volume of test specimen is dispensed into the sample pad of the strip, the specimen migrates by capillary action across the strip. HBsAg, if present in the specimen, will bind to the HBsAg conjugate. The immunocomplex is captured on the membrane by already coated anti-HBsAg antibody, forming a burgundy-colored band (Bottéro et al., 2013). The appearance of two red lines on the control line and on the determinant(test) line of the test strip indicates a positive result, while the appearance of a single red line on the control line indicates a negative result.

### Detection of immunological response to HBV infection

The sera of the Positive samples were further screened using HBV Combo test cassette for HBeAb, HBeAg, HBcAb, and HBsAb.

### Data Analysis

Statistical Package for Social Sciences (SPSS) Version 25.0 was used to enter the gathered

data. Univariate analysis was carried out, which entails descriptive statistics like frequencies and an examination of the distribution of all relevant variables. The result was presented using tables, percentage and the degree of confidence was set at 95% (*p*-value of 0.05). The association between the variables was tested using chi-square test, i.e., association between HBsAg and possible risk factors, including multiple sexual partners, sexual activity history of blood transfusion, Protected sexual practice, and use of already used sharp object. A *p*-value of 0.05 or below was used as the threshold for statistical significance.

## RESULT

**Table 1** shows the Seroprevalence of Hepatitis B virus infection using the HBsAg marker among 139 students of Usmanu Danfodiyo University Sokoto. It shows that 17 students are positive, which presents the prevalence of HBsAg as 12.2%.

**Table 2** shows the sociodemographic characteristics of the study population By age group, students 15-20 age have no positive sample, students 20-25 age have 11(64.7%) positive samples, students 25-30 age have 6(35.3%) positive samples while students 30-35

age have no positive sample. The infection is more common among males, with 112 (91.8%) Positive Samples, while females have only had 10 (7.2%) Positive Samples. It also shows that infection is more common among single with 116(95.1%) Positive samples, than married students that have 6(4.9%) Positive samples.

**Table 3** presents the Association of risk factors and HBV infections among students of UDUS. There is a statistically significant association with Sexual activity 63.6% (*P*=0.01), Hepatitis B virus vaccine 2.9% (*P*=0.01), Multiple sexual partners 75% (*P*=0.01), awareness of the risk factors with only 2.3% (*P*=0.01) and also use of sharp object 53.1% (*P*=0.05). There is no statistically significant difference between the association of HBV infection and History of blood transfusion 17.6% (*P*=0.345).

**Table 4** Shows the Association of HBV infection and its immune markers among the students of Usmanu Danfodiyo University Sokoto. There is no statistically significant difference between HBV infection (HBsAg) and HBsAb because All the HBsAg-positive subjects were negative for HbsAb and HBeAg. There is statistically significant Association between the HBsAg and HBeAb (88.2%) and HBcAb.(88.2%). *P*=0.01.

**Table 1. Sero Prevalence of HBsAg Among students of Usmanu Danfodiyo University Sokoto**

Variable	Frequency (%)
Seropositive	17(12.2)
Seronegative	122(87.8)
Total	139(100)

**Table 2. Sociodemographic characteristics and prevalence of Hepatitis B virus infection**

Demographic Factors	No of samples examined	No of positive HBsAg(%)	No of Negative HBsAg(%)
<b>Age</b>			
18-20 years	10	0(0.0)	10(8.2)
20-25 years	87	11(64.7)	76(62.3)
25-30 years	38	6(35.3)	32(26.2)
30-35 years	4	0 (0.0)	4(3.3)
<b>Gender</b>			
Male	129	17(100)	112(91.8)
Female	10	0	10(7.2)
<b>Marital Status</b>			
Married	132(95%)	1(5.7)	6(4.9)
Single	7(5%)	16(94.3)	116(95.1)

**Table 3** The prevalence of HBsAg infection among students in connection to lifestyle choices and risk factors that may be linked to the Hepatitis B virus

Possible risk factors	No of sample examined	No of positive HBsAg (%)	No of negative HBsAg (%)	X2	P-value
<b>Hepatitis vaccine</b>					
Yes	35	1(5.9)	34(15.4)	3.829	0.39
No	104	16(94.1)	88(84.6)		
<b>Sexually active</b>					
Yes	118	14(82.4)	8(6.6)	64.343	0.01
No	11	3(17.6)	114(93.4)		
<b>Unprotected sexual practice</b>					
Yes	96	16(94.1)	80(68.3)	48.613	0.01
No	38	1(5.9)	37(31.7)		
<b>Use of already sharp objects</b>					
Yes	32	15(11.8)	17(13.9)	46.481	0.01
No	107	2(88.2)	105(86.1)		
<b>Awareness of risk factors</b>					
Yes	86	2(11.8)	84(68.9)	20.613	0.01
No	53	15(88.2)	38(31.1)		
<b>Multiple sexual partners</b>					
Yes	43	12(70.6)	31(36.0)	52.495	0.01
No	60	5(29.4)	55(64.0)		
<b>History of blood Transfusion</b>					
Yes	40	9(52.9)	31(26.3)	0.529	0.345
No	95	8(47.1)	87(73.7)		

**Table 4.** The Association of HBV infection and its immune markers among HBsAg Positives students of Usmanu Danfodiyo University Sokoto.

Serologic marker	No. of positive (%)	No. of negative (%)
HBsAb	0	17(100)
HBeAg	0	17(100)
HBeAb	15(88.2)	2(11.8)
HBcAb	15(88.2)	2(11.8)

**KEY:**

HBsAb: Hepatitis B surface antibodies  
HBeAg: Hepatitis B envelope Antigen  
HBeAb: Hepatitis B envelope Antibodies  
HBcAb: Hepatitis B core Antibodies

**DISCUSSION.**

This study reported 12.2% prevalence of HBV infection among students Of Usmanu Danfodiyo University Sokoto. This prevalence is considered endemic. According to the World Health Organization, prevalence of <2% is considered low, prevalence of 2-8% is considered moderate, while Prevalence>8% is considered High (WHO, 2016).

The seroprevalence in this study is higher than 6.5%, as reported by (Lado *et al.*, 2018) at Nile University. It is also slightly higher but in line with prevalence among medical students in

Ethiopia (11.5%) (Tesfa *et al.*, 2021b) and (12.5%) (Aminu *et al.*, 2013) in Ahmadu Bello University Zaria. It is also lower compared to a study report from a study by (Tula *et al.*, 2015) among North-eastern Nigeria (31.5%). This variation may be attributed to the differences in the awareness of the Infection and the lack of proper practice of infection prevention packages(vaccines). The Infection is higher among students who have no vaccine although it is not statistically significant. The finding is the same as the research conducted by (Tesfa *et al.*, 2021b).in eastern Ethiopia and Cameroon (Ndongo *et al.*, 2018).

66.7% of students who practice unprotected sex are positive for HBsAg with statistically significant association. These findings are similar to (Isa *et al.*, 2017) and (Otori *et al.*, 2013) but with no significant association. This explains that during protected sex if the condom is not properly utilized, the risk of the infection increases. The use of condoms among couples in Nigeria may show a lack of trust (Isa *et al.*, 2017).

The Infection is higher among students who use already used sharp objects (53.1%) although there is no significant association. These findings are the same as those of (Isa *et al.*, 2017) at Ahmadu Bello University Zaria and (Ndako *et al.*, 2011), who reported a significant association. This adds affirmations to the fact that these factors don't determine the infection, but they expose one to body fluids and secretions, which might eventually lead to the acquisition of the infection. Awareness of risk factors.

This study also finds that most of the positive students are not aware of the risk factors associated with the infection, as also found by (Okeke *et al.*, 2008), a study conducted among Nigerian university students. However, studies conducted in Bhatia Medical and Dental College, Mirpur Khasas, show that despite the awareness of risk factors, the high occurrence is possibly due to the Low knowledge of the preventive measures during needle recapping, owing to a lack of clinical expertise and experience (Tesfa *et al.*, 2021b).

The prevalence is higher in students with multiple sexual partners. The result is similar to the report of (Otori *et al.*, 2014). HBsAg seropositivity was found to be higher in those practicing polygamy compared to those with monogamous relationships. The study contradicts (Isa *et al.*, 2017), who explained that this might be because the students have learned to use various protective sexual practices.

There is no significant association between the history of blood transfusion and Hbv infection, probably because of the strict screening of transfusion transmissible infections of blood donors In Udu. These findings are not the same with (Isa *et al.*, 2017) in Ahmadu Bello University Zaria, who explained that it may be attributed to the fact that most Nigerian hospitals have no adequate modalities to effectively screen blood.

None of the HBsAg reactive students had developed anti-HBs, based on the distribution of

HBV markers in this group of pupils. Anti-HBs are typically considered a sign of hepatitis B virus infection recovery and immunity. A person who has received a successful hepatitis B vaccination will also develop anti-HBs. Given that none of the study participants had produced anti-HBs, likely they are still recovering from a natural illness. Furthermore, none of the HBsAg reactive students had HBeAg. Its presence indicates that the virus is replicating, and the infected students had high levels of HBV. The prevalence was lower as compared to the study by (Otegbayo *et al.*, 2008), who reported 2.3% positive for HBeAg. This may be attributed to the fact that all the subjects may be in the early phase of the acute infection. In addition, there is a high risk of developing liver cirrhosis or hepatocellular carcinoma later in life as the virus keeps on replicating.

Further analysis indicated that 88.2% of the individuals had developed the envelope antibody (anti-HBe). When an acute HBV infection occurs, the immune system often produces this antibody momentarily, signifying a reduction in HBV levels or the end of the infection. The first antibody to appear is anti-HBc. (Park *et al.*, 2015), Demonstration of anti-HBc in serum indicates HBV infection, current or past. The IgM anti-HBc is present in high titre during acute infection and usually disappears within 6 months, although it can persist in some cases of chronic hepatitis. IgG anti-HBc generally remains detectable for a lifetime (Deng *et al.*, 2022). As a result, the exact period these students contracted the HBV could not be ascertained.

## CONCLUSION

This study found the seroprevalence of HBV infection among students in Usmanu Danfodiyo University Sokoto to be 12.2%. Based on WHO criteria, this suggests that the virus is endemic among the students. The study found that Unprotected Sexual Activity, Lack of awareness, and Multiple sexual partners use of already-used sharp objects were significantly associated with the HBV infection. All the HBsAg positive students harbour the virus, indicating that they were mostly still in the early stages of infection. As a result, these students may act as possible reservoirs of the virus and can transmit it.

## RECOMMENDATION

The university management should initiate strong campaigns that will create awareness of HBV infection. Furthermore, HBV vaccination



should be mandatory for all newly admitted students.

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