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Prevalence of Hepatitis B Surface Antigen, Human Immunodeficiency Virus and Malaria among Pregnant Women Attending Antenatal Clinic at Eku Baptist Government Hospital, Eku, Delta State, Nigeria.Akpo, C.O.^{1*}, Edonmi, T. J¹ and Okoye, L. C.²Department of Pharmaceutical Microbiology, Delta State University, Abraka¹, Department of Program Management, Achieving Health Nigeria Initiative².Author for Correspondence *: mummypure637@gmail.com/+234- 803- 225- 5534**Abstract**

Hepatitis B, Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome and malaria are global threats to public health because of their prevalence and tendency to affect offspring when transmitted vertically. The aim of this study was to determine the prevalence of hepatitis B (HBsAg), HIV and malaria among antenatal women in Eku Baptist Government Hospital, Delta State, Nigeria. A questionnaire was administered to each voluntary participant to obtain information on their socio-demographic characteristics. The presence of HBsAg, and HIV were determined from the serum sample of each study subject using SD Bioline HBsAg serological kit and ABON-HIV™ 1/2/0 Tri-line Human Immunodeficiency Virus Rapid Test Device respectively. The presence of malaria was determined using whole blood from each study subject by rapid diagnostic testing and further confirmed using microscopic technique. A total of 100 samples were tested and the prevalence of malaria, HBsAg and HIV among the pregnant women were 23%, 7% and 2% respectively. The HBsAg/malaria co-infection prevalence rate was 2%. There was no HBsAg/HIV and HIV/malaria co-infection. Age group 25-30 years had highest prevalence 2(5.4%) and 10 (27%) for HIV and malaria respectively while the age group 31-35 years had the highest prevalence 4(13.8%) for HBsAg. The age group 43-49 years tested negative to all three infections. The prevalence of hepatitis B, HIV and malaria is still a challenge in Eku and surrounding towns. This report calls urgently for effective health interventions and health education at the national and state levels

on prevention of mother-to-child transmission of HBV and HIV. Malaria should be addressed by incorporating regular periodic prophylaxis for women attending antenatal clinics to resolve asymptomatic infections and save the lives of the women.

Keywords: *Prevalence, Hepatitis B Surface Antigen, Human Immunodeficiency Virus, Malaria, Pregnant women, antenatal.*

Introduction

Maternal health care is the total well-being of a woman of childbearing age from the time of pregnancy till the child is five years old. This is very important since the state of the mother will determine the survival of the foetus and to an extent, the neonate. Maternal health care services include an extensive scope of health services mothers are given before pregnancy, during pregnancy, delivery and post-natal. Antenatal care can help women prepare for delivery and understand warning signs during pregnancy and childbirth. It can be a source of micronutrient supplementation, treatment of hypertension to prevent eclampsia, immunization against tetanus, HIV testing, in addition to medications to prevent mother-to-child transmission of HIV in cases of HIV-positive pregnant women. In areas where malaria is endemic, health personnel can also provide pregnant women with medications and insecticide-treated mosquito nets to help prevent this debilitating and sometimes deadly disease (UNICEF, 2019). Pregnant women with hepatitis B infections can also be monitored so that in line with CDC (2010) recommendations, the neonate would be

given the HBIG shot and the first dose of Hepatitis B vaccine within 12 hours of being born.

Hepatitis B is a liver disease which results when one is infected with Hepatitis B virus. It is contagious, potentially life-threatening and a global health challenge. Terrault *et al.* (2018) reported that all those living in Africa should be screened for Hepatitis B since the continent is a high-risk zone. Pregnant women, when infected with hepatitis B surface antigen (HBsAg) are a risk group for transmission of the virus to the newborn. This risk is amplified with positivity of hepatitis B envelope antigen, HBeAg (Gentile and Borgia, 2014). Although pregnancy is generally well tolerated by women living with HBV, it is associated with increased rates of ante-partum haemorrhage, gestational diabetes, and preterm labour, as well as premature and low birth weight babies (Gasim *et al.*, 2013). In Africa, studies have shown that pregnant women with Human Immunodeficiency Virus (HIV) are three times more likely to have Hepatitis B Virus (HBV) than pregnant women without HIV (Kourtis *et al.*, 2012). It has been reported that Nigeria has a hepatitis B prevalence of 8.1%. There are an estimated 19 million people living with hepatitis B (Hepatitis B foundation, 2020).

The Human Immunodeficiency Virus (HIV) causes Acquired Immunodeficiency Syndrome (AIDS). HIV/AIDS is a global health challenge. The United Nations Programme on HIV/AIDS (UNAIDS, 2019) reported that there were approximately 37.9 million people across the globe with HIV/AIDS in 2018. Of these, 36.2 million were adults and 1.7 million were children (<15 years old). Two million, two hundred thousand persons were living with HIV in Western and Central Europe and North America while in Asia and the Pacific, 5.9 million. A prevalence of about 1% of HIV among pregnant women attending antenatal clinics in the six states of India where the disease is concentrated, have been reported by Vidyaraj *et al.* (2017). In Eastern and Southern Africa, 20.6 million people had HIV in 2018. However, in Western and Central Africa, the number is remarkably low (5.0 million). In Nigeria, 1.9 million people were living with HIV in 2018. Of these, 1.5% were adults; 130,000 were newly infected and 53 000 died from AIDS-related diseases (UNAIDS, 2019).

Malaria infection during pregnancy is a significant public health problem with substantial risks for the pregnant woman, her foetus, and the newborn child. Pregnant women are three times more likely to develop severe disease than non-pregnant women acquiring infections from the same area. Malaria infection during pregnancy can lead to miscarriage, premature delivery, low birth weight, congenital infection, and/or perinatal death (CDC, 2019). Malaria-associated maternal illness and low birth weight is mostly the result of *Plasmodium falciparum* infection and occurs predominantly in Africa (WHO, 2017). Malaria in pregnancy causes a decrease in birth weight by intra-uterine growth restriction, preterm delivery or both (Umbers *et al.*, 2011). In the first half of pregnancy, malaria infection is associated with a reduction in foetal head diameter (Rijken *et al.*, 2012).

The complications of hepatitis B Virus (HBV), human immunodeficiency virus (HIV) and malaria are more pronounced among immune-compromised patients such as pregnant women. These three infectious diseases cause similar adverse pregnancy outcomes which include spontaneous abortion, stillbirth or death of the pregnant women. Therefore, early diagnosis of these deadly infections through screening among the pregnant women is crucial to the ongoing efforts and campaign on the reduction of maternal and child mortality in Nigeria. This study, thus presents the results of prevalence of Hepatitis B surface antigen (HBsAg), HIV and Malaria among Pregnant Women attending antenatal clinic at Eku Baptist Government Hospital, Delta State, Nigeria. This aim of this study is to determine the prevalence of Hepatitis B surface antigen (HBsAg), HIV and Malaria among Pregnant Women attending antenatal clinic at Eku Baptist Government Hospital, Eku, Delta State, Nigeria.

Materials and Methods

Ethical Clearance

Ethical clearance was obtained from the hospital management and written informed consent was also sought and obtained from the participants.

Study Area

This study was carried out in Eku, a town located in Delta State, Nigeria. Eku Baptist Government Hospital is the most utilized health facility in

Ethiopia East Local government area of the state. The hospital was chosen to have more representative study subjects since women from surrounding towns and villages as well as areas such as Warri attend antenatal clinic there. All pregnant women who consented to the screening after a counselling session were enrolled into the study.

Materials

The materials used in the course of this research include: sterile disposable syringes and needles, cotton wool, light microscope, glass slide, Giemsa stain, SD Bioline HBsAg serological kit, ABON-HIV 1/2/0 Tri-line serological kit, CareStart™ malaria diagnostic kit and centrifuge, among others.

Administration of Questionnaire

The participants were interviewed by using a structured questionnaire to obtain information on demographic characteristics. The questionnaire was typed in English language.

Collection of Samples

After questionnaire administration, sterile disposable syringes and needles were used to collect blood (6ml) by venepuncture. About 3ml of the blood samples were placed in labelled sterile tubes, allowed to clot and then centrifuged to obtain serum for hepatitis B surface antigen (HBsAg) and human immune deficiency virus (HIV). The remaining 3ml whole blood was placed in labelled heparinized tubes for malaria parasite tests. Rapid serological kits were used for HBsAg and HIV, while malaria was tested using rapid diagnostic kits and confirmed by microscopic technique in whole blood.

Method of Testing for HBsAg

SD Bioline HBsAg serological kit manufactured by J. Mitra and Co. Rit. Ltd, was used for the rapid diagnosis of HBsAg. The package of SD Bioline HBsAg serological kit was opened and checked for the test device and desiccant in individual packs; and instructions for use. Having read the instructions for using the SD Bioline HBsAg WB test, as given by the manufacturer, the expiration date was checked at the back of each pouch to avoid the use of expired and impotent kit. Each pouch was opened and the test device, labelled with the patient's identifier.

Using a pre-set micropipette, 100µl of serum was drawn from each test tube and dispensed into the specimen well marked 's' in each test device. Results were read, interpreted and recorded at 20 minutes of dispensing. The presence of a test line 'T' and a control line 'C' within the result window, indicates a reactive result. On the other hand, the presence of only the control line 'C' within the result window, indicates a non-reactive result.

Method of Testing for HIV

Human Immunodeficiency Virus was tested using ABON-HIV™ 1/2/0 Tri-line Human Immunodeficiency Virus Rapid Test Device manufactured by ABON Biopharm (Hangzhou) Co. Ltd., China. The materials provided in each Kit are: 40x test devices, 40x specimen droppers, 2x buffers (2x 3 mL) and 1 instruction for use. The instructions for use was studied carefully before carrying out the test as directed by the manufacturer. The test device, buffer and specimen were removed from the refrigerator and allowed to reach room temperature (15-30 °C) prior to testing. Each test device was removed from the foil pouch and used as soon as possible (within one hour). Afterwards, it was placed on a clean and level surface and labelled with specimen ID. The dropper was held vertically to transfer a drop of serum to the specimen well (S) of the test device, then a drop of buffer was added. Results were read at 10 minutes. A reactive result is indicated when two or three distinct coloured lines appear in the control line region (C), the test line region(s) (T1 and/or T2). A non-reactive result is indicated when one coloured line appears in the control region (C) and no apparent coloured lines appear in the test line regions (T1 and/or T2).

Methods of Testing for Malaria

Rapid Diagnostic Test: Rapid diagnostic test for malaria parasite was done using CareStart™ Malaria HRP₂ (*Pf*) manufactured by Access Bio, Inc, USA. Following the manufacturer's instructions, the expiry date on the test packet was checked. Each packet was opened and a test pack, a pipette and desiccant were removed. The patient's name was written on the test pack while with the pipette, whole blood was collected from each heparinized bottle. The tip of the pipette was touched to the sample hole marked "S" and

squeezed gently to transfer the blood. After use, the pipette was discarded. Thereafter, two (2) drops of buffer were placed into the assay hole marked “A”. Results were read 20 minutes after adding the buffer. A positive result for *Plasmodium falciparum* is shown by a line in “C” and a line in “T”. A line in “C” and no line in “T” means a negative result for *Plasmodium falciparum*.

Giemsa Staining Technique: The heparinized blood samples was collected from each bottle with a sterile Pasteur pipette and used to make a smear on a clean, dry microscopic glass slide. The staining procedure recommended by WHO, 2016 was employed. The smear was air-dried for 1 hour on a staining tray. This was closely followed by pouring the stain gently into the staining tray, to cover the slides. Stain was left on the slides for 1 hour and afterwards, slides were

flooded gently with buffered water to float off the iridescent “scum” on the surface of the stain. Finally, the slides were removed one by one and placed, thick film downwards, in a drying rack to drain and dry, making sure that the thick film does not touch the edge of the rack.

Dried slides were examined using a light microscope at a magnification of x100 under oil immersion objective. *P. falciparum* presented as a ring with a reddish nucleus and a blue cytoplasm, within a red blood cell. Negative and positive controls was included in every set of stained films.

Data Analysis

The one hundred questionnaires were coded and entered into SPSS version 20 spread sheet for descriptive and inferential statistics.

Results

Table.1: Socio-demographics distribution of subjects

| Variable | | Frequency | Percent |
|-------------------|------------------|-----------|---------|
| Age group | 18-24 | 20 | 20.0 |
| | 25-30 | 37 | 37.0 |
| | 31-36 | 29 | 29.0 |
| | 37-42 | 13 | 13.0 |
| | 43-49 | 1 | 1.0 |
| Educational level | Primary | 14 | 14.0 |
| | Secondary | 49 | 49.0 |
| | Tertiary | 37 | 37.0 |
| Marital status | Single | 11 | 11.0 |
| | Married | 88 | 88.0 |
| | Co-habiting | 1 | 1.0 |
| Occupation | Student | 7 | 7.0 |
| | Unemployed | 7 | 7.0 |
| | House wife | 6 | 6.0 |
| | Farmer | 7 | 7.0 |
| | Petty trader | 47 | 47.0 |
| | Self employed | 25 | 25.0 |
| | Clergy | 1 | 1.0 |
| Gestation | First trimester | 23 | 23.0 |
| | Second trimester | 61 | 61.0 |
| | Third trimester | 16 | 16.0 |
| Status gravid | Primigravid | 31 | 31.0 |
| | Multigravid | 69 | 69.0 |

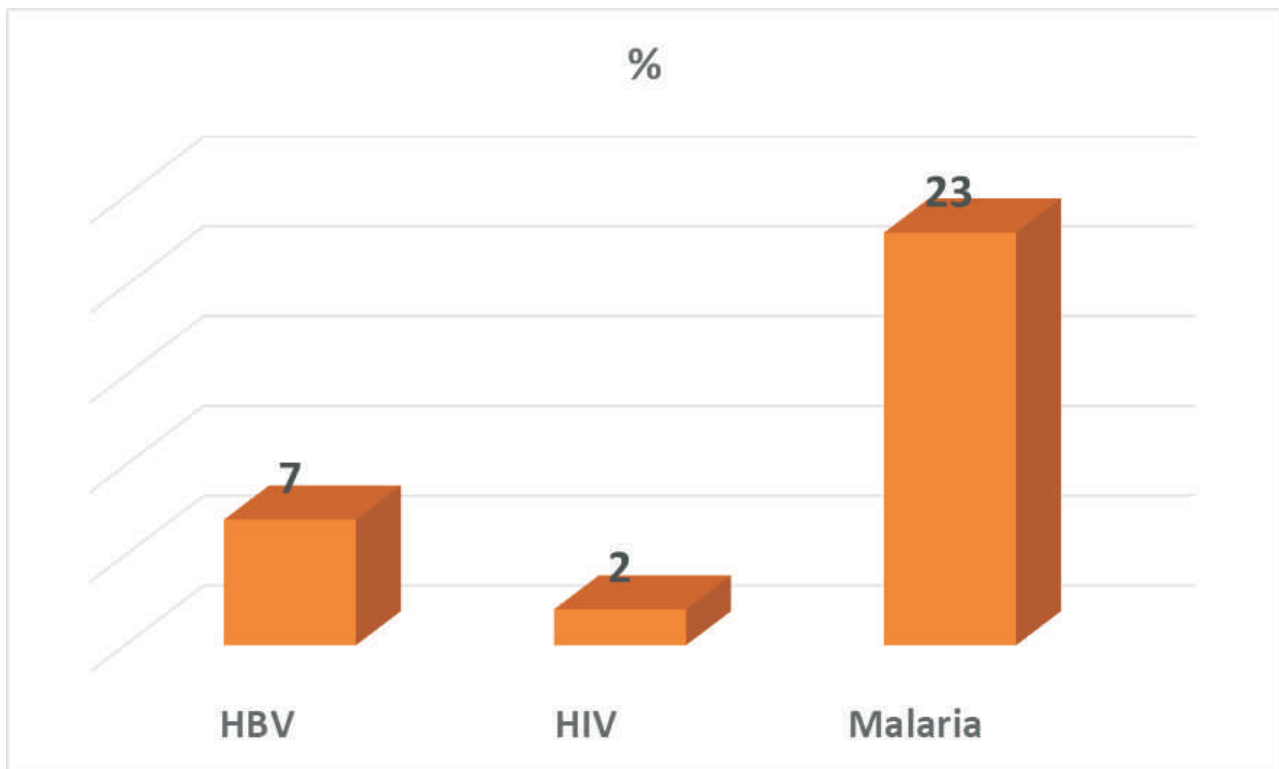


Figure 1: Prevalence of HBV, HIV and Malaria among the Subjects

Table 2: Prevalence of HBsAg, HIV, Malaria and HBsAg/Malaria Coinfection among Pregnant women.

| Parameter | HBV | HIV | Malaria | HIV and HBV | HBV and malaria | HIV and Malaria |
|-----------------|--------|-------|---------|-------------|-----------------|-----------------|
| No positive (%) | 7(7) | 2(2) | 23(23) | 0(0) | 2(2) | 0(0) |
| No negative (%) | 93(93) | 98(8) | 77(77) | 100(100) | 98(98) | 100(100) |

Table 3: Prevalence of HBsAg, HIV and malaria among the women based on age-groups.

| Age-group | No screened | No positive for HBV (%) | No positive for malaria (%) | No positive for HIV (%) |
|-----------|-------------|-------------------------|-----------------------------|-------------------------|
| 18-24 | 20 | 1(5) | 3(15) | 0(0) |
| 25-30 | 37 | 2(5.4) | 10(27) | 2(5.4) |
| 31-36 | 29 | 4(13.8) | 7(24.1) | 0(0) |
| 37-42 | 13 | 0(0) | 3(23) | 0(0) |
| 43-49 | 1 | 0(0) | 0(0) | 0(0) |

Discussion

The demographics of the subjects is shown in Table 1. The mean age of the 100 pregnant women was 30 ± 0.63 years. The highest number of the respondents (37%) are between the ages of 25 – 30, followed by 31 - 36 and the least 1%, between 43 – 49 years. This is most likely because at 43 - 49 years, most women would be approaching menopause.

The respondents had primary education as the least qualification (14%) while the highest number had secondary education (49%), followed by tertiary education (37%). With their level of education, the subjects would have known the risks associated with the infections under study. Most of the subjects were married (88%). The unmarried women represent 11% of the subjects in this study and 1% of the participants was cohabiting. These are at risk of HBV and HIV since they may end up with multiple partners.

Petty trading is observed as the occupation with the highest frequency (47%) of respondents, followed by the 25% self - employed (such as tailoring, hairdressing, etc.). The unemployed, student and farming attendees were 7% respectively while the Clergy is 1%. The unemployed group have been shown to have the highest seroprevalence of HIV and HBV (Ikeako *et al.*, 2014).

The highest number (Sixty one percent) of the women were in the second trimester of pregnancy followed by 23% in the first trimester while 16% were in the third trimester. The multigravid women accounted for 65% while the primigravid women represent 31%.

In this research, the prevalence of malaria, Hepatitis B and HIV diseases among gravid women attending antenatal clinic at Eku Baptist Government Hospital are 23%, 7% and 2% respectively. There was no malaria /HIV coinfection and no HBV/HIV coinfection. However, a 2% coinfection of HBsAg and malaria was observed (Table 2). This result differs from that obtained by Helegbe *et al.* (2018) who recorded a prevalence of 11.6% for malaria, 4.2% for HBV infection and 1.0 % for

HIV mono infection; and HBsAg/Malaria coinfection of 0.7% among pregnant women attending antenatal clinics (ANC) in the Tamale Metropolis, Ghana. In a previous research, a prevalence of 13%, 3% and 1% were reported for malaria, hepatitis B virus and malaria/hepatitis B coinfection respectively, in pregnant women, in Osogbo, Nigeria (Adeleke *et al.*, 2013).

The prevalence of HBsAg in this research is 7% (Fig. 1). This is the same with the 7% seroprevalence reported among antenatal attendees at the University of Abuja Teaching Hospital (Nongo *et al.*, 2016). Recently, Mustapha *et al.* (2020) reported a 6.7% prevalence among antenatal clinic attendees in Gamawa Local Government Area of Bauchi State, Nigeria. The findings are also comparable to the 6% among antenatal clinic attendees at Nnewi as reported by Oluboyo *et al.* (2014). Recently, Brittaye *et al.* (2019) reported a 9.2% prevalence among antenatal subjects at the Edward Francis Small Teaching Hospital, Banjul, The Gambia. In contrast, previous reports have shown lower HBsAg prevalence. Kemebradikumo and Isa (2013) reported a prevalence rate of 3.67% among antenatal attendees at a tertiary health facility in Bayelsa, 2.2% was reported by Oladeinde *et al.* (2013) in Benin City and 2.5% reported by Ugbebor *et al.* (2011). Studies from the Middle East reported 4.3% of HBV in Jordan (Batayneh and Bdour, 2012).

The highest prevalence of 13.8% for HBsAg was observed among the age group 31 – 36 years followed by 25-30 years (Table 2). This is similar, to an extent to the age group of 25 -29 years, reported by Nongo *et al.* (2016) and Anaedobe *et al.* (2015). The members of this group are sexually active and mostly multigravid and as such more exposed to risk factors, which may account for the high prevalence. However, a more recent report shows that the age group of 46 – 55 years had the highest prevalence of HBsAg (Mac *et al.*, 2019). From the findings in this work, the least prevalence in age group 18 -24 years could be attributed to improved education and immunization programs for children which most parents have keyed into.

This research has shown the prevalence rate of Human Immunodeficiency virus (HIV) as 2%. This complies with the drop in HIV prevalence in West Africa from 4.3% to 2.9% overall as reported by Eaton *et al.* (2014). In the same vein, a study in Port Harcourt, Rivers state, Nigeria recorded a prevalence of 3.0% among expectant mothers attending antenatal clinic (Okereantugba *et al.*, 2015). Recently, a 5.2% prevalence was reported in South Western Nigeria (Atilola *et al.*, 2018). On the contrary, higher prevalence rates have been reported. A study in Jos, Plateau state, Nigeria among 216 antenatal clinic participants observed a prevalence of 8.3% (Anyaka *et al.*, 2016). In South Western Nigeria, a recent study reveals HIV prevalence 26.4% in a cohort of antenatal attendees of a tertiary hospital in Ibadan (Okonko *et al.*, 2019), with the age group 25 -31 showing the highest prevalence of 37%. The result of this research also reveals that this age group are the ones infected with the disease (Table 3). This agrees with previous studies which reveal that women in their sexually active years and at the peak of their child bearing years are the most affected (Duru *et al.*, 2009).

Malaria prevalence in this study is 23%. Our finding is consistent with that previously reported in Mozambique by Saute *et al.* (2002). Similarly, Nduka and Wogu (2012) reported 26% malaria prevalence among antenatal subjects in Port Harcourt, Rivers State, Nigeria. On the contrary, a prevalence rate of 7.7% was reported in Lagos by Agomo *et al.* (2009). Frank *et al.* (2016) reported a high prevalence of 65% among Pregnant Women Attending Three Selected Health Centres in Ideato South Local Government Area, Imo State. A similar result was reported recently by Onyemaechi and Malann (2020) in the Lugbe and Gosa areas of Abuja, Nigeria. Their study revealed a high incidence rate of malaria in pregnancy (70.5%). In a recent research, the prevalence of asymptomatic malaria infection was reported as 5.7% among antenatal attendees in North-Shoa, Ethiopia (Feleke *et al.*, 2020). The highest prevalence (27%) of malaria in this study is among the age group of 25 – 30, followed by 24% of the age group, 31 – 36 (Table 2). This result is consistent with report by Erhabor *et al.* (2019), who reported a prevalence of 36.7%

among this age group; and 73% reported among this age group by Frank and Colleagues (2016).

The HBsAg/malaria coinfection prevalence in this research is similar to the work of Anabire *et al.* (2019) who reported a prevalence of 1.9% HBsAg/malaria coinfection in pregnant women attending antenatal clinic, in northern Ghana. Similarly, in Nigeria, Abah *et al.* (2019) reported HBsAg/malaria coinfection of 3% among antenatal women in Federal Medical Centre, Owerri. On the contrary, a 14% coinfection of HBsAg/malaria has been reported among pregnant women in Northern Ghana. Their findings suggest that chronic hepatitis *B and malaria* could exacerbate inflammatory cytokine responses and increase susceptibility to liver injury among pregnant women in endemic settings. It has been reported that malaria and hepatitis B virus (HBV) infections are endemic in most parts of Nigeria (Lavanchy, 2004). Cases of gestational diabetes, antepartum haemorrhage and preterm delivery have been observed to be more frequent in pregnant women with chronic hepatitis B (Jonas, 2009; Tse *et al.*, 2005).

Nigeria is one of the countries with severe cases of maternal mortality in the world (Nwokocha, 2012). The result of this research has shown that hepatitis B, HIV and malaria remain a threat to the lives of pregnant women. HBsAg as assessed in this work is a marker to liver infection which may be transmitted to the unborn child. The low rate of promiscuity in this study area may be responsible for the low HIV prevalence rate recorded in this research. The HIV prevalence, though low in this research, still gives room for fear of further spread to vulnerable individuals. A study has shown that asymptomatic malaria is prevalent in pregnant women and it affects their haemoglobin level (Feleke *et al.*, 2020). It is good that most women in the Niger Delta region attend antenatal clinics during pregnancy thereby having access to skilled health professionals, a strategy for reducing maternal and newborn morbidity and mortality. Globally in 2019, 81 per cent of births were assisted by skilled health professionals (UNICEF, 2020). The inclusion of skilled birth attendance in the Sustainable Development Goal (SDG) Framework is expected to spur efforts to reach

universal coverage with skilled delivery care by 2030 and hold us all to account for progress (UNICEF, 2019).

Conclusion

This study shows that HBsAg, HIV and malaria infections are still a health threat to pregnant women in Eku and her surrounding towns, and Nigeria in general. The age group 18-36 years were most infected while the age group 43-49 years were not infected with any of the disease. This report calls urgently for effective health interventions and health education at the national and state levels on prevention of mother-to-child transmission of HBV and HIV. For malaria which is endemic in Sub Saharan Africa, regular periodic prophylaxis should be incorporated in antenatal clinics to resolve asymptomatic infections and save the lives of the women.

Pregnant women should be mandatorily and routinely screened for hepatitis B, HIV and malaria infections as part of antenatal care services at their booking. Those who test positive to any of the infections should be treated and monitored (as the case may be), at subsequent antenatal visits. It is recommended that immunization programs for women of child-bearing age is reintroduced to prevent mother- to-child transmission of HBsAg. Neonates of infected mothers should be given a dose of Hepatitis B vaccine and Hepatitis B Immunoglobulin within 12 hours after being born, to protect them from the infection. HBsAg positive subjects should be tested for HBeAg/HBcAg and treated appropriately. While HIV-positive subjects should be counselled and referred to centres where their CD-4 cells can be counted for appropriate treatment. They should also be monitored to avoid mother to child transmission during delivery and educated to avoid breastfeeding the infant and how to care for the baby to avoid transmission of infection.

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