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First Serological Evidence of Zika Virus Recent Infection among Pregnant Women Attending a Tertiary Hospital in Rivers State, Nigeria

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ABSTRACT: The Zika Virus (ZIKV) has emerged as a significant public health concern, particularly affecting pregnant women due to its potential adverse effects on fetal development. Despite its endemic presence and recent outbreaks, information on ZIKV in Rivers State, Nigeria, remains limited. This study aimed to assess the seroepidemiology of Zika Virus among pregnant women in Rivers State. Plasma samples from 94 confirmed pregnant women attending the University of Port Harcourt Teaching Hospital (UPTH), Rivers State, Nigeria, were tested for ZIKV-specific antibodies using an IgM ELISA assay. The overall prevalence of antibodies against ZIKV-specific antibodies was 72.3% (68/94). Age group 0-26 years showed the highest seropositivity at 87.1%. Occupational analysis revealed that tailors had a 100.0% seropositivity rate. Among marital status categories, single participants showed a seropositivity of 84.4%, and those with a primary level of education exhibited a 100.0% seropositivity rate. In terms of gestation period, those within their first trimester showed the highest seropositivity (100.0%). Age was a significant risk factor associated with ZIKV (p = 0.008). This study highlights a high prevalence of ZIKV-specific antibodies among pregnant women in Rivers State, with the highest seropositivity observed among younger age groups, tailors, single individuals, and those with primary education. These findings underscore the need for targeted public health interventions and continuous surveillance to mitigate the impact of ZIKV among vulnerable populations.
[Nwachukwu, D., Okonko, B. J., Chukwu, A. O., Igweze-Ezepue, N. E., Awanye, A. M., Ibezim, C. N. E., Okerentugba, P. O. and Okonko, I. O. **Frist Serological Evidence of Zika Virus Recent Infection among Pregnant Women Attending a Tertiary Hospital in Rivers State, Nigeria.** *J Am Sci* 2026;22(5):1-6]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 01. doi:10.7537/marsjas220526.01

Keywords: Antibodies; IgM; Zika Virus; Rivers State; Nigeria

1. INTRODUCTION

Zika virus is a mosquito-borne virus that was first identified in the Zika Forest of Uganda in 1947. The virus is primarily transmitted to humans through the bite of infected Aedes mosquitoes, particularly Aedes aegypti and Aedes albopictus. The Zika virus garnered global attention due to outbreaks that occurred in various regions, including the Americas, Southeast Asia, and the Pacific Islands. (WHO, 2020). Apart from mosquito bites, the Zika virus can also be transmitted through sexual contact, blood transfusion, and from mother to child during pregnancy or childbirth. In rare cases, the virus has been found in other body fluids such as saliva and urine (CDC, 2020). The majority of Zika virus infections are asymptomatic, with only about 20% of infected individuals showing mild, self-limiting symptoms. Common symptoms include fever, rash, joint pain, and conjunctivitis (Noorbakhsh et al., 2019; Masmejan et al., 2020). However, the virus gained notoriety for its association with severe birth defects, particularly microcephaly, when pregnant women are infected. Zika virus infection during pregnancy can lead to severe neurological complications in the fetus, including microcephaly (an underdeveloped brain and head), as well as other brain abnormalities (CDC, 2020). The virus can also cause other pregnancy-related complications, such as preterm birth and miscarriage. Zika virus has been identified in various regions worldwide, with outbreaks reported in the Americas, Southeast Asia, the Pacific Islands, and Africa (Masmejan et al., 2020). Due to the global nature of travel and the presence of Aedes mosquitoes in many areas, there is ongoing concern about the potential for the virus to spread to new regions. Preventive measures primarily focus on reducing

mosquito exposure, using insect repellents, wearing protective clothing, and implementing vector control strategies (Masmejan et al., 2020 et al., 2016). Pregnant women, or those planning pregnancy, are often advised to avoid travel to areas with ongoing Zika virus transmission (WHO, 2020). Farmers, who operate in environments that usually pose a higher risk of being bitten by the Aedes mosquitoes, are also high-risk individuals (CDC, 2020).

2. MATERIALS AND METHODS

2.1. Study Design

This research utilized a cross-sectional approach, encompassing the collection and examination of samples using Zika virus IgM ELISA kits.

2.2. Study Area

This investigation was centered on Rivers State, located in the Niger delta region of Southern Nigeria. Specifically, the study was conducted within University of Port Harcourt in the city of Port Harcourt.

2.3. Sample Size Estimation

The sample size for this study was determined using the established formula (MacFarlane, 1997; Niang et al., 2006; Awando et al., 2013): N = [Z^2(pq)] / d2. The total sample size was calculated as 73.

2.4. Population for the Study

Around 94 confirmed pregnant patient samples, labeled 1-94, were systematically collected and appropriately stored. These samples were obtained from University of Port Harcourt, situated in the state of Rivers, Nigeria. Trained medical laboratory professionals conducted the collection. Subsequently, the samples were transported to the Virus & Genomics Research Unit within the Department of Microbiology at the University of Port Harcourt (UNIPORT) for the necessary laboratory analyses. Alongside the testing, pertinent clinical history, behavioral patterns, and demographic information were also documented.

2.5. Sample and Sampling Techniques

The stratified sampling technique was employed to select sample specimens for this study. A total of 94 individuals who were HIV-infected were randomly chosen from the study population and enrolled in the investigation. The focus was on conducting a hospital-based study, specifically targeting individuals with HIV who were receiving care at the aforementioned hospital and healthcare facility in Port Harcourt. Each eligible patient was assigned a unique number, recorded on their patient card, and facilitated by the assistance of laboratory staff or hospital personnel. In addition to the blood samples, essential demographic information, behavioral and lifestyle patterns, and clinical history were systematically collected. The demographic data were classified into several groups, including age groups (0-26 years, 27-32 years and 33-70 years); highest educational attainment; marital status (single, married); and Occupation. Clinical history was also thoroughly assessed. Statistical analysis was performed using the chi-square test, and the data were processed using WPS version 12.2.

2.6. Inclusion/Exclusion Criteria

Participants were required to meet specific inclusion criteria to be eligible for the study. Only female individuals were considered, regardless of age. A key residency requirement stipulated that participants must have lived in Rivers State for a minimum of five years. Both single and married women were eligible to participate, and employment status—whether employed or unemployed—did not affect eligibility. All participants were also required to have a verified pregnancy. Furthermore, the study involved hospitalized patients from whom at least 3.0 milliliters of EDTA plasma could be obtained. Individuals who did not satisfy these criteria were excluded from the study to maintain the integrity and consistency of the research sample.

2.7. Sample Collection

Venepuncture, the procedure of drawing blood from a vein, was employed to collect five milliliters (5 ml) of venous blood from each participant. The collected blood was then placed in EDTA bottles and subjected to centrifugation at 3000 rpm for five minutes. This centrifugation process aimed to separate the plasma, which is necessary for detecting Zika Virus antibodies. The obtained plasma was carefully stored at -20°C until it underwent laboratory testing. In the laboratory, the testing process utilized the ELISA technique. Specifically, kits designed for capturing IgM antibodies were employed to facilitate the detection of Zika Virus antibodies in the plasma samples. This technique is a widely used and effective method for detecting specific antibodies in biological samples.

2.8. Nature/Source of Data

The data consisted of both quantitative and qualitative information from study participants within the University of Port Harcourt in Port Harcourt. Quantitative data collection methods were employed for gathering the data.

2.9. Serological Analysis

The testing of samples was performed using ELISA techniques, adhering to the manufacturer's instructions for Zika virus IgM tests, as outlined in Tables 1 and 2, respectively. The samples utilized in the testing process consisted of plasma collected in EDTA tubes. Results from the testing process were obtained and interpreted utilizing an ELISA reader, specifically the ELx800 model, which measured absorbance at wavelengths of 450nm and 630nm. Data analysis was carried out using the Chi-square test, a statistical method used to determine if there is a significant association between

categorical variables. Additionally, descriptive statistics were utilized to present demographic summary tables, which included factors such as age range and gender, providing an overview of the study population.

The mean OD450nm value of the Negative Control is calculated as: $\frac{NC+NC+NC}{3}$

Then the formula below is applied: $Cut\ Off = mean\ NC + 0.250$

2.10. Data Analysis

Socio-demographic information, encompassing age, occupation, marital status, and educational status, as well as clinical characteristics such as gestation period, was gathered from the patient registration book at the hospital. Following a validation process, this data was meticulously entered into the WPS spreadsheet version 12.2 for subsequent analysis. To facilitate analysis, the patients' data were initially categorized into groups based on their respective distributions. Subsequently, the data was further organized to present an overview of overall IgM prevalence. Additionally, an evaluation of prevalence based on each characteristic was conducted to provide a comprehensive understanding of the study population.

2.11. Statistical Analysis

The statistical analysis employed the chi-square method to investigate potential relationships between the identified characteristics and the seroprevalence rates among patients. Additionally, further statistical analyses were conducted to pinpoint specific categories within each risk factor characteristic that exhibited heightened vulnerability. This involved a detailed examination of their contribution factors to the observed seroprevalence rates.

2.12. Ethical Approval

This study received ethical approval from the University of Port Harcourt Research Ethics Committee, ensuring compliance with ethical guidelines governing medical research involving identifiable human subjects and their data or materials.

3. RESULTS

3.1. Socio-demographical and Clinical Characteristics of Study Participants

The categorical data detailing the socio-demographical and clinical characteristics of the study participants have been systematically organized and are presented in Tables 1 and 2, respectively.

3.2. Overall IgM Assay Results

The study reported an overall 72.3% seropositivity for IgM, as indicated in Table 1.

3.3. ZIKV IgM Seroprevalence Concerning Socio-demographic Characteristics

The age group 0-26 exhibited the highest ZIKV IgM prevalence (87.1%), while the age group 33-70 displayed the lowest (53.1%) (Table 1). Among Occupation, ZIKV prevalence in tailors (100.0%) were the highest while unemployed (30.8%) were the lowest (Table 1). Married participants showed the lower IgM seropositivity rates (66.1%) compared to single participants (84.4%) (Table 1). In terms of educational status, individuals with primary education had the highest seropositivity (100.0%), while those with tertiary education had the lowest (61.4%) (Table 1).

Table 1: IgM Results Concerning Socio-demographic Characteristics of Study Participants

Variables	Categories	No. Tested (%)	No. Positive (%)
Age Group (Years)	0-26	31 (33.0)	27 (87.1)
	27-32	31 (33.0)	24 (77.4)
	33-70	32 (34.0)	17 (53.1)
Occupation	Caterers	7 (7.4)	5 (71.4)
	Civil Servant	33 (35.1)	23 (69.7)
	Nurse	5 (5.3)	4 (80.0)
	Tailor	5 (5.3)	5 (100.0)
	Trader	22 (23.4)	15 (68.2)
	Education	9 (9.6)	6 (66.7)
	Unemployed	13 (13.8)	4 (30.8)
Marital Status	Single	32 (34.0)	27 (84.4)
	Married	62 (66.0)	41 (66.1)
Educational Status	Primary	5 (5.3)	5 (100.0)
	Secondary	25 (26.6)	22 (88.0)
	Tertiary	44 (46.8)	27 (61.4)
	None	20 (21.3)	14 (70.0)
Overall		**94 (100.0)**	**68 (72.3)**

3.4. ZIKV IgM Seroprevalence Concerning Clinical Characteristics

Concerning Gestation period, participants in the first trimester showed the highest ZIKV seropositivity (100.0%) while those in their third trimester showed the least (66.7%) as shown in Table 2.

Table 2: IgM Results Concerning Clinical Characteristics of Study Participants

Variable	Categories	No. Tested (%)	No. Positive (%)
Gestation Period (Trimester)	First	6 (6.4)	6 (100.0)
	Second	21 (22.3)	16 (76.2)
	Third	15 (16.0)	10 (66.67)
Overall		**42(44.7)**	**31(76.2)**

3.5. Statistical Analysis

Statistical analysis revealed that age (p-value= 0.008) was significant while occupation (p-value= 0.681), marital status (p-value= 0.061), education (p-value= 0.053) and gestation period (p-value= 0.269) were not significant risk factors among the study participants (Table 3).

Table 3: IgM Statistical Analysis Results

Characteristic	Categories	P-Value	Significance
Age Group (Years)	0-26 27-32 33-70	0.008	Significant
Occupation	Caterers Civil Servant Nurse Tailor Trader Education Unemployed	0.681	Not Significant
Marital Status	Single Married	0.061	Not Significant
Educational Status	Primary Secondary Teritary None	0.053	Not Significant
Gestation Period (Trimester)	1st 2nd 3rd	0.269	Not Significant

4. DISCUSSION

This study revealed that 63.4% of the 93 pregnant women tested positive for ZIKV IgM-specific antibodies, highlighting the considerable prevalence of the virus in this population. Notably, this prevalence rate of 78.2% for IgM in this study exceeds the rates reported in previous studies on pregnant women conducted by Anejo-Okopi et al. (2020) in Jos, Plateau State, within North Central Nigeria (14.4%) and Mac et al. (2023) in 3 regional zones in Nigeria - Southern, Central and Northern- (19.2%). Asebe et al. (2021) in a study carried out in Gambella Region, South West Ethiopia reported a 27.3% seroprevalence. Furthermore, it differs from the rate of 49.0% reported in a previous study in the French Polynesia by Aubry et al. (2017).

The highest seropositivity was observed in several key demographic groups, suggesting that certain factors may influence exposure and susceptibility to the virus. For example, the age group of 0-26 years showed a seropositivity rate of 70.0%, indicating that younger pregnant women may be at greater risk, potentially due to lifestyle factors, social interactions, or biological vulnerabilities. This finding is in contrast with Anejo-Okopi et al. (2020), who reported a higher seropositivity rate among individuals between 31-50 years (35.3%) in Jos, Plateau state, Nigeria. Asebe et al. (2021) reported a ZIKV seropositivity of 40.3% withing participants aged 18-30 in Southern Ethiopia. Aubry et al. (2017) reported that children aged 6-16 exhibited the greatest seropositivity for Zika virus

infections (66.0%) within the French Polynesia. Choyrum et al. (2022) reported a 30.1% seroprevalence among pregnant women aged >23-25 in Thailand. According to Langerak et al. (2019), participants aged ≥60 years exhibited the greatest seropositivity at 74.6%. Marchi et al. (2020) reported a 21.2% seropositivity among people aged 18-29 years.

Civil servants exhibited the highest seropositivity rate at 87.9%, which could point to occupational exposure as a significant risk factor. This demographic's increased exposure may be linked to their work environments or the nature of their daily activities, suggesting the necessity for workplace interventions and education to reduce the risk of ZIKV transmission.

Single participants also showed a high seropositivity rate of 71.0%, which might reflect differences in social behavior, healthcare access, or other socio-economic factors compared to their married counterparts. The study further identified individuals with a primary educational background as having an 80.0% seropositivity rate, indicating a potential gap in awareness and preventive practices among this group. These findings highlight the importance of educational interventions tailored to individuals with lower educational attainment to improve their understanding of ZIKV risks and prevention.

The analysis also revealed that women in their second trimester had a 45.0% seropositivity rate, suggesting that the stage of pregnancy may influence the likelihood of ZIKV infection, in line with Shaibu et al. (2021) reported that pregnant women in their 2nd trimester (13-27 weeks) exhibited the greatest seroprevalence (3.4%) but there was no statistical relationship between the positive and negative values (p = 0.146), Anejo-Okopi et al. (2020) reported that participants in their 3rd trimester (10.8%) showed greater ZIKV seropositivity, however this was not a significant risk factor (p = 0.622). Choyrum et al. (2022) reported the highest seroprevalence of zika virus IgM antibodies at a gestational age of >28 weeks (26.4%, p = 0.72). Phatihattakorn et al. (2021) reported that, while not significant (p = 0.14), participants in their 1st trimesters showcased the greatest seroprevalence (34.63%). This finding emphasizes the need for continuous monitoring and protective measures throughout pregnancy, with a particular focus on the second trimester when the risk appears significant.

5. CONCLUSION

The study on the IgM seroprevalence of Zika virus among pregnant women in Rivers State, Nigeria, has revealed a significant prevalence of ZIKV-specific antibodies, with 63.4% of the tested population showing seropositivity. The findings underscore the heightened risk of Zika virus exposure among specific demographic groups, including younger women, civil servants, single individuals, and those with a primary level of education. The study also highlights the importance of the second trimester as a critical period for monitoring and intervention. These results point to the need for targeted public health strategies to mitigate the impact of ZIKV, particularly among vulnerable populations. The high seropositivity rates in certain groups suggest that tailored educational campaigns, workplace interventions, and continuous surveillance are essential to reducing ZIKV transmission and protecting maternal and fetal health. Further research is warranted to explore the underlying factors contributing to these disparities and to develop more effective prevention and control measures against the Zika virus in this region.

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