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Prevalence of Hepatitis B Surface Antigen among Healthy Assymptomatic Students of a Tertiary Institution in South Eastern, Nigeria

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Abstract

Hepatitis B (HBV) affects both women and men irrespective of age and race. Despite the existence of a safe and effective vaccine, Nigeria has remained a hyper-endemic area for hepatitis B virus infection, with an estimated 12% of the total population being chronic carriers. This study determined the prevalence of hepatitis B surface antigen (HBsAg) amongst healthy, asymptomatic students of Federal University of Technology, Owerri (FUTO), and assessed the possible associated risk factors. Structured questionnaires were administered to obtain information on socio-demographic, clinical data, and potential risk factors that might be associated with HBV. Blood samples were collected within the university from 100 consenting students aged between 15 and 32. Sera were screened for HBsAg using ELISA kit (Monalisa HBs Ag ULTRA kit, BIO-RAD, France). Among the 100 students tested for HBsAg, 10.0% (10/100) tested positive for HBV infection. This seroprevalence indicates high endemicity according to WHO classification. The study responses indicated that multiple sexual relationships, pedicure, manicure, barbers' clipper, sharing of unsterilized sharp instruments, and blood transfusion, might be the risk factors that predisposed participants to the infection. About 9.1% of the students claimed ignorant of HBV infection. There is need to create awareness on risk factors of HBV infection among the student population, to enlighten them on the complications associated with this viral infection and the preventive strategies for its control.

Keyword: Multiple sex partners**,** blood transfusion, ELISA kit, seroprevalence, HBV infection, students, hyper-endemic

1. Introduction

Hepatitis B virus (HBV) infection, one of the leading killers among infectious diseases across the globe, is a world-wide public health problem, accounting for about 80% of cancer-related deaths, annually. At the early stages, the disease is asymptomatic, and, as it progresses, chronic stage, liver cirrhosis and hepatocellular carcinoma (liver cancer) may result at the later stages if left untreated (Kowdley, Wang & Welch, 2012). Data from the World Health Organization estimates that worldwide, 325 million people are living with chronic HBV infection, of which roughly one million die annually from HBV-related liver diseases. Globally,

it is estimated that HBV infections and their complications led to about 1.34 million deaths in 2015. Only about 9% of the global population are aware of their HBV status, while the death tolls from this silent killer have continued to rise unabatedly (WHO, 2017).

HBV causes both acute and chronic infections in humans, with varying clinical manifestations (Kao, 2008). During the acute phase, manifestations range from subclinical or anicteric hepatitis to icteric hepatitis and, in some cases, fulminant hepatitis, while in the chronic phase, manifestations range from an asymptomatic carrier state to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Eng-Kiong & Lok, 2013). Most HBV deaths recorded in 2015 were due to chronic liver disease (720 000 deaths due to cirrhosis) and primary liver cancer (470 000 deaths due to hepatocellular carcinoma) (WHO, 2017).

HBV can be transmitted through percutaneous or mucosal exposure to infected blood or other body fluids. The transmission has been observed with numerous forms of human contact including perinatal / mother-to-child; household (nonsexual); sexual; needle-sharing; and occupational/health-care-related. The highest concentrations of infectious HBV are found in blood. However, other body fluids, such as semen and saliva could also be infectious. Persons with chronic HBV infection are the major reservoir for transmission, although any person testing positive for hepatitis B surface antigen (HBsAg) is potentially infectious to both household and sexual contacts (Shepard, Simard, Finelli, Fiore & Bell, 2006).

HBV is a partially double stranded DNA virus belonging to the *Hepadnaviridae* family. The viral genome is a circular DNA molecule of 3.2 kb organized in a compact manner with four partially-overlapping open reading frames (ORF) including surface (S), core (C), polymerase (P) and X genes. These viral genes can be translated to different viral proteins from four major transcripts, such as surface antigen (HBsAg), envelop antigen (HBeAg) and core antigen (HBcAg).The host immune system produces corresponding antibodies against these viral proteins, including anti-HBs, anti-HBe and anti-HBc respectively. These antigens and antibody biomarkers lay the basis for serological diagnosis of HBV infection (Kao, 2008). HBV shows a high degree of species specificity and infects only humans and higher primates (Li, Miao, Qi, ZengLiang, & Liang, 2010).

HBV produces several antigens that can be detected in the blood and disappear as the body produce antibodies against them, which normally will bring about antigen-antibody reaction resulting to the elimination of the antigens from the body. The patterns of these and other markers provide clues to the phase of infection. HBV surface antigen and HBV DNA are often the first detectable markers of acute infection, appearing before the onset of symptoms or before elevation of alanine amino transferase (ALT) occurs. By definition, an HBV infection is chronic if surface antigen persists longer than 6 months. HBV e antigen**,** derived from pre-core protein, is considered a marker of HBV replication and infectivity. In chronic infection, e antigen can persist for years or decades. HBV core antigen cannot be detected in the serum, but antibodies against it (anti-HBc) can, first, immunoglobulin M (IgM) and later, immunoglobulin G (IgG) (Chen, 2009).

Between 18 million and 22 million Nigerians are currently infected and approximately 5 will die of causes related to HBV infection. HBV infection is indeed hyper endemic in Nigeria and may be the highest in Sub-Sahara Africa (Musa, Bussell, Borodo, Samaila & Femi, 2015). Over 70% of the population shows evidence of past infection with the virus and 7.3–24% has serological evidence of current infection (average 13.7%) (Nwokediuko, 2011).

The risk of contracting HBV in Nigeria is substantial, and given that as many as 75% of the population (Ola & Odaibo, 2007) are unaware of their HBV status, it became absolutely necessary to conduct this study among, apparently healthy undergraduate students of Federal University of Technology, Owerri (FUTO), with a view to determining the level of HBV exposure among the students of this tertiary institution.

2. Materials and Methods

2.1. Study Design

This is a cross-sectional prospective study in which blood samples were collected from consenting male and female undergraduate students of Federal University of Technology, Owerri (FUTO).

2.2. Study Site

This study was carried out at the Federal University of Technology, Owerri, Imo State, South Eastern Nigeria.

2.3. Study Population

The study population included randomly selected 60 male and 40 female, students of FUTO, aged between 15 and 32 years. The purpose of the study was fully explained to them and their informed consent obtained prior to collection of samples. The specimen size was determined using the formula:

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

where n = sample size, z = standard deviation (1.96), p = prevalence, q = 1-p and d = degrees of freedom (0.05), based on the most recent anti hepatitis B virus antibodies prevalence done in Lagos (Adesina, Akinyemi, & Adewale, 2010). The least number of specimens to be collected for the study was calculated to be 89; hence 100 specimens were collected.

2.4. Ethical consideration

Ethical clearance was obtained from the Medical Ethical and Scientific Research Committee of the School of Biological Sciences, Federal University of Technology, Owerri, before the commencement of study. Also, a consent form was given to each student after explanation, to obtain permission.

2.5. Inclusion and exclusion criteria

Those excluded from the study included non-students and students who did not give consent to participate in the study. All consenting undergraduate students were enrolled for the study.

2.6. Administration of questionnaire

A structured questionnaire was administered to the consenting students in order to obtain information on their socio-demographic, clinical data, and potential risk factors that might be associated with HB.

2.7. Sample Collection

Using a sterile syringe, 5 ml of blood was collected via vein puncture and transferred into plain properly labelled vacutainer test tubes, and transported on ice packs in a cold box to the Laboratory of the Department of Microbiology, FUTO. The blood samples were allowed to stand for 60 minutes in an attempt to allow coagulation. The samples were then

centrifuged at 3000 rpm for 5 minutes. The supernatant (serum) was transferred safely into 2 ml cryovial and stored at -20°C until tested. Samples used for this were collected between October, and November, 2017.

2.8. Surface Antigen (HBsAg) Detection

The serum samples were screened for the presence of HBsAg using Monalisa HBs Ag ULTRA kit, obtained from BIO-RAD, France. The test was carried out based on the manufacturer's instructions. Check value of the control or patient sample **/** extinction value of calibrator 3 = Ratio. The results were interpreted as described by the manufacturers' kit insert.

Ratio <0.07: was considered negative
Ratio ≥0.07 to <0.09: was considered borderline
Ratio ≥1.1: was considered positive

2.9. Statistical Analysis

Paired T test was used to compare rate of HBV infection between male and female undergraduates of FUTO using Graph Pad Prism software version 5.01 linear regressions (Graph pad Software Inc., U.S.A): $P < 0.05$ at 95% confidence interval was considered significant.

3. Results and Discussion

Results showed that a total of 10 (10%) of the blood samples were positive for the HBs antigen (Table 1). The result also showed that the infection was more pronounced in the age group of 21-23 (21.4%). When the rate of infection in male and female students was compared, it was observed, to be 13.3% in males and 5.0% in females as shown in Figure 1. The potential risk factors, of which HBV was associated with, are represented in Table 2. The most prevalent risk factor associated with HBV infection in FUTO was multiple and indiscriminate sexual relationships (30.8%), while the least was from patronage of public barbers' shops (8.0%). The Table also showed that about 10.9% of the students have not heard of HBV infection before this study, while about 9.1% claimed ignorance of their HBV status.

Table 1. Prevalence of HBsAg among undergraduate students of FUTO based on age.

Age interval	Total screened	HBSAg –ve	HBSAg +ve	% +ve
15-17	9	9	0	0.0
18-20	29	27	2	6.9
21-23	33	27	6	18.2
24-26	17	15	2	11.8
27-29	8	8	0	0.0
30-32	4	4	0	0.0
Total	**100**	**90**	**10**	**36.9**

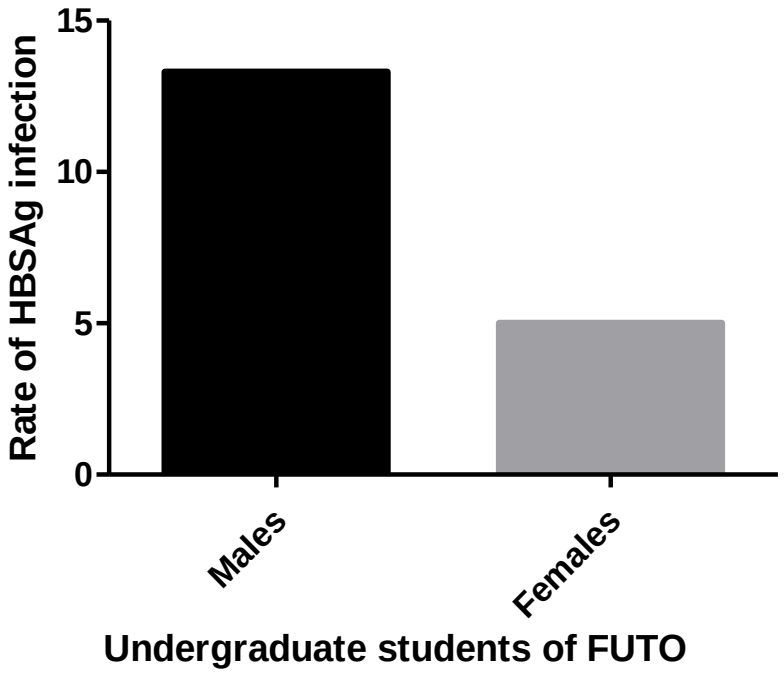

Fig. 1. Rate of HBsAg infection among male and female students

Table 2. Risk factors associated with HBV infection among study participants

Risk Factors		Total Screened	HBSAg –ve	HBSAg +ve	% +ve
Blood Transfusion	(Yes)	11	8	3	27.2
	(No)	89	82	7	7.9
Multiple Sex partners	(Yes)	13	9	4	30.8
	(No)	87	81	6	6.9
Share of Sharp Objects	(Yes)	24	19	5	20.8
	(No)	76	71	5	6.6
Barber clipper	(Yes)	50	46	4	8.0
	(No)	50	44	6	12.0
Pedicure	(Yes)	23	18	5	21.7
	(No)	77	72	5	6.5
Manicure	(Yes)	28	22	6	21.4
	(No)	72	68	4	5.6
HBV awareness	(Yes)	27	25	2	7.4
	(No)	73	65	8	10.9
HBV status	(Yes)	12	10	2	16.7
	(No)	88	80	8	9.1

This study determined the prevalence of HBsAg amongst healthy, asymptomatic students of Federal University of Technology, Owerri (FUTO), Nigeria, and assessed the possible associated risk factors. The result (Table 1) indicated that the total prevalence of HBsAg infection in FUTO among students, was 10.0%. This rate was found to be higher in the age groups of 21-23 (18.2%), and 24-26 (11.8%). This could be due to the fact that, these age groups, as adolescents and due to peer pressure indulge in most of the risk associated with HBV infection (Khan, Shams, Qureshi, Israr, Khan, Sarwar & Ilyas, 2011). Our study is in conformity with previous studies conducted on age group carrier rates of HBsAg which indicated that the infection was more prevalent in the young (18-25) than in older age (35-40) groups (Khan *et al*., 2011; Isa, Aminu, Abdulahi, Sani & Akafyi, 2015; Musa *et al*., 2015; Kolou, Katawa, Salou, Gozo-Akakpo, Dossim, Kwarteng & Prince-David, 2017).

This study also observed that the rate of HBsAg infection in FUTO was higher in male (13.3%) than in female students (5.0%) as shown in Figure 1. The reason that could simply be adduced for this observation was that, males indulge in more of the risk factors that predispose to HBV infection than females. It was observed that more of the male students consented to having multiple sexual partners, as well patronizing local pedicure and manicure vendors, than the females. The trend of gender distribution of HBsAg infection rate in FUTO was not different from that found in other parts of the world (London & Drew, 1977; Khan *et al*., 2011; Kolou *et al*., 2017), and Nigeria (Okonko, Okerentugba & Akinpelu, 2012; Isa *et al*., 2015; Musa *et al*., 2015; Tewase & Chima, 2015). Besides risk factors, studies have shown that, the reason for gender differences in response to HBsAg, is that, females are more likely than males, to produce antibody known as anti-HBs against HBsAg (London & Drew, 1977; Blumberg, 1979). In another study however, Wei-Cheng and Quan-Yan, (2014) identified abnormal forms of apolipoprotein A-I (Apo A-I), a protein involved in fighting inflammation, in the livers of infected males but not infected females.

The risk factors of HBV infection were found to be more prevalent in FUTO, with multiple sexual relationships accounting for the highest rate of 30.8%, while the least, 8.0%, was found among those that patronize commercial barbers' shops. This indicated that, the rate of indulgence in sexual activities among students of FUTO, was very high. This observation was in direct agreement with that made in previous studies about the high rate of sexually transmitted HBV infection among young adults and adolescents (Judson, 1981; Risbud, Mehendale, Basu, Kulkarni, Walimbe, Arankalle, Gangakhedkar, Divekar, Gadkari & Paranjape, 2002; CDC, 2017). Other risk factors such as blood transfusion (27.2%), patronage of commercial pedicure (21.7%) and manicure practitioners (21.4%) were equally prevalent in FUTO, and the fact that about 10.9% of the respondents were either unaware of HBV infection or ignorant of their HBV status (9.1%). Based on the findings of this study, the entire university community is at risk of uncontrolled spread of HBV infection. According to WHO standard, FUTO could be classified as a highly endemic community for HBsAg (Isa *et al*., 2015), as a result of the seroprevalence rate of 10.0% identified among its students.

4. Conclusion and Recommendation

This study implies that HBV infection is highly endemic among the students. The students who claimed ignorant of HBV infection, and yet are HBsAg positive, could serve as potential reservoirs for transmission of the virus. There is urgent need to create awareness about HBV infection among the student population, to enlighten them on the complications associated with this viral infection and the preventive strategies for its control.

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