



## RESEARCH ARTICLE

### PREVALENCE OF HEPATITIS B AND C VIRUS ANTIBODIES AMONG STUDENTS INVOLVED IN RISKY SEXUAL BEHAVIOR IN A NIGERIAN TERTIARY INSTITUTION.

**Oluboyo Bernard Oluwapelumi, Sunday Samuel Iko-Ojo, Akinseye Janet Funmilayo, Akele Richard Yomi. Oluboyo Adeola Olanrewaju<sup>1</sup>, Adewumi Funmilayo.**

1. Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti.
2. Department of Microbiology and Parasitology, College of Medicine, Ekiti State University, Ado-Ekiti.

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#### Abstract

Sexual contact is one of the ways hepatitis B and C can be transmitted. Limited investigations have been recorded on the prevalence of HBV and HCV antibodies among students involved in risky sexual behavior in tertiary institutions in Nigeria. This study was conducted to determine the prevalence of Hepatitis B and C antibodies in such group of people. Structured questionnaires were administered to obtain data on sexual behavior of the participants. A total of 91 students consisting of 46 males and 45 females between ages 16 and 25 years were examined using enzyme linked immunosorbent assay (ELISA) technique. Liver enzymes of ELISA positive samples were assayed. Prevalence of HBV and HCV antibodies among students involved in risky sexual behavior were 23.1% and 0% respectively. There was no significant difference between the prevalence of HBV antibodies among gender. Although higher prevalence was recorded among students between ages 16-20, in relation to students in ages 21-25, the difference is not statistically significant ( $>0.05$ ). The prevalence of HBV antibodies among students involved in multiple sex partnership was significantly higher ( $p < 0.05$ ) than those involved in single sex partnership. The prevalence was significantly higher ( $p < 0.05$ ) in non-condom users than in condom users. Six (6.0%) of the students recorded raised liver enzymes. Students involved in risky sexual behavior are at high risk of HBV infection especially in cases of multiple sex partnership. Condom usage may not provide risk-free sex for users. Enlightenment campaign against risky sexual behaviors in higher institutions is advocated.

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#### Introduction:-

Hepatitis endangers people's life because of the risk of death from liver cirrhosis and liver cancer in chronic infection (WHO, 2017). Hepatitis B is the most frequent cause of chronic liver disease in Nigeria. In southern parts of the country, up to 58.1% of patients with chronic liver disease were reported positive for hepatitis B surface antigen (HBsAg) test (Lesi *et al.*, 2004).

**Corresponding Author:-Oluboyo Bernard Oluwapelumi.**

Address:-Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti.

Hepatitis B is frequently acquired when blood, semen, or other body fluids from an infected person gain entrance into the body of an uninfected person (WHO, 2017). Workers in healthcare centers, blood transfusions centers, dialysis rooms, people living with an infected person and travelers to countries where the infection rate is high are all classified as risk group to the viral infection (CDC, 2011). Hepatitis B virus is said to survive outside the body for at least 7 days and remains highly infective if it enters the body of a person who is not protected by the vaccine (WHO, 2017). Hepatitis B virus (HBV) and hepatitis C virus (HCV) are spread mainly through contaminated blood and blood products, sexual contact and contaminated needles (Rikabi *et al.*, 2009). The virus replicates profusely and produces high titre in the blood such that any parenteral or mucosal exposure to infected blood poses a high risk of infection (Pennap *et al.*, 2011). HBV and HCV are both associated with jaundice, liver cancer, and liver damage (Ayolabi *et al.*, 2006).

Although much is known about the epidemiology of HBV in Nigeria, limited investigations have been published on HBV among students in tertiary institutions in Nigeria involved in risky sexual activities. Studies carried out by various authors have shown that HBV infections are highly prevalent among Nigerians (Imarenezor *et al.*, 2016). Researchers focused on infections in various groups such health care workers such as blood donors and medical waste handlers; there is little information on the prevalence of the virus among the youths at risk of infection because of their sexually active stage. This work is therefore aimed at determining the seroprevalence of Hepatitis B and C viral infection among students involved in risky sexual activities in a Nigerian tertiary institution of learning.

Sexual transmission of hepatitis C has been a subject of debate, however, some researchers opined that sexual practices that involve higher levels of trauma to the anogenital mucosa, such as anal penetrative sex, or that occur when there is a concurrent sexually transmitted infection, including HIV or genital ulceration, do present a risk (Tohme and Holmberg, 2010). There is scarce information on the epidemiology of HCV in Nigeria; however, it has long been suspected that it may be endemic. Some studies in Nigeria reported prevalence of 12.3% among adult blood donors (Ejiofor *et al.*, 2010), 11.0% among doctors and dentists (Olubuyide *et al.*, 1997).

Youth sexual behavior is highly significant to different public health problems. Unprotected vaginal sexual intercourse among youth has contributed to unwanted pregnancies, abortions, pregnancy related complications, and sexually transmitted infections (Tang *et al.*, 2011). Substantial increase in the proportion of undergraduate students who report sexual activity while at school in Nigeria has been observed. Studies have reported this among the adolescents (Owuamanam 1995; Ugoji 2008). Sexual behaviors particularly among undergraduate students has become alarming and worrisome (Ugoji 2014) in view of the associated short and long term effects. Risky sexual behaviors among undergraduates involve the practice of multiple sex partnership, group sex, sex without condom, anal and oral sex. These actions expose students to sexually transmitted infections.

The prevalence of chronic HBV ranges from less than 2 percent in low-prevalence areas (e.g. United States, Canada, Western Europe) to 2-7 percent in intermediate-prevalence areas (e.g. Mediterranean countries, Japan, Central Asia, Middle East, and parts of South America) to  $\geq 8$  percent in high-prevalence areas (e.g. Western Africa, South Sudan) (Ott *et al.*, 2012; Zhang *et al.*, 2016). Nigeria is classified among the high-prevalence areas.

In Nigeria, the risk of acquiring HBV infection is considerable; as many as 75% of the population may be exposed (Ola *et al.*, 2002). Some reports have shown varying risk group-specific estimates. A prevalence of 10-15% in the average risk Nigerian population has been reported (Emechebe *et al.*, 2009), researchers have reported high HBV prevalence among surgeons (25.7%), voluntary blood donors (23.4%), and infants (16.3%) (Musa *et al.*, 2015). Information on the prevalence of HBV and HCV among students in tertiary institution involved in risky sexual behavior will be an added contribution to reports on risk specific groups.

## Materials And Methods:-

### Study area

This study was carried out among students a tertiary institution in Ado-Ekiti, Nigeria. Ado-Ekiti is a city in southwest Nigeria that lies between latitude  $7^{\circ}35'$  and  $7^{\circ}38'$  North of the equator and Longitude  $5^{\circ}10'$  and  $5^{\circ}15'$  East of the Greenwich Meridian (Adebayo and Jegede, 2010),

### Eligibility criteria

Students between ages 15 and 35 years involved in sexual acts who consented to the study were eligible for the study. Students who have been transfused with blood, intravenous drug users and those with tattoo marks on their body were excluded. Students without sexual experience were also excluded from the study.

#### **Ethical consideration**

Ethical clearance was obtained from the research and ethics committee of the institution under study. The study participants were informed about the purpose of the study and consented to the study.

#### **Sample size**

The sample size was determined using the following formula (Smith, 2013) using an estimated prevalence of HBV of 6.0% in Nigeria tertiary institution (Imarenezor *et al.*, 2016) at a confidence level of 95 and desired precision of 0.05. Ninety one (91) students were enrolled for the study. These comprised of 45 females and 46 males.

#### **Sample collection**

A semi structured questionnaire was used to obtain information on student's sexual behaviors. Blood was collected aseptically from consenting individuals by venipuncture, allowed to clot and the serum was separated by centrifugation at room temperature for 3000 revolution per minute (rpm) for 10 minutes into screw-capped plain bottles, stored at -20°C, until ready for analysis (Tammen *et al.*, 2005).

#### **Sample analysis**

The serum samples were analyzed using ELISA technique for identification of antibodies to Hepatitis B and C.

#### **Hepatitis B surface antibody (HBsAb) ELISA test**

AccuDiag™ HBsAb ELISA (DIAGNOSTIC AUTOMATION INC., Woodland Hills, CA) is a solid phase enzyme linked immunosorbent assay based on the principle of double antigen sandwich technique for the detection of antibodies to HBsAg in human serum or plasma (Hoofnagle, and Di-Bisceglie, 1991).

During the assay, the test specimen and horseradish peroxidase-ABsAg (HRP-HBsAg) conjugates are incubated simultaneously with the coated microwells. HBsAb, if present in the specimen, reacts to the HBsAg coated on the microwell surface as well as the HRP-HBsAg conjugate, forming sandwich complex conjugates. Unbound conjugates are then removed by washing. The presence of the complexed conjugates is shown by a blue color upon additional incubation with 3, 3', 5', 5'-tetramethylebenzidine (TMB). The manufacturer's procedure was strictly followed. The optical density was measured at 450nm wavelength using a plate reader (MARVOTECH PLATE READER, China).

#### **Hepatitis C virus (HCV) antibody ELISA test**

The test principle is based on indirect enzyme immunoassay. Micro titer wells are coated with certain amount of HCV recombinant antigens. Then serum samples are allowed to react with solid phase antigens. If HCV-specific antibodies (IgG and IgM) are presented in the serum they will bind to HCV antigens through their individual Fab section. After incubation, the wells are washed to remove unbound antibodies and anti-human antibodies (IgG/IgM) conjugated with HRP is added into the wells following another incubation and wash step. A solution of chromogen 3,3',5',5'-tetramethylebenzidine (TMB) is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm in a MARVOTECH PLATE READER (China).

#### **Alanine aminotransferase (ALT) estimation**

Alanine aminotransferase (ALT) is incubated at 37 °C for exactly 30 minutes in a pH 7.4 buffered substrate containing alanine and alpha-ketoglutarate. ALT catalyzes the transfer of the amino group from alanine to ketoglutarate, forming pyruvate and glutamate. The pyruvate reacts with 2, 4-dinitrophenylhydrazine (DNPH) to form 2, 4-dinitrophenylhydrazone which in an alkaline medium gives a red-brown colour. The absorbance of the colour produced is measured in a spectrophotometer (PIOWAY, China) at 546 nm wavelength (Young, 1997). The corresponding concentration of the optical density was recorded from the plotted graph of ALT activity chart. Reference range of ALT is 7-56 international units/Litre (IU/L) (Shivraj *et al.*, 2009).

#### **Aspartate aminotransferase (AST) estimation**

The enzyme aspartate aminotransferase (AST/GOT) catalyzes the transfer of an amino group from L-aspartate to 2-oxoglutarate to give L-glutamate and oxalacetate. The oxalacetate reacts with reduced Nicotinamide adenine dinucleotide (NADH) in the presence of Malate dehydrogenase (MDH) to produce Malate and NAD. The decrease

in NADH concentration, measured at 340 nm, is proportional to the AST/GOT activity. Lactate dehydrogenase (LDH) is present in the substrate to convert endogenous pyruvate to lactate prior to measuring the AST/GOT level (Young, 1997). The concentration of AST in the serum was obtained from the plotted graph of AST activity chart. Serum AST reference range is 0-35 IU/L (Shivaraj *et al.*, 2009).

### Result:-

Students having multiple sex partners numbered 30 (33%) as against 61(67%) recorded by students having a single sex partner. Condom users were 59 (64.8%) while none condom users were 32 (35.2%). Of the 91 participants, 21 (23.1%) were positive and 70 (76.9%) were negative for Hepatitis B virus (HBV) antibody. None (0.0%) of the participants was positive for HCV antibody.

Prevalence of HBV antibody was significantly higher ( $p < 0.05$ ) among multiple sex partners in relation to single sex partners (Table 1). Non-condom users recorded a significantly ( $p < 0.05$ ) higher rate of infection than condom users (Table 2). There was no significant difference ( $p > 0.05$ ) between HBV infectivity among the gender (Table 3). There was also no significant difference ( $p > 0.05$ ) between the age ranges of the student in relation to HBV infectivity (Table 4). Six of the ELISA positive samples recorded ALT and AST above the upper limits of the reference ranges giving a total prevalence of suspected active infection as 6.0%.

**Table 1:-HBV antibody against number of sex partners.**

No. of sex partnership	HBV antibody			$\chi^2$	P-value
	No. Positive (%)	No. Negative (%)	Total (%)		
Multiple sex partners	21(70)	9 (30)	30 (100)	55.510	0.000**
Single sex partners	0 (0.0)	61 (100)	61(100)		
Total	21 (23.1)	70 (76.9)	91 (100)		

\*\*P-value < 0.05 (Significant)

**Table 2:-HBV antibody against condom usage among the students.**

Condom usage	HBV antibody			$\chi^2$	P-value
	No. positive (%)	No. negative (%)	Total (%)		
Condom users	1 (1.7)	58 (98.3)	59 (100)	43.212	0.000**
Non-condom users	20 (62.5)	12 (37.5)	32 (100)		
Total	21 (23.1)	70 (76.9)	91 (100)		

\*\*P-value < 0.05 (Significant)

**Table 3:-HBV antibody against gender distribution of the students.**

Gender	HBV antibody			$\chi^2$	P-value
	No. Positive (%)	No. Negative (%)	Total (%)		
Female	13 (28.9)	32 (71.1)	45 (100)	1.694	0.193
Male	8 (17.4)	38 (82.6)	46 (100)		
Total	21 (23.1)	70 (76.9)	91 (100)		

P-value > 0.05 (Not significant)

**Table 4:-HBV antibody against age range of the students.**

Age range	HBV antibody			$\chi^2$	P-value
	No. Positive (%)	No. Negative (%)	Total (%)		
16-20	13 (27.1)	35 (72.9)	48 (100)	0.919	0.338
21-25	8 (18.6)	35 (81.4)	43 (100)		
Total	21 (23.1)	70 (76.9)	91 (100)		

P-value > 0.05 (Not significant)

### Discussion:-

An overall prevalence rate of 23.1% was recorded for HBV antibody and 0% was recorded against HCV antibody in this research. This result for HBV may be regarded as high according to WHO classification for assessing severity of HBV infections in HBV endemic countries. WHO defines low prevalence to be < 2%, moderate to be 2-8% and high prevalence to be > 8% HBV positivity (WHO, 2010). Records are scanty on the prevalence of HBV antibody among tertiary students involved in sexual activities. However, seroprevalence rate for HBV antibodies recorded in random sampling of students irrespective of their sexual status are more available. Such records include prevalence of 6.0% recorded among students of the Federal university of wukari, Taraba state, Nigeria (Imarenezor *et al.*, 2016), 4.5% recorded among students of university of Maiduguri in Nigeria (Dawurang *et al.*, 2012) and 12.5% reported among asymptomatic students in Ahmadu Bello University, Zaria (Aminu *et al.*, 2013). Also, the prevalence rate of 9.5% reported in a population of students at Ladoke Akintola University of Technology in south west Nigeria (Mabayoje *et al.*, 2010), 9.2% recorded in Ahmadu Bello university, Zaria, Kaduna State, Nigeria (Isa *et al.*, 2015); and 13.2% recorded in Nassarawa State University in Keffi, Nigeria (Pennap *et al.*, 2010) for HBsAg are all lower than the prevalence rate recorded in this study for HBV antibodies. The reason is that while these researchers investigated randomly selected subjects irrespective of risk exposure, this study focused only on a group of subjects with sexual risk behavior only. Thus, risky sexual behavior is an important risk factor in the transmission of HBV virus in tertiary institution of learning in Nigeria.

No case of anti-HCV antibody was recorded in this study. This is consistent with WHO report that transmission of HCV through sex is uncommon (WHO, 2017). As this study excluded other risk factors for HCV infection such as intravenous drug use, blood transfusion, sharing of blades and contaminated sharp objects, reuse of contaminated needles were excluded from the study. These factors may be responsible for the prevalence of HCV recorded previously by other researchers.

Risky sexual behavior among the students (and perhaps, youth generally) is an important factor in the transmission of HBV and other sexually transmitted diseases. A study found out that 80% of the males and 73% of the females had experienced heterosexual intercourse in United States (Reinisch *et al.*, 1995). Another study showed that 74% of university students reported ever having had sexual intercourse in Turkey (Gokengin *et al.*, 2003). The situation is the same among youth irrespective of their race. Young people are at the age of greatest sexual activities thus enhancing sexual transmission of microorganisms (Dawaki and Kawo 2006). The focus on sexual transmission as the sole risk factor in the prevalence of HBV and HCV among students revealed risky sexual behavior among students as a major risk factor in the transmission of HBV.

Of the 30 students who admitted having multiple sex partners, 21 (70 %) tested positive to HBV antibodies. This supports the reports published in other literature that involvement in multiple sex partnership increases the risk of HBV infection (Adekanle *et al.*, 2010). Having unprotected vagina sex may also be a reason for the relatively higher prevalence recorded in this study. Condom usage was the preferred means of prevention of pregnancy and infection by some students investigated in this. Kabir *et al* (2004) reported in a study on sexual behavior among students in tertiary institutions in Kano, northern Nigeria that 71.7% of students preferred the use of condoms as a means of conferring protection against unwanted pregnancy and the risk of sexually transmitted infections. However, 32 of the investigated participants in this study were non-condom users and of these, 30 (94%) tested positive to HBV

antibodies. Among the condom users only 4.8% tested positive to HBV antibodies. The prevalence of HBV infection among non-condom users is significantly higher ( $p < 0.05$ ) than condom users. This means that the risk of acquiring HBV infection is higher among non-condom users as compared to condom users. This is consistent with the findings from other studies that unprotected sexual intercourse is a major route of transmitting the infection (Okonkwo *et al.*, 2010; Dawaki and Kawo, 2006). In an attempt to determine the level of protection conferred on an individual against HBV through the use of condoms in this study, the risk percentage was calculated among condom users (1 in 59). The risk percentage was 1.7%. This means that out of every 100 students that make use of condoms in an attempt to protect against acquiring HBV infection, about 2 of them will contract the infection. Though significant numbers of students were protected from HBV through condom usage, it does not confer absolute protection against HBV infection.

Age or gender has no direct relationship with HBV infectivity. Both sexes are infected at the same rate. No particular age appeared to be more susceptible to HBV infection than the other.

A raised liver enzyme (ALT and AST) is suggestive of active infection in some of the students. The percentage of students having raised liver enzymes (6.0%) is of serious concern as they may develop into liver diseases. More studies focusing on risk group of students such as those involved in risky sexual behavior in many more tertiary institutions of learning will further expose the danger confronting this group of students and help in campus campaign against such behavior.

### Conclusion:-

This study found the prevalence of HBV and HCV antibodies among students involved in sexual risk behavior to be 23.1% and 0% respectively. Seventy percent (70%, 21 out of 30) of students involved in multiple sex partnership recorded positive result to HBV antibodies while none of the students involved in single sex partnership tested positive to HBV antibodies. Only 1.7% of condom users tested positive to HBV antibodies as against 62.5% of non-condom users that recorded positive result to HBV antibodies. 28.9% of females tested positive to HBV antibodies while 17.4% of the males tested positive to the virus. This difference is however not statistically significant. 27.1% of students in ages between 16 and 20 tested positive to HBV infection as against 18.6% in ages between 21 and 25. This difference is equally not statistically significant. 6.6 % of the students recorded increased liver enzymes suggestive of active infection.

This study has provided information on the burden of HBV among students involved in risky sexual behavior in a Nigerian tertiary institution of learning. Campaign against risky sexual behavior in Nigerian tertiary institutions of learning will help in reducing the rate of spread of HBV.

### Conflict of interest

The authors declare no conflict of interest in this work.

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