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SEROLOGICAL DETECTION OF ZIKA VIRUS INFECTION AMONG HIV INFECTED PREGNANT WOMEN IN DUTSE, JIGAWA STATE, NIGERIA

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ABSTRACT
Zika virus infection is one of the emerging often neglected viral infections in Africa. A study to determine the seroprevalence of Zika Virus (ZIKV) infection among HIV Positive Pregnant Women in Dutse, Jigawa state, Nigeria was conducted. A total of 89 HIV positive pregnant women were randomly selected for the study. Samples were collected between February, 2020 and March, 2021 from Rashid Shekoni specialist Hospital and Dutse General Hospital respectively. A standard rapid immuno-chromatographic technique that is; rapid ELISA was used to screen all the sera for IgM antibodies to Zika virus in addition to haematological analysis using Automated Haematological Analyser (Sysmex, KX-21N). Result has shown that out of the 89 participants enrolled, 4 (4.5%) of them were infected with Zika virus. Seventy-five Percent (75%) of the study participants had PCV below 30% and Lymphocytes below 20%. It was also found that, 75% of the participants that have Zika virus infection were in their first trimester of pregnancy. It can be concluded that, there is a low sero-prevalence of ZIKV infection in the study population. However, the need for public health authorities to provide quick response for the prevention of transmission of Zika virus in the state cannot be overemphasized. Hospital delivery among HIV positive pregnant women co-infected with Zika virus should be encouraged in Jigawa State to prevent vertical transmission to their babies.
Keywords: Zika Virus (ZIKV), Human Immunodeficiency Virus (HIV), Prevalence, Jigawa.

INTRODUCTION

Zika virus (ZIKV) is a mosquito – borne virus associated with neurological disorders such as Guillain –Barre Syndrome and microcephaly (Oyarzuri–Arrau *et al.,* 2020). It is an emerging mosquito borne virus that was first identified in Uganda in 1947 in Rhesus monkeys through a monitoring network of yellow fever. It was subsequently identified in humans in 1952 in Uganda and the United Republic of Tanzania, it was later identified in Humans in 1968 for the first time in Nigeria (Haddow *et al.,* 2012).

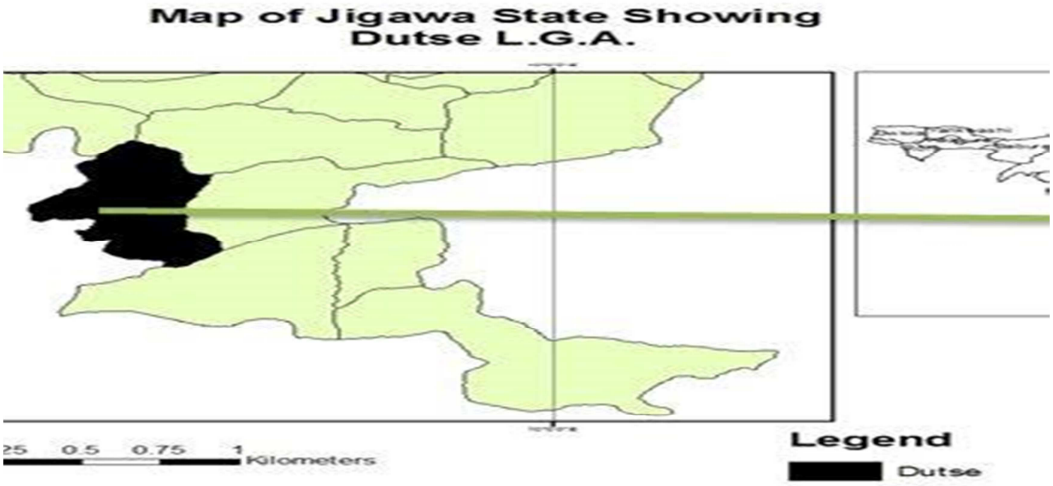
A unique characteristic of ZIKV is its diversity in terms of transmission. Infection is primarily from mosquito bites but can also occur by sexual intercourse, blood transfusion, accidental blood exposure, transplacentally, and possibly via breast milk (Siqueira *et al.,* 2016*;* CDC, 2019). ZIKV can be spread through mosquito bites from Aedes spp., (*Aedes aegypti* and *Aedes albopictus)* (Alonso-Palomares *et al.,* 2019). During pregnancy, ZIKV infection may induce congenital Zika Syndrome (CZS) in foetuses and new-borns (Schwartz *et al.,* 2017). Identification of effective strategies to prevent ZIKV transmission, especially to pregnant women is an obvious priority. Since there is no vaccine or specific treatment for ZIKV, prevention and education are key factors in controlling and combating this infection (Petersen *et al.,* 2016; Thomas *et al.,* 2016).

ZIKV complications associated with HIV positive pregnant mothers are rarely investigated during Antenatal Care in order to attain management and to prevent vertical transmission. This study was therefore, carried out to determine the relationship between HIV infection and ZIKV prevalence in pregnant mothers.

MATERIALS AND METHODS

Study Design and Study Area

This was a hospital based study conducted between February, 2020 and March, 2021, at Dutse General Hospital and Rasheed Shekoni Teaching Hospital both of which are located in Dutse Jigawa state Nigeria. Dutse is located on Latitude 11.7023^0 N, and Longitude 9.3340^0 E. General Hospital Dutse is located along Sani Abacha way Kiyawa road opposite Kandahar (old Government House) and Rasheed Shekoni Teaching Hospital is located along Sani Abacha Way, Kiyawa road in a village called Danmasara, a little South from the state Headquarter.



Source: GIS Lab Federal University Dutse (FUD, 2019).

Study Population and Sample Collection

The study population comprised of pregnant mothers living with HIV attending Highly Active Antiretroviral Treatments (HAART) clinics and Antenatal Clinics (ANC) in Dutse General Hospital and Rasheed Shekoni Teaching Hospital. Eighty-nine (89) participants were randomly selected based on Random Sampling method. Socio-demographic data were collected using a structured questionnaire which includes information on pregnancy, knowledge of Zika virus risk factors and other related data such as Haemoglobin (HB) count, Packed Cell Volume (PCV), White Blood Cells (WBC) and Lymphocytes count.

About 5ml of venous blood was drawn aseptically with the help of sterile syringe and transferred into appropriately labelled screw capped plain tubes. Two millilitres (2ml) of the sample was then transferred into EDTA bottle for the haematological analysis using the Automated Haematological Analyser (Sysmex, KX-21N).

Laboratory Analysis

ELISA Procedure (IgM and IgG) for the Detection of ZIKV Infection

In order to determine the prevalence of ZIKV infection, general screening was carried out for all the HIV positive pregnant mothers using IgM ELISA Test kit from (Melsin Medical Co., limited CAT. NO. 1881). The bench was cleansed using paper towel before placing the materials. The cassette/micro Elisa strip plate was placed on the bench, the location of positive and negative controls of the sample on the plate were marked. Fifty (50) µL of the positive and negative control reagents were added to the positive and negative control wells respectively. Ten (10) µL of the patient's serum was pipetted using micropipette and added to the corresponding labelled sample wells on the micro-ELISA strip plate. 40µl of the Diluent Reagent were again added to the sample well containing the sample. A 100µl of the HRP-Conjugate reagent was then added to each well, covered with an adhesive strip, and then finally incubated at 37^0C for 60 minutes. The strip plate was then decanted and washed by pipetting and filling each well with 400µl of the wash solution. The washing procedure was repeated 4 times. After the last wash, the strip plate was blotted against a clean cellulose paper. Fifty 50µl of the chromogen solution A and chromogen solution B were added to each sample well. The setup was then gently mixed and incubated at 37^0C for 15 minutes. A 50µl of stop solution was added to each well.

A colour change was then finally observed from blue to yellow in positive samples. Finally, the Optical Density (OD) was measured at 450nm using an ELISA Microplate Reader. An O.D. of greater or equals 0.162 was considered positive.

Full Blood Count (FBC)

Haematological Parameters of the study participants were assessed using full blood count with the aid of automated blood analyser (KX-

21N) as described by the manufacturer (Sysmex Corporation, Japan).

Statistical Analysis

Data generated were subjected to chi-square test using statistical package of social sciences (IBM SPSS statistics 23, IBM Corporation and Armonk, N. Y, USA).

RESULTS

The result of the study revealed that 4 (4.9%) of the pregnant women studied had ZIKV infection and there was no significant difference in the prevalent of the infection among the two different Hospitals studied (P=0.305) (Table 1).

Table 1: Prevalence of Zika Virus among pregnant women

Facility Attended	No Examined	No Negative (%)	No Positive (%)	P-value
Dutse General Hospital	44	45 (50.6)	1 (1.1)	**0.350**
Rashid Shekoni Teaching Hospital	45	40 (44.9)	3 (3.4)	
Total		**85 (95.5)**	**4 (4.5)**	

From the table below results showed 75% of the infected women had PCV level below 30%. The result also indicated that 75% of the infected women had less than 20% Lymphocytes counts, and 100% of the women who tested positive had Haemoglobin less than 12%.

Table 2: Prevalence of Zika Virus based on haematological profile of the participants

Parameters	**ZIKV Infection Status** (IgM Assay) Negative (%)	Positive (%)	P-value
PCV (%)	63 (71.6)	3 (3.4)	**0.197**
<33			
33- 38	18 (20.5)	0 (0.0)	
>38	3 (3.4)	1 (1.1)	
Total	**84 (95.5)**	**4 (4.5)**	
Lymphocytes (%)			
<20	14 (15.7)	3 (3.4)	**0.021***
20-40	32 (36.0)	0 (0.0)	
>40%	39 (43.8)	1 (1.1)	
Total	**85 (95.5)**	**4 (4.5)**	
Haemoglobin (g/dL)			
<12	84 (94.4)	4 (4.5)	**1.00**
12.1-15.1	1 (1.1)	0 (0.0)	
Total	**85 (95.5)**	**4 (4.5)**	

Table 3 contain data indicating result based on risk factors that predispose women to Zika infection, data showed no association between presence of water bodies, use of sharp object and improper dumping of waste, but 75% (3 cases) of the positive cases were indicated to be within first trimester of pregnancy.

Table 4 indicates relationship between socio-demographic characteristics and Zika infection. From the table below it is indicated that 100% of the positive cases had lower educational level ranging from informal (1), primary (1), and secondary education (2), while based on age distribution, the data shows that women with age below 30years are at high risk of Zika infection.

Table 3: Prevalence of virus based on possible risk factors

Risk factors	ZIKV Infection Status (IgM Assay) Negative (%)	Positive (%)	P-value
Stagnant water near home			
Yes	36 (40.4)	1 (1.1)	0.638
No	49 (55.1)	3 (3.4)	
Total	**85(95.5)**	**4 (4.5)**	
Dumping Site Near Home			
Yes	33 (37.1)	1 (1.1)	1.00
No	52 (58.4)	3 (3.4)	
Total	**85 (95.5)**	**4 (4.5)**	
Use of Sharp Objects			
Yes	3 (3.4)	0 (0.0)	1.00
No	82 (92.1)	4 (4.5)	
Total	**85 (95.5)**	**4 (4.5)**	
Trimester			
1st	32 (36.0)	3 (3.4)	0.380
2nd	26 (29.2)	1 (1.1)	
3rd	27 (30.3)	0 (0.0)	
Total	**85 (95.5)**	**4 (4.5)**	

Table 4: Prevalence of Virus based on Socio-demographic status of the participants

Demographic factors	ZIKV Infection Status (IgM assay) Negative (%)	Positive (%)	P-value
Educational Status			
Degree	3(3.4)	0(0.0)	0.819
Diploma	9(10.1)	0(0.0)	
Non formal	25(28.1)	1(1.1)	
Primary	29(32.6)	1(1.1)	
Secondary	19(21.3)	2(2.2)	
Total	85(95.5)	4(4.5)	
Age Group (Years)			
18-25	22(24.7)	2(2.2)	0.293
>25	63(70.8)	2(2.2)	
Total	85(95.5)	4(4.5)	

DISCUSSION

The ELISA result from table 1 above revealed the prevalence of Zika IgM antibodies in the sera of 4 participants (4.5%) which account for approximately one out of every 22 pregnant infected women. This result varies with the one obtained by Anejo-Okopi *et al.* (2020) who reported the sero-prevalence rate of 20% among HIV positive pregnant women in Jos, Nigeria. Sherman *et al.* (2018) reported 12.3% sero-prevalence rate of Zika virus antibodies in 236 samples collected and analysed from HIV/HBV co-infected individuals in Accra, Ghana using ELISA technique. In all the respondents that were found positive to Zika antibodies in this study, low CD4, PCV, Haemoglobin as well as white blood cells were recorded which could be as a result of HIV co-infection. The differences in the data gathered in this research and that of other researchers can be attributed to so many factors such as: differences in temperature and geographical locations (Jos, Dutse and Accra), strain of the virus, travel history of the respondents, hygienic practices or even the respondent's Anti-Retroviral Therapy (ART) combinations. The hot temperature prevailing in Dutse is enough to inactivate most enveloped viruses (to which Zika virus happens to be one of them), because they are more temperature sensitive than naked viruses (Howie *et al.,* 2010). However, the result obtained in this study is in agreement with the findings of Mathe' *et al.* (2018) who reported 5% seroprevalence of Zika IgG antibodies in Cameroun.

In this study, it was gathered that there is higher occurrence of Zika antibodies from the sera of women living in rural areas than city/urban areas. This could be due to high outdoor activities during farming and household chores. Also, by nature of the rural environment, little or no attention is given to environmental sanitation. This is worrisome as both HIV and Zika can be transmitted vertically.

Globally it is believed that many socio-demographic factors are associated with so

many infectious diseases contraction and spread (Amugsi *et al.,* 2015). In this research work it was observed and found that low educational background is directly related to ZIKV infection as seen in table 4 where the data indicated that out of the 4 tested positive women 1 had non-formal education, 1 attended primary only and another 2 attended secondary school only which cumulatively shows that all the positive tested subjects with educational background not higher than secondary education.

On the age however, the results revealed that 22 of the HIV-infected pregnant women representing 24.7% were within the age of 18 – 25, while 63 of the women representing 70.8% were above 25 years of age. Furthermore, the results revealed that 2 of the HIV-infected pregnant women representing 2.2% were tested ZIKV-negative, and also 2 women representing 2.2% were ZIKV-positive. The overall results on age revealed ZIKV has low prevalence among HIV-positive women above 25 years (>25).

CONCLUSION

In this study it can be concluded that there is low seroprevalence of ZIKV infection in the sera of pregnant mothers living with HIV attending secondary health facilities in Dutse, Jigawa state. Therefore, HIV positive pregnant women co- infected with ZIKV should deliver at hospital and also to adopt personal protection measures to avoid mosquito bites and subsequent transmission of the virus to uninfected individuals.

Akcnowlegdements

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Conflict of Interest

The authors declare that no conflict of interest exists.

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