Confirmation of Hepatitis B Surface Antigen (HBsAg) Among Selected Tertiary Institution Students in Ogun State, Nigeria

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ABSTRACT: The aim of this study was to confirm the presence of HBsAg among students of Crawford University. Students were selected using the stratified random sampling method with a response rate of 100.0%. A total of ninety six samples were also collected from students. Screening was carried using HBsAg rapid test strips and confirmation was by the use of a fourth generation Enzyme-Linked Immunosorbent Assay (ELISA). All 4(100.0%) positive samples were confirmed positives and 1(1.1%) of the negative serum samples came out positive on ELISA establishing the fact that ELISA has a greater sensitivity and it is the standard for HBV diagnosis. Results showed an overall prevalence of 5.2%. The seroprevalence of HBsAg was higher in females [5(10.9%)] than in their male counterparts [0(0.0%)]. The age-specific distribution of HBsAg among the subjects showed that those in the age group of 21-25 years had a higher prevalence rate of (7.8%) compared to those in age group 16-20 years which had a lower prevalence rate of 2.2%. There was significant association ($p<0.05$) between age, sex and HBsAg seropositivity in the study. Only 10.0% of the subjects had a previous knowledge of Hepatitis B virus. About 3.0% of the subjects with positive serum had had contact with body fluids of other individuals (infected or not). There was poor knowledge about Hepatitis B infection among Crawford University students. Majority had no idea if they had been immunized or not. This study confirms the presence of HBsAg among students of Crawford University, Igbesa, Ogun State, Nigeria. It further confirms that ELISA is the most acceptable and conclusive method for diagnosing HBsAg compared to the use of rapid test strips. The importance of routine HBsAg screening among students especially the young and singles is advocated.

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1. INTRODUCTION

Hepatitis B is a serious liver disease caused by hepatitis B virus (HBV). It is extremely infectious and is transmitted through sexual intercourse or contact with blood or body fluids. It is one of the major leading diseases of human and has caused serious health problems (CDC, 2007). Although HBV can infect people of all ages, young adults and teenagers are the most exposed. It has been discovered that about one million people die each year and two billion people have been infected with an estimated worldwide carrier state of more than 350million. Hepatitis B disease is widely spread and needs much attention from the medical community. It is highly contagious and has been discovered to be 50 to 100 times more contagious than the HIV virus and can resist any antibody produced by our defense system (CDC, 2007).

Only people who have been vaccinated successfully or those who have developed anti-HBs antibodies after HBV infection are immune to HBV infection (Mahony, 1999). Following acute HBV infection, the risk of developing chronic infection varies inversely with age. Chronic HBV infection occurs among about 90% of infants infected at birth, 25-50% of children infected at 1-5 years of age and about 1-5% of persons infected as older children and adults. Chronic HBV infection is also common in persons with immunodeficiency (Robinson et al., 1995).

There are many factors that influence the probability of developing a chronic infection. Age is important and transmission from mother to infant at birth or infection while very young nearly always results in chronic infection (90%). In children the rate is lower (25%) and in healthy adults the risk of developing chronic infection is much reduced (2 - 5%) (CDC, 2007).

The prevalence of HBV infection varies widely, with rates ranging from 0.1% to 20% in different parts of the world (Lavanchy, 2004). The prevalence of HBV infection, according to the geographical area,

may be high (8%), intermediate (2%-7%) or low (<2%) (Maddrey, 2000). In several studies from different regions of Nigeria, the prevalence of hepatitis B surface antigen (HBsAg) among normal population was reported from a minimum of 2% to a maximum of 14.3%-average 6.8 % (Mistik and Balik, 2001). In Europe and America, chronic HBV carriers are found in <2% of the population (Kane, 1995). In endemic areas, most individuals are infected by vertical transmission (Wright, 2006). In Africa, more than half of the population becomes HBV infected during their life time and about 8% of inhabitants become chronic carriers; most of the infections take place during delivery or infancy (Kane, 1995).

The global burden of disease attributable to hepatitis B remains enormous, and this is due largely to the lack of universal vaccination. Because of this, hepatitis B vaccine has been effective in reducing the incidence of infection in endemic areas where it has been adopted for universal immunization. There should no longer be any reason to avoid vaccination especially among the people of the Northern part of Nigeria. In Taiwan, one of the earliest countries to adopt universal immunization, the prevalence of HBsAg positivity decreased from 15%-20% to 7% among children and adolescents (Chen et al, 2006). Furthermore, a reduction in the incidence of HCC was observed after initiation of large-scale vaccination against hepatitis B (Chen et al, 2006).

In Nigeria, following the adoption of universal infant vaccination in 1995, the incidence of acute hepatitis B in children and adolescents has decreased, and the ethnic possibility in the prevalence of chronic HBV infection have narrowed down (Umolu et al, 2005). Prevention of vertical transmission is extremely important because HBV infection in early life usually results in a chronic carrier state. HBV infection does not appear to be teratogenic.

The aim of this study was to confirm the presence of HBsAg among students of Crawford University, Igbesa, Ogun State, Nigeria to compare the sensitivity and specificity of HBsAg rapid strips with that of the standard ELISA. It also aimed at ascertaining the level of awareness of hepatitis B virus infection among students.

2. MATERIALS AND METHODS

2.1. Study population

This study was carried out in Crawford University, a tertiary institution situated in Faith City, Igbesa, Ogun State, Nigeria. The study population used were both male and female within the ages 16 to 25years using random sampling method. Ninety six (96) serum samples were collected and analysed with the use of HBsAg strips and Enzyme-Linked Immuno Sorbent Assay (ELISA) technique with questionnaires being distributed as blood samples were collected.

2.2. Detection of HBsAg

Sera were tested for hepatitis B virus by ELISA (HBV; 0003463 Hepalisa kit), in a stepwise order for the detection of HBsAg in the blood. These methods which are immunochromatographic and qualitative in nature, detect the presence of HBsAg in human blood and can be read in-vitro having more than 99.9% sensitivity and 99.75% specificity. The interpretation of test results was performed according to the manufacturer's specifications.

2.3. Confirmation of HBsAg

This was carried out at Nigerian Institute of Medical Research using the enzyme linked immunosorbent assay. Using a calibrated pipette, 100microlitre of each serum sample was dispensed into each well of a 96-well microtiter plate. With a multipipette, 50microlitre of conjugate was dispensed into each serum sample in the well. The plate was covered with a seal and was incubated for 1hour 30minutes. After this, the plate was washed in an autostrip washer. It was washed for at least five times and the wells were damped to remove debris. About 50microlitre of substrate was added into each well, if sample is positive, that particular well turned from pink to purple but if negative it remained pink. The wells were then incubated in the dark for 30minutes. This was done to keep samples away from light because light can affect the reaction. It is washed in an auto ELISA plate washer. Stop solutions were added and left for 4minutes after which positive samples changed from purple to yellow and negative samples turned colorless. The wells were placed in a universal microplate reader and results were printed out.

2.4. Calculation of Cut-off values

Control samples of both confirmed positive and negative individuals were used. The values were calculated from the figures given by the autoread ELISAN machine. Four control samples were used of which 3 were negative and 1 positive.

Negative Control (NC)=
Sum of the negative control
Total number of negatives

Positive Control (PC)=
Sum of the positive control
Total number of positives

Cut Off (CO) = NC + 0.050 (constant). Any result greater than the cut off is then recorded as positive.

2.5. Data analysis

The prevalence for HBsAg was calculated by using patients with positive samples as numerator and the total numbers of patients enrolled in this study as denominator. The data generated from this study were presented using descriptive statistics. The data was subjected to statistical analysis using SPSS computer software version 19.0 for Windows to determine any significant relationship between infection rate, age and gender.

3. RESULTS ANALYSIS

A total of 96 blood samples and questionnaires were distributed of which all were analyzed giving a response rate of 100 percent.

3.1. Gender Distribution

A total of 50 males (52.0%) and 46 females (48.0%). Therefore, majority of the respondents were male (52.0%). Figure 1 shows gender distribution of respondents.

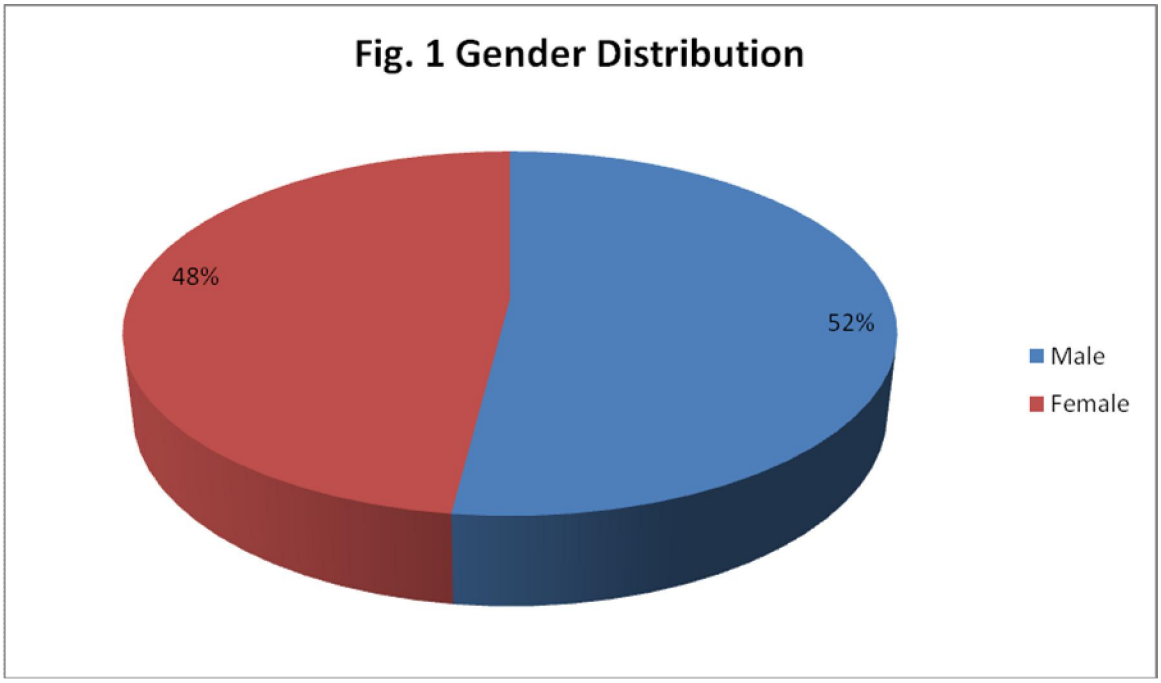

Figure 1: Gender Distribution of Respondents

3.1. Age Distribution

Table 1 shows age distribution of respondents. The respondents ranged from ages 16 to 25 years with highest population ranging between 18 and 19 years.

Table 1: Age Distribution of Respondents

Age groups (years)	No. Tested (%)
16-20	45(46.9)
21-25	51(53.1)
Total	96(100.0)

3.3. Respondents ideas on the factors that promote the spread of the disease

Table 2 shows the respondents ideas on the factors that promote the spread of the disease. Majority of the respondents had not undergone blood transfusion, been in an accident, were not sexually active, have not been exposed to body fluid, do not share sharp objects and do not share toothbrush while the minority had been exposed at one time or the other to one or all the factors. Sharing of sharp objects is one of the highest ways by which the infection can be acquired 39.6% followed by being sexually active individuals which had 2% positives out of the 32.3% individuals and exposure to body fluid such as saliva had 3% positive out of 30.2% individuals exposed. The other three possible causes which are blood transfusion accident and sharing of toothbrush also could be a major cause but were not so significant ($p>0.05$) in this study (Table 2).

Table 2: Respondents ideas on the factors that promote the spread of the disease

Risk factors	**No. (%)**
Sexually active risky life style/unprotected sex	
Yes	31(32.3)
No	65(67.7)
Blood transfusion	
Yes	10(10.4)

No	86(89.6)
Unsterilized/sharp Instruments	
Yes	38(39.6)
No	58(60.4)
Accident	
Yes	12(12.5)
No	84(87.5)
Other body fluid	
Yes	29(30.2)
No	67(69.8)
Sharing of tooth brush	
Yes	5(5.2)
No	91(94.8)
Total	**96(100.0**

3.4. Respondents' Place of Birth, Place of Residence and State of Origin

Table 3 shows the geographical distribution of respondents. Lagos had the highest population for place of birth (58.3%) and place of residence (62.5%) while majority are indigenes of Ogun state (26.0%).

Table 3: Respondents' Place of Birth, Place of Residence and State of Origin

States	Birth (%)	Residence (%)	Origin (%)
Lagos	56 (58.3)	60 (62.5)	19 (19.8)
Ogun	10 (10.4)	25 (26.0)	25 (26.0)
Plateau	3 (3.1)	2 (2.1)	
Oyo	5 (5.2)	5 (5.2)	4 (4.2)
Osun	9 (9.4)		17 (17.7)
Enugu	2 (2.1)		
Rivers	3 (3.1)	4 (4.2)	
Imo	2 (2.1)		5 (5.2%)
Sokoto	1 (1.0)		
Edo	4 (4.2)		6 (6.3)
Ebonyi			1 (1.0)
Ondo			7 (7.3)
Delta			6 (6.3)
Kogi			3 (3.1)
Abia			3 (3.1)
Total	96(100.0)	96(100.0)	96(100.0)

3.5. Detection and Confirmation of HBsAg

All the positive serum samples gotten by the use of HBsAg rapid test strips were confirmed positive by ELISA however, one of the negative samples by HBsAg rapid test strip was confirmed positive by ELISA. Table 4 shows the prevalence of HBsAg in relation to gender and age of the participants. It showed that all males tested none (0.0%) were positive for HBsAg while 10.9% of the females tested were positive for HBsAg. There was a significant association ($p<0.05$) between sex and HBsAg seropositivity.

Table 4 also showed the prevalence of HBsAg in relation to the ages of the subjects. The age-specific distribution of HBsAg among subjects in the study showed that those in the age group of 21-25 years had a higher prevalence rate of 4(7.8%) compared to those in age group 16-20 which had a lower prevalence rate of 1(2.2%). There was a significant association ($p<0.05$) between age groups and HBsAg seropositivity.

Table 4: Prevalence of HBsAg in relation to sexes and ages of subjects

Demographic characteristics	No. Tested (%)	No. Positive for HBsAg (%)
Sex		
Males	50(52.0)	0(0.0)
Females	46(48.0)	5(10.9)
Age groups (years)		
16-20	45(46.9)	1(2.2)
21-25	51(53.1)	4(7.8)
Total	**96(100.0)**	**5(5.2)**

4. DISCUSSION

Hepatitis B as infectious as it has an extremely low awareness in this part of the country. Most people analysed had no knowledge of the virus and those who had a little knowledge had not been vaccinated or are not sure if they had been vaccinated. Only about 3.0% knew that they had been vaccinated while the others did not even know about the virus nor its vaccination. All the positive serum samples gotten by the use of HBsAg rapid test strips were confirmed positive by ELISA however, one of the negative samples by HBsAg rapid test strip was confirmed positive by ELISA. This proved that ELISA is far more sensitive than the rapid test strips and ELISA is the standard and most acceptable method for the diagnosis of Hepatitis B virus. Therefore, HBsAg diagnosis should not be limited to rapid test strips alone.

Out of every twenty four students, one is positive for the virus. In total 5(5.2%) were positive while 91(94.8%) were negative which is higher compared to the work done previously by the Institute of Health Surveillance (2003) where the prevalence of the virus among adult population varies from 0.65% to 0.70% (age range 18-80 years). This shows that the infection pattern is still on the low rate but has the capacity of increasing if no awareness is created. The presence of HBsAg indicates ongoing HBV infection, and in newly infected persons, HBsAg is the only serologic marker detected during the first 3-5 weeks after infection. In persons who recover from HBV infection, HBsAg is usually eliminated from the blood in 3-4 months, and anti-HBs develop (Mast et al., 2005).

The age-specific prevalence of HBsAg in this study showed an association of age with HBsAg seropositivity. This observation is consistent with the report by Uneke et al. (2005) who claimed that higher prevalence occurs among less than 30 years of age. Pennap et al. (2010) reported a higher age-related prevalence for HBsAg among those aged 1 - 40 years (13.8%) and above 40 years (11.5%). However, this observation is inconsistent with the report of Motta-castro *et al* (2003) who reported no association of age with HBsAg seropositivity.

The sex-related prevalence of HBsAg in this study showed an association of sex with HBsAg seropositivity. It was discovered in this study that females had the highest prevalence of HBsAg than their male counterparts with zero prevalence. This is consistent with findings of Harry et al (1994); from Northern part of Nigeria 22.0% and 11.6% respectively. It is also in consonance with what was reported by Okonko et al. (2010, 2012a). It is not consistent with the findings of Okonko et al. (2012b,c) who reported higher prevalence in males than females. Sule et al. (2010) reported no association with gender and HBsAg seropositivity. The gender related prevalence of HBsAg was 9.5% in females and 24.1% in males in a study by Pennap et al. (2010). The HBV carrying rate of pregnant women in Wuhan City, China has reportedly decreased in recent years (Yu et al., 2005). The survey by Guo et al. (2010) showed that from 2004 to 2006, the HBV carrying rate was 7.8% for in Wuhan City, China. This 0.0% rate reported for males in this study was lower than that of developed countries, such as European countries and the United States (Denis et al., 2004) and that of many developing countries (Akani et al., 2005; Bertolini et al., 2006). This might be attributed, at least in part, to the increase in HBV screening (Guo et al., 2010).

The location-specific prevalence showed that subjects positive for HBsAg either born in Lagos or resides in Lagos which means the endemic rate in Lagos may probably be high. From the response of two (2.1%) of the positive participants, there was no direct cause in the sense that they had not been exposed to any of the possible causes, so infection may be due to transmission from mother-to-child. The risk is as high as 90percent if the mother is also positive for HBeAg (Hollinger et al., 2001). The other two were either sexually active or had been exposed to an infected person's blood or body fluid. This means that exposure to infected fluids such as blood or sexually active individuals who do not take precaution and are not vaccinated are at greatest risk of acquiring the infection as stated by Duguid et al. (1978). Sharing of toothbrush is one of the least ways by which infection can occur because hardly in this area do people share toothbrush and those who share toothbrush all came out negative.

Several workers have detected different HBsAg antigenemia rates in different parts of the country. The 5.2% seropositivity reported for HBsAg in this study is far lower than the 12.4% reported by Alikor and Erhabor (2007) in children attending tertiary health institution in Niger Delta of Nigeria; and the 12.0% reported among pregnant women attending ante-natal clinic at Central Hospital, Warri, Delta State (Ophori et al., 2004). It is also slightly lower than the 7.0% among Taiwanes adolescents (Ni et al., 2001). However, it is higher than the 4.1% seropositivity reported Ugwuja and Ugwu (2010) among apparently healthy adolescents in Abakaliki, South Eastern Nigeria; and the 2.19% maternal seroprevalence reported by Onakewhor et al. (2001). Vardase et al. (1999) detected a prevalence of 10.4% HBsAg antigenemia in South African children in a pre-immunization, community-based investigation. Nasidi et al. (1986) found HBsAg prevalence of 10.3% in children from Lagos and Bauchi states while Akenami et al. (1997), in Calabar, detected HBsAg antigenemia of 20% and 26% in healthy and malnourished children, respectively. Bukbuk et al. (2005) found HBsAg antigenemia of 44.7% among pupils in primary school in rural Borno state. Onakewhor et al. (2001) reported the neonatal seroprevalence and the vertical transmission rate to be 0·96% and 42·86%, respectively. In a study by Guo et al. (2010), the intrauterine infection rate of newborns was 6.7% and the chronic HBV rate of children was 4.0%. The prevalence rates are not the same but they firmly place Nigerian children in the highly endemic group.

Two analytical methods were used, HBsAg rapid test strips and ELISA. It was discovered that the use of ELISA is the standard method for analyzing hepatitis B because ELISA is a more sensitive method than the rapid test strips. Out of the 96 samples analyzed, 4.2% came out positive on HBsAg rapid tests strips while others were negative. Those same samples were further screened using ELISA and it was discovered that one of the negatives on strip came out positive on ELISA giving a frequency rate of 5.2%. This could be as a result of recent infection in which the virus has not gotten to a significant level therefore they could not appear on the strips but ELISA being a very sensitive kit can detect even the slightest of virus in the serum. Therefore ELISA is recognized to be the standard diagnostic technique for the detection of HBV in serum.

5. CONCLUSION

HBV is still by far deadly and 50 to 100 times more infectious than HIV (CDC, 2007) and could

result in liver cirrhosis and cancer if it is not treated early enough. Vaccination is the surest way to safety from HBV (Doylestown, 2009). This study has shown that the prevalence of HBV is high among students of Crawford University. Care has to be taken to avoid the spread of HBV. The knowledge of HBV is very poor in Crawford University having just 10.0% awareness. However, during the course of this study, most students got to know about the virus and the possible ways of contacting it.

In conclusion, this study confirms the presence of HBsAg among students of Crawford University, Igbesa, Ogun State, Nigeria. It further confirms that ELISA is the most acceptable and conclusive method for diagnosing HBsAg compared to the use of rapid test strips. Care has to be taken because as hope has been given for those infected with HBV. The importance of routine HBsAg screening among students especially the young and singles is advocated.

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