Detection of Hepatits B surface Antigen (HBsAg) among pregnant women attending Antenatal Clinic at O.L.A. Catholic Hospital, Oluyoro, Ibadan, Oyo State, Southwestern Nigeria

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Abstract: This study was carried out to determine the seroprevalence of HBsAg among pregnant women attending antenatal clinic at Oluyoro Catholic Hospital in Ibadan and to generate data which may be useful for appropriate health authorities. Two hundred pregnant women who consented voluntarily after thorough explanation of the purpose of the study were recruited for this study. The samples were tested for the presence of antibodies using rapid Dia Spot HBsAg rapid test strip developed by Dia Spot Diagnostics, USA and Clinotech HBsAg (Clinotech diagnostics, Richmond, Canada). Analysis of the result shows that 23 (11.5%) of the pregnant women had HBsAg. Statistical analysis, however, showed no significant difference (p=0.05) between the prevalence and the age of patients, parity, trimester and type of family. The frequency of HBV carriers did not vary significantly with scarification and the use of condoms and contraceptives. However, there was a significant frequency variation in the distribution of HBsAg between those that have previous history of transfusion, operation, circumcision and those who never had history of operation, transfusion or circumcision.Therefore, the need to institute public health measures to reduce disease burden and transmission, including routine screening of all pregnant mothers for HBV infection and early passive-active immunization of babies born to HB_SAg-positive mothers are advocated.
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1.0. Introduction

Hepatitis B is an infectious illness caused by hepatitis B virus (HBV) (Engy, 2011); an enveloped virus with double-stranded circular DNA. Viral hepatitis is a systemic disease primarily involving the liver. Most cases of acute viral hepatitis are caused by Hepatitis A Virus, Hepatitis B Virus (HBV) or Hepatitis C virus (HCV). The complex antigen found on the surface of HBV called HBsAg. Previous designation included the Australia or Au antigen (Blumberg, 1971). The Australia antigen was quickly associated with serum hepatitis, including a significant fraction of post transfusion hepatitis cases which at the time occurred in 10% to 30% or more of multiply transfused individuals (Seeger *et al*., 2007).

The presence of HBsAg in serum or plasma is an indication of active Hepatitis B infection, either acute or chronic. Viral hepatitis is now known to be the most common complication of blood transfusion (Stevens *et al*., 1990). The hepatitis agents known to be transmitted through blood and blood products include hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis G virus (HGV) (Goodnough *et al*., 1999). Hitherto, only hepatitis A virus (HAV), and HBV were characterized, and hepatitis not caused by these two agents was then referred to as non-A non-B hepatitis (Choo *et al*., 1989). Both HBV and HIV share similar mode of transmission and risk factors (Ansa *et al*., 2002), HIV-infected people are frequently co-infected with HBV (Ezegbudo *et al*., 2004). Hepatitis B virus infection is associated with significant morbidity and mortality in patients with HIV infection (Thio *et al*., 2002). Co-infection of HIV with HBV affects number of patients worldwide (Nelson, 2002). Although, very few co-infection studies have been carried out in Africa but since sub-Saharan Africa is a home of about 29.4 million HIV infected people, high HIV/HBV co-infection is expected. However results are contradictory. While in Kenya, 32(78%) out of 41 patients with AIDS had serological evidence of exposure to HBV7, a study among pregnant women attending ante-natal clinics in Burkina Faso, showed a low co-infection rate of 0.88% (Dao *et al*., 2001).

The HBsAg One Step Hepatitis B Surface Antigen Test Strip (Serum/Plasma) is a rapid test to qualitatively detect the presence of HBsAg in serum or plasma specimen. The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBsAg in serum or plasma. However, universal vaccination still remains a goal rather than an accomplished fact, and the World Health Organization (WHO) estimates that there are currently 400,000,000 individuals worldwide who are

chronically infected with HBV, of whom 25% will die of chronic liver disease or hepatocellular carcinoma (Seeger *et al*., 2007).

In Nigeria, several reports have established the endemic nature of HBV by the presence of HBsAg in different population groups from different parts of the country. Akani *et al.* (2005) reported a prevalence of 4.3% in pregnant women in Port Harcourt, Nigeria. The prevalence of HBsAg among blood donors was 5.4% in Benin City (Umolu *et al.,* 2005) and 14.3% in Jos, Nigeria (Uneke *et al.,* 2005). Among HIV-infected individuals in Jos, Nigeria 25.0% prevalence was reported, indicating a higher HBV infection among this group (Uneke et al., 2005). A 4.4% HBsAg seroprevalence was reported in Lagos among healthy pregnant women. A study carried out on 100 healthy blood donors in Benin City showed 11.0% prevalence, while another at Central Hospital, Warri in Delta State, Nigeria showed a seroprevalence of 12.0%.

This study therefore, is designed to determine the prevalence and incidence of HBsAg among pregnant women attending antenatal clinic at O.L.A. Catholic Hospital, Oluyoro, Oke-Ofa, Ibadan, Oyo State, Nigeria.

2.0. MATERIALS AND METHODS

2.1. Study area

The study area is the O.L.A. Catholic Hospital, Oluyoro, Oke-Ofa located at the municipal area of Ibadan, which is made up of five local government areas. Ibadan is the capital city of Oyo State located in the forest zone of southwestern Nigeria. Ibadan city lies on the longitude 3°5' East of Greenwich meridian and latitude 7°23' North of the Equator. Besides being the largest indigenous city in Africa south of Sahara, the city is an important trade and educational centre. It also houses one of the largest and foremost teaching hospitals in Africa. However, the city is characterized by low level of environmental sanitation, poor housing, and lack of potable water and improper management of wastes especially in the indigenous core areas characterized by high density and low income populations.

2.2. Study population

After informed consent was obtained, a total of 200 pregnant women blood samples were collected from these pregnant women attending the Antenatal Clinic, of the O.L.A. Catholic Hospital, Oluyoro, Oke-Offa, Ibadan, South-Western, Nigeria. Ethical approval for this study was obtained from the O.L.A. Catholic Hospital ethical committee. Demographic and clinical information of the subjects were obtained by chart abstraction and recorded on a prepared data collection form. The study groups were also stratified by age distribution. Information were collected on the women's age, occupation, menstrual and obstetric histories, gestational age, and parity, perceived gynaecological symptoms, health care-seeking behavior and contraceptive practices.

2.3. *Study Design*

The study was carried out between March 2010 and August, 2010. The location of the present study was O.L.A. Catholic Hospital, Oluyoro, Oke-Ofa, Ibadan, Oyo State, Nigeria; and the participants were also those attending the hospital. We obtained permission to carry out the study from the Management of the hospital. Subsequently, the objective and procedures of the study were explained to all consecutive adult patients visiting the hospital and consenting individuals recruited into the study. With well-structured questionnaire forms, pertinent demographic data were obtained from the participants. The data collected included age, gender, patient and their spouses' occupation. About 5ml blood sample was aseptically collected by venipuncture from each participant into sterile plane bottle. The blood samples were left to form clots at room temperature, after which they were centrifuged for 10 minutes at 200 revolutions per minute (rpm) to separate serum from clot. Each blood sample was screened using third generation immunoassay based test strips for qualitative detection of HBsAg in serum (relative sensitivity and specificity of > 99% and 97.0% respectively with accuracy of 98.5%); the test and result interpretations were done according to the test kit manufacturer's instruction. The assay was carried out using Dia Spot HBsAg rapid test strip developed by Dia Spot Diagnostics, USA and Clinotech HBsAg (Clinotech diagnostics, Richmond, Canada). These serum/plasma test strips are rapid, one step test for the qualitative detection of Hepatitis B surface Antigen (HBsAg) in serum or plasma. The HBsAg one step Hepatitis B Surface Antigen Test Strip (serum/plasma) is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B Surface Antigen in serum or plasma. The relative sensitivity of the HBsAg test strip is over 99.0% and the relative specificity was 97.0%. The accuracy is 98.5%.

2.4. DATA ANALYSIS

The data generated in this study were analyzed at 5% level of significance by Chi-square statistical test using contingency table. Data was presented using descriptive statistics for HBsAg.

We presented the results of this study with descriptive statistics. In addition, we used CHI^2 statistical test to establish difference or absence thereof of HBsAg seroprevalence between farming and non-

farming groups and between other categories; $P \leq 0.05$ was used as indicator of statistical significance. We used SPSS 13.0 for the analysis.

3.0. RESULTS

Of the total 200 samples tested for HBsAg, 23 tested positive giving HBsAg prevalence of 11.5%. In the age group 30-39years, a total of 72 samples were tested, out of which 11 tested positive thus, giving the prevalence of 15.3%. In the age group 20-29years, a total of 125 samples were tested and 11(8.8%) tested positive and in the age group 40-49 years, one sample was tested and the sample tested positive giving a prevalence of 100% as shown in table 1. There exists a significant difference ($P<0.05$) in the association between age and prevalence of HBsAg.

Table 1: Distribution of HBsAg by age

Age Group (Years)	No. Tested (%)	No. (%) Positive for HBsAg
Less than 20	2(1.0)	0 (0.0)
20-29	125(62.5)	11 (8.8)
30-39	72(36.0)	11 (15.3)
40-49	1(0.5)	1 (100.0)
Total	**200(100.0)**	**23(11.5)**

Distribution of HBsAg in relation to parity (No. of pregnancy) is shown in Table 2. 9(20.9%) of 43 women who were in their 3rd pregnancy and above or who have had more than 3 children had HBsAg, 2 (4.7%) of 43 women were in their 2nd pregnancy also had HBsAg while 12 (8.3%) of the 114 women who were in their 1st pregnancy had antibodies to HBsAg. However, there is significant difference ($P<0.05$) between the number of parity and HBsAg.

Table 2: Distribution of HBsAg by Parity (No. of Pregnancy)

Parity	No. Tested (%)	No. Positive for HBsAg (%)
1st pregnancy	114(57.0)	12 (8.3)
2nd pregnancy	43(21.3)	2 (4.7)
3rd pregnancy and above	43(21.5)	9 (20.9)
Total	**200(100.0)**	**23 (11.5)**

Table 3 shows the distribution of HBsAg in relation to trimester (a period of three months, especially one of the three three-month periods into which human pregnancy is divided for medical purposes) as at the time of this study. This revealed that women in their 3rd trimester of their pregnancy had the highest prevalence of HBsAg [104], with 14(13.5%) having HBsAg followed by women in their 2nd trimester [81] with 8(9.9%) having HBsAg while women in their first trimester of their pregnancy, (15) with 1(6.7%) having HBsAg. Statistical analysis showed no significant difference ($P>0.05$) between Prevalence of HBsAg and trimester.

Table 3: Distribution of HBsAg by Trimester (Period of 3 Three-Months of Pregnancy)

Trimester (period of 3 three-months)	No. Tested (%)	No. Positive for HBsAg (%)
First trimester (1st 3 months)	15(7.5)	1 (6.7)
Second trimester (2nd 3 months)	81(40.5)	8 (9.9)
Third trimester (3rd 3 months)	104(52.0)	14 (13.5)
Total	**200(100.0)**	**23 (11.5)**

Table 4 shows the distribution of HBsAg in relation to type of family. This revealed that out of the 6 single pregnant women tested, none had HBsAg. Out of the 178 women in monogamous family, 21(11.8%) tested positive to HBsAg while 16 women in polygamous family were tested and 2 (12.5%) were positive to HBsAg. Statistically, there is no significant difference between the type of family and prevalence of HBsAg.

Table 4: Distribution of HBsAg by the type of family

Type of family	No. Tested (%)	No. Positive (%)
Single	6(3.0)	0(0.0)
Monogamous	178(89.0)	21 (11.8)
Polygamous	16(8.0)	2 (12.5)
Total	**200(100.0)**	**23 (11.5)**

Table 5 shows distribution of HBsAg in relation to other associated risk factors. The results showed that women who had previous history of blood transfusion had the highest prevalence of 57.0% while those who had no history of blood transfusion have a prevalence of 8.1%. The frequency of HBsAg carriers did not vary significantly with scarification/tribal marks/tattooing/incisions, the use of condoms and contraceptives, history of previous surgical operation, and female circumcision ($P>0.05$). However, the frequency of HBsAg carriers vary significantly with previous history of transfusion ($P<0.05$).

Table 5: Distribution of HBsAg in relation to associated risk factors

Risk factors	No. Tested (%)	No. Positive for HBsAg (%)
History of blood transfusion		
Yes	14(7.0)	8(57.0)
No	186(93.0)	15(8.1)
Use of condoms and contraceptives		
Yes	22 (11.0)	2(9.0)
No	178(89.0)	21(11.8)
Female circumcision		
Yes	20(10.0)	2(10.0)
No	180(90.0)	21(11.7)
Scarification/tribal marks/tattooing/incisions		
Yes	37(18.5)	4(10.8)
No	163(81.5)	19(11.7)
History of previous surgical operation		
Yes	20(10.0)	2(10.0)
No	180(90.0)	21(11.7)
Total	**200 (100.0)**	**23(11.5)**

4.0. DISCUSION

The overall prevalence of HBsAg in pregnant women attending antenatal clinic at Oluyoro Catholic Hospital, Ibadan was 11.5%. The distribution of HBsAg by age groups shows that women in the age group 40-49years have the highest prevalence of 100%; Osmond (1994) reported that age is a co-factor for disease susceptibility and progression. Therefore age is an indicator of the prevalence of HBV among the pregnant women. The result showed that the age range between 40-40years which is found to be the oldest among the age group examined had the highest prevalence (1 out of 1; 100%), though statistical analysis showed no significant difference. The classification of high endemicity from HBV infection has been defined as HBsAg greater than 7% in an adult population (Uneke *et al,* 2005). The HBsAg seropositivity of 11.5% among pregnant women in Ibadan shows that Ibadan like other areas in Nigeria is endemic for HBV Infection. This result is in conformity with an earlier report that sub-Saharan Africa has HBV carrier rate range of 9 – 20% (Kiire, 1996). The HBsAg prevalence of 11.5% is similar to the finding of Harry *et al,* (1994) who found 11.6% among pregnant women in Maiduguri, Borno State, Nigeria. The prevalence of 11.5% in comparison with 12.6% obtained by Jombo *et al,* (2005) in a study carried out in a rural community in North central Nigeria, confirmed that, though the research was hospital and urban based, it has a high tendency of reporting accurate figures of the community. This is because the subjects were not patients but pregnant women attending their routine antenatal health care clinics.

Several studies have been conducted on hepatitis B and pregnancy. In the U.S., HBsAg positivity was reported in 5.8% of the Asians, 1.0% in non-Hispanic blacks, 0.6% of non-Hispanic whites and 0.1% of Hispanics (Euler, *et al.*, 2003). HBsAg was found in 1.7% of pregnant women in Brazil (Bertolini, *et al*., 2006). In Africa, HBsAg was positive in 4.6% of pregnant women in Nigeria (Obi, *et al*., 2006) and in 5.6% of pregnant women of Sudan (Elsheikh, *et al*., 2007). In another study, the seroprevalence of HBsAg among the pregnant women in the countries of the Persian Gulf territory revealed a rate of 7.1% in Oman, 1.0% in Qatar and 1.5% in UAE (Al Awaidy, *et al*., 2006). In France, HBsAg was positive in 0.29% of the pregnant women of French origin, 7.15% of Southeast Asian origin, and 6.52% for Sub Saharan African origin (Denis, *et al*., 2004). In a study conducted in six regions of Italy, HBsAg was positive in 1.1% of pregnant women born in Italy, but it was 5.9% among immigrants (Stroffolini *et al*., 2003)

Analysis of the prevalence of HBV in relation to the type of family revealed a higher prevalence rate among women who are in polygamous families. This may be due to a high number of individuals in the family and since most HBV infections are subclinical, infection of a member of the family predispose others to the infection. Statistically, there is no significant difference between the type of family and prevalence of HBsAg among the pregnant women. Higher prevalence among the subjects from polygamous families concurs with the finding of Adoga *et al*. (2009) among prison inmates in Nasarawa state, Nigeria. Higher household contacts and sharing of sharp objects such as razor blades for finger-nail cutting with the carriers might account for the higher prevalence found among the subjects from polygamous family.

On the other hand, the prevalence of 11.5% is lower than 16.0% and 36.0% prevalence reported by Bada *et al.* (1996) among ante-natal clinic patients and patients of sexually transmitted diseases respectively. Higher prevalence among patients of sexually transmitted diseases is not unexpected since sexual intercourse is a route of hepatitis B virus transmission. However, the reason for higher prevalence reported among pregnant women attending ante-natal clinic is not immediately known to this study since the subjects were not patients but pregnant women attending their routine antenatal health care clinics.

However, in relation to other risk factors, history of previous blood transfusion accounted for a high proportion of HBsAg infection. This may be due

to improper screening of blood and blood products before transfusion. It has been previously reported that human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) have similar routes of transmission namely through blood and blood products, intravenous drug abuse, unsafe injections and sexual activity, shared needle, other body fluids such as semen, virginal fluid and breast milk; intravenous drug abuse, from mother to child, needle stick injury, ear piercing, tattooing and other tribal ceremonies (scarification), barbers razors etc. (Agbede et al., 2007; Olokoba *et al*., 2008, 2009). This suggests that prior to the advent of HIV/AIDS in Nigeria; there was no routine screening for HBsAg before blood transfusion. Patients transfused with blood from the seropositive donors are subject to direct transmission of the hepatitis B virus.

In this study, it was found that the age range of the pregnant women who tested positive to HBsAg infection fell between 18-49 years and ages 40-49 years of age had the highest prevalence for HBsAg seropositivity. This similar to the report of Motta-castro et al. (2003) that age was not significantly associated with HBsAg seropositivity among Afro-descendant community in Brazil. However, the age of acquiring infection is the major determinant of the incidence and prevalence rates (Ezegbudo et al., 2004). Again serological evidence of previous HBV infections varies depending on age and socioeconomic class (Ugwuja and Ugwu, 2010). The high prevalence rate of HBV among relatively older people in this study indicates that most of these subjects may have acquired the infection through sex and transfusion of unscreened infected blood while others may have acquired any of these infections prior to transfusion. However, the incidence of HBV transmission through sex and transfusion of unscreened infected blood could be reduced with the introduction of HBV vaccines, screening of blood donors and better sterilization procedures for all blood products (Hollinger and Liang, 2001).

The 10.8% and 10.0% prevalence due to scarification and female circumcision could be attributed to the use of unsterilized instruments in which the same instrument are used on many individuals without proper sterilization of the equipments. The 10.0% prevalence due to History of previous surgical operation may be due to improper screening of blood and blood products before transfusion, following surgical operations. It could also be due to the use of contaminated instruments during an operation in which reusable instruments and surfaces contaminated with HBV positive blood were not properly sterilized and disinfected before such instruments were used on a new individual. The frequency of HBV carriers did not vary significantly with scarification and the use of condoms and contraceptives. However, there was a significant frequency variation in the distribution of HBsAg between those that have previous history of blood transfusion and those who never had history of blood transfusion.

Transfusion of blood/blood products is a very significant route of HBV transmission in the present study and calls for the strengthening of the national policy on blood transfusion with the view of curtailing transmission through this route (Ugwuja and Ugwu, 2010). This observation was similar to an earlier report in Anyigba, Dekina local government area of Kogi State in which pregnant women with history of blood transfusion had significantly higher HBsAg prevalence compared to those without such history (Sule et al., 2007) and Ugwuja and Ugwu (2010), who reported that the major routes of HBV transmission in the population used in their study were unsafe injection, tribal marks/circumcision/ scarification and blood/blood products transfusions. Percutaneous exposures to infected blood and blood products were reported to be the major route of transmission of HCV. Cultural practices such as tattooing, ear piercing, circumcision, face marking (tribal marks) are widely practiced in underdeveloped countries including Nigeria (Odaibo et al., 2003).

A similar observation was also made by Motta-Castro et al. (2003) among Afro-descendant community in Brazil. Awole and Gebre-Selassie (2005) however, reported no association of blood transfusion with HBV infection among pregnant women of Ethiopia. In Nigeria, studies on the prevalence of HBsAg among blood donors have been documented. Opaleye et al. (2010) reported a prevalence rate of 5.4% among blood donors in Benin City, Nigeria. These showed that transfusion of HBV-infected blood was possible in Nigeria.

Pregnancy is not a contraindication for vaccination for HBV (Gambarin, 2007). Therefore, those pregnant women who are identified at risk for HBV infection during pregnancy should be vaccinated (Gambarin, 2007). World Health Organization (WHO) and Center for Disease Control and Prevention (CDC) advise that HBsAg should be examined in all pregnant women and that infants born to HBsAg-positive mothers should receive hepatitis B vaccine and 0.5 mL HBIG within 12 hours of birth. This offer has begun to be applied widely all around the world. In addition, viral hepatitis preventive board (VHPB) suggests routine screening of pregnant women for HBsAg; if one is found positive, active and passive prophylaxis, and vaccination of the newborn to prevent the perinatally transmission of HBV (Van Damme, 1998) are recommended.

5.0. CONCLUSION

This study has further provided information on the prevalence of HBsAg infection among pregnant women in Ibadan, Nigeria. General surveillance, mass immunization and public health education to stop the spread of the infection on campus and indeed the whole society is advocated. Free screening and immunization of all pregnant women and their infants should be incorporated in the antenatal and postnatal programmes in hospital to prevent postnatal infection of the infants by their infected mothers. The use of other HBV infection serological markers such as anti HBs, anti HBc and anti HBe is advocated for future studies among the population under study.

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