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INVESTIGATING ANTIBODY RESPONSES TO ZIKA VIRUS IN PREGNANT WOMEN IN SOUTHWEST NIGERIA

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ABSTRACT

Zika virus (ZIKV), a member of the flavivirus family is primarily spread through mosquito bites.. In recent times, the virus has been associated with fetal anomalies and pregnancy complications due to its ability to cross the placental barrier and infect the developing fetus. Nigeria is at high risk of Zika virus transmission due to the presence of mosquitoes that can transmit the virus. The study recruited 92 pregnant women attending a tertiary hospital in Southwest Nigeria from whom blood samples were obtained and analyzed for Zika virus antibodies using an enzyme-linked immunoassay. Thirty-one samples were positive for ZIKV IgG, and 8 samples were positive for ZIKV IgM, with only 4 samples testing positive for both IgG and IgM. Demographic and medical history information was gathered using structured questionnaires to identify potential risk factors. Participants over the age of 30, those living in urban areas, and Unemployed participants had the highest prevalence for both IgM and IgG. Participants with tertiary education showed a higher prevalence rate of IgM, and those with primary education had the highest prevalence of IgG. Higher prevalence rate of both IgG and IgM was reported in multiparous participants with single pregnancy and in their first trimester. The most reported symptoms of Zika virus infection were headaches, fever, muscle pain and joint pain. This study presents evidence of the presence of Zika virus in pregnant women in the study location, underscoring the significance of including Zika virus testing as part of routine diagnostics during antenatal check-ups.

Keywords: ZIKV; Antibody; Pregnancy; Seroprevalence, IgG, IgM

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INTRODUCTION

Zika virus (ZIKV), a recently re-emerging virus, is transmitted by mosquitoes and belongs to the flavivirus genus (Singh *et al.*,

2018; Noorbakhsh *et al.,* 2019). Zika virus, previously thought to be a benign virus with limited clinical severity, has received a lot of attention in recent years due to a series of outbreaks, particularly in the Western Hemisphere (Bradley & Nagamine, 2017). The virus is antigenically and phylogenetically related to the Spondweii virus and has been linked to a variety of human health complications, including fetal microcephaly, autoimmune disorders such as Guillain-Barre syndrome, and neurological complications (Singh *et al.,* 2018; Noorbakhsh *et al.,* 2019).

ZIKV was discovered in 1947 during a geographic mapping of the yellow fever virus in Uganda, but it remained unknown until a small outbreak occurred on Yap Island in 2007 (Maslow, 2017; Senjuti *et al*., 2021). George W. A. Dick, a Scottish virologist, discovered the virus in the blood of a febrile monkey in Uganda's Zika forest (Dick *et al.,* 1952; White *et al.,* 2016). Mosquito species were first identified as vectors when the same virus was discovered in a pulverized mosquito suspension later in the same area; the first human cases were reported a few years later in 1952 (White *et al*., 2016).

Most people with ZIKV infection are asymptomatic, however typical symptoms include maculopapular rash, fever, myalgia, arthralgia, headache, non-purulent conjunctivitis, and retro-orbital pain (Charrel *et al*., 2016; Vue & Tang, 2021). The virus is transmitted in an urban cycle involving humans and peridomestic or domestic Aedes spp mosquitoes (Aliota *et al*., 2017). The virus is primarily transmitted through the bite of an infected Aedes mosquito, but other routes of transmission have also been reported (Musso & Gubler, 2016; Vue & Tang, 2021). The Zika virus has been reported to spread through sexual contact, breast milk, during pregnancy, and saliva (Malone *et al*., 2016). Although it has been reported that approximately 80% of infected individuals are asymptomatic, this lack of symptoms does not imply protection from infection-related complications such as microcephaly in infants and Guillain-Barre Syndrome in adults (Haby *et al*., 2018).

Due to the fact that there are currently no ZIKV vaccines or treatments available, the best way to avoid Zika virus infection is to avoid mosquito bites (Belaunzarán-Zamudio *et al*., 2021). The Pan American Health Organization predicts that the virus will continue to spread to Aedes mosquito-endemic areas (Aliota *et al*., 2017; Malone *et al*., 2016). Screening for Zika virus, particularly among pregnant women, is now being advocated to prevent the spread of Zika virus infection, and also protect the growing fetus, particularly in areas with high population of its mosquito vectors- *Aedes sp*, potentially resulting in epidemics. Diagnosis can be made through the investigation of antibody responses with the aid of enzyme-linked immunoassay in serum, plasm and urine samples (Shiu *et al*., 2018). Despite recent studies on the ZIKV, little research has been conducted in Nigeria, particularly in areas endemic to the dengue virus, which share vectors with the ZIKV. Research gaps exist in virus surveillance, vector control, and the social factors responsible for local infection transmission. The purpose of this study was to investigate the seroprevalence among pregnant women attending a tertiary hospital in southwest Nigeria.

METHODS

***Ethics approval and consent to participate*:**

The Olabisi Onabanjo University Teaching Hospital Health Research Ethics Committee granted ethical approval to conduct this study (OOUTH/HREC/463/2021AP). All participants provided informed consent in accordance with human experimentation standards and the Helsinki Declaration of 1975, revised in 2000. This was accomplished by the completion of informed consent forms by all participants recruited for the study.

Sample Population and Size

The sample size was calculated using Fischer's formula for cross-sectional study design. Using the 4% reported by Mathé *et al.*,

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Where n = sample size of subjects required for the study

Z-statistic for a level of 95% confidence interval = 1.96

P = Prevalence

d = precision (allowable error) = 5%=0.05

$$\text{Thus } n = \frac{Z^2 (1 - P) P}{(0.05)^2}$$

$$n = \frac{1.96 \text{ X } 1.96 \text{ X } 0.96 \text{ X } 0.04}{0.0025} = 59$$

The sample size was calculated using Fischer's formula for cross-sectional study design to be 59 but increased to 92

Data collection

Participants' socio-demographic (sex, age, educational level, occupation, and residential area) and medical information were collected using structured questionnaires (gravidae, gestational age, ZIKV infection-related symptoms, history of mosquito bites, and history of arboviral infection). Face-to-face interviews were used to collect data.

Sample collection

Using aseptic techniques, Blood samples were gently obtained from participants by a trained phlebotomist and gently displaced in to plain sterile tubes. Each tube was then labeled with the participant's identification number. Serum was then separated from the blood samples by allowing the blood samples to clot at room temperature, before subjecting it to centrifugation at 2500 rpm for 10 min. The obtained serum was thereafter transferred in to cryovials and stored at -20 ° C until laboratory analysis.

Sample analysis

Zika Virus Immunoglobulin M (IgM) and Immunoglobulin G (IgG) Enzyme Linked Immunosorbent Assay (ELISA)

Source of ELISA kit

The VIRCELL Microbiologists Zika ELISA (enzyme-linked immunosorbent assay) IgM

and 4.4% had both ZIKV IgM and IgG (Table 1). Regarding age and residence, participants over 30 years and those living in urban areas had higher seroprevalence rates in all categories. Regarding education, individuals with secondary school education had the highest seroprevalence of 33.3% for ZIKV IgG, while those with tertiary education had higher seroprevalence rates of 9.3% and 7.4% for ZIKV IgM and both IgM and IgG, respectively. Self-employed participants had the highest seroprevalence for IgG, whereas unemployed individuals had the highest rates for the other two categories (Table 2).

Table 1: The prevalence of ZIKV IgM seropositivity in the study population

Status	IgG	IgM	Both IgM & IgG
Positive	31 (33.7%)	8 (8.7%)	4 (4.4%)
Negative	61 (66.3%)	84 (91.3%)	88 (95.6)
Total	92 (100%)	92 (100%)	92(100%)

Footnote: Table 1 shows the prevalence of ZIKV IgM and IgG as well as the Co-occurrence of both antibodies in the study participants.

Table 2: Seroprevalence of ZIKV IgM and socio-demographic variables

Variables	No tested (%)	Seropositive (prevalence)			Significance (p-value)
		IgG	IgM	Both IgM & IgG	
Age					
< 30	44 (47.8%)	11 (25%)	3 (6.8%)	1 (2.3%)	0.5358
≥30-44	48 (52.2%)	20 (41.7%)	5 (10.4%)	3 (6.25)	
Residence					
Rural	25 (28.3%)	8 (32%)	0 (0%)	0 (0%)	0.2323
Urban	67 (71.7%)	23 (34.3%)	8 (11.9%)	4 (6.0%)	
Education					
None	0 (3.3%)	0 (0%)	0 (0%)	0 (0%)	0.8097
Primary	5 (6.1%)	3 (60%)	0 (0%)	0 (0%)	
Secondary	33 (21.7%)	11 (33.3%)	3 (9.1%)	0 (0%)	
Tertiary	54 (68.9%)	17 (31.5%)	5 (9.3%)	4 (7.4%)	
Employment Status					
Employed	30 (32.6%)	9 (30%)	1 (3.3%)	2 (6.7%)	0.2150
Self Employed	59 (64.1%)	21 (35.6%)	6 (10.2%)	1 (1.7%)	
Unemployed	3 (3.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	
Total	**92 (100%)**	**31**	**8**	**4**	

IgG– Immunoglobulin G, IgM—Immunoglobulin M

(catalogue number: M1023) and Zika ELISA IgG (catalogue number: G1023) were used to screen the separated serum samples for the presence of ZIKV IgM and IgG.

Assay procedure

The incubator was initially set to 37°C and all reagents were brought to room temperature of 25°C for about an hour before use without removing the plates from the bag. The plates were removed from their packaging, and the four control wells were determined: two for the cut-off controls and one for each of the negative and positive controls. The control wells were prepared by first adding 100 µl of serum diluent to the corresponding wells, followed by 5 µl of the positive, negative, and cut-off controls. The remaining wells were thereafter filled with 5 µl of the samples and 100 µl of the serum diluent. To achieve a homogeneous mixture, the reagents were mixed with a pipette before shaking the plate for two minutes. Following that, the plates were sealed with a sealing sheet and incubated for 45 minutes at 37°C. After incubation, the seals were removed. The liquid in each well was aspirated, and the wells were washed five times with 0.3 ml of washing solution per well. Any residual liquid in the wells was drained. Each well was immediately filled with 100 µl of the IgM conjugate solution. For another 30 minutes, the plates were covered and incubated at 37°C.

The seals were removed once again after incubation. The liquid in each well was aspirated, and the wells were washed five times with 0.3 ml of washing solution per well. Any residual liquid in the wells was drained. Immediately, 100 µl of the substrate solution was added to each well and incubated at room temperature for 20 minutes, protected from light. Following incubation, 50 µl of the stopping solution was added to each well. The optical densities of the plates were determined using an ELISA plate reader at 450/620 nm within an hour of stopping.

Samples with indexes greater than 11 were considered positive; those with indexes less than 9 were considered negative, and samples with indexes between 9 and 11 were retested.

Statistical Analysis

The questionnaire data and laboratory analysis results were entered into Microsoft Excel and analyzed using GraphPad Prism 5. Tables were used to describe study findings; chi -square and p-values were calculated for statistical significance. The statistical significance level was set at $P \leq 0.05$.

RESULTS

The 92 pregnant women participating in the study were divided into two age groups: under 30 years (47.8%) and 52.2% of 30 years and older (Table 2). The study included more urban residents (71.7%) than rural participants (28.3%), and most of the women had tertiary education (68.1%) and were self-employed (64.9%). Table 2. Nearly all the women were carrying a single baby (94.4%), with the majority being in their second trimester (46.7%) and had already given birth to two or more children (62.8%). Table 3.

When asked about mosquito bites in the past month, most participants reported being unaware of any recent mosquito bite (38.7%). Among those experiencing symptoms, headaches were the most reported. Other symptoms reported were fever, joint pain and muscle pain (Table 3)

Analysis of 92 serum samples showed that 33.7% had ZIKV IgG, 8.7% had ZIKV IgM,

The study found that the highest seroprevalence across all categories was observed among pregnant women with a single pregnancy (Table 3). Additionally, the highest seroprevalence rates, based on parity and gestational age, were found among primiparous par and those in their first trimester. Those who claimed they were not bitten by mosquitoes had the highest prevalence across all categories (Table 3).

Table 3: Seroprevalence of ZIKV IgM and Medical Variables

Variables	No tested (%)	Seropositive IgG	IgM	Both IgM & IgG	Significance (p-value)
Type of Pregnancy					
Single	89 (94.4%)	31 (34.8%	8 (9.0%)	4 (4.5%)	0.3800
Twin	2 (2.8%)	0 (0%)	0 (0%)	0 (0%)	
Do not Know	1	0 (0%)	0 (0%)	0 (0%)	
Other	0 (2.2%)	0 (0%)	0 (0%)	0 (0%)	
Gestational Age					
0-13	16 (16.7%)	7 (43.8%)	2 (12.5%)	2 (12.5%)	0.4211
14-26	42 (46.7%)	12 (28.6%)	3 (7.1%)	0 (0%)	
27-40	34 (36.7%)	13 (38.2%)	3 (8.8%)	2 (5.9%)	
Parity					
Nulliparous	24 (8.9%)	9 (37.5%)	1 (4.16%)	1 (4.16%)	0.3743
Primiparous	42 (28.3%)	16 (38.1%)	6 (14.3%)	3 (7.1%)	
Multiparous	21 (62.8%)	6 (28.6%)	1 (4.8%)	0 (0%)	
History of Mosquito Bite					
Yes	29 (37.2%)	9 (31.0%)	1 (3.5%)	1 (3.5%)	0.4446
No	42 (23.9%)	16 (38.1%)	6 (14.3%)	3 (7.1%)	
Do not know	21 (38.9%)	6 (28.8%)	1 (4.8%)	0 (0%)	
Total	**92 (100%)**	**31**	**8**	**4**	

IgM—Immunoglobulin M, IgG– Immunoglobulin G

Out of the 92 participants, 50 did not display any symptoms associated with ZIKV infection. Among the remaining participants, 19 individuals solely experienced headaches, while 7 participants exclusively reported joint pain. Additionally, one participant each reported fever or muscle pain as their singular symptom. Some participants encountered a combination of symptoms. Specifically, 8 participants had both joint pain and headaches, while 3 participants had simultaneous symptoms of headaches and muscle pain. Two participants endured a trio of symptoms, including joint pain, headache, and muscle pain. Lastly, one participant reported a combination of fever, joint pain, and headaches. Notably, none of the participants reported experiencing a rash or conjunctivitis. (Table 4).

Among participants who did not exhibit any symptoms related to the Zika virus (ZIKV), 20 tested positive for IgG, and 3 tested positive for IgM. In two participants, both antibodies were detected simultaneously. Among those who reported fever as their only symptom, one participant tested positive for IgM, while two participants tested positive for IgG. For participants who solely experienced headaches, 4 were IgG positive, and 2 were IgM positive, with co-occurrence of both antibodies observed in 2 participants. Among those who reported joint pain and headache as their symptoms, 3 participants were IgG positive, and 2 were IgM positive. Additionally, one participant each tested positive for IgG among those who experienced headache and muscle pain, as well as joint pain, headache, and muscle pain. (Table 4).

Table 4: Observed Symptoms among Participants

Variables	No tested (%)	Seropositive		
		IgG	IgM	Both IgG & IgM
None	50	20	3	2
Fever	1	0	1	
Rash	0	0	0	
Conjunctivitis	0	0	0	
Joint pain	7	2	0	
Headache	19	4	2	2
Muscle pain	1	0	0	
Fever +joint pain + headache	1	0	0	
Joint pain + headache	8	3	2	
Joint pain + headache + muscle pain	2	1	0	
Headache + muscle pain	3	1	0	
Total	**92**	**31**	**8**	**4**

IgG– Immunoglobulin G, IgM—Immunoglobulin M

DISCUSSION

While prior viral infections usually confer natural immunity, arboviral infections can be an exception due to their genetic relatedness, potentially leading to more severe forms of subsequent infections through antibody-dependent enhancement. Thus, even individuals with prior ZIKV infections, indicated by the presence of ZIKV IgG, may still be susceptible to future infections and should take preventive measures against arbovirus vectors. This study aimed to determine the prevalence of ZIKV IgG and IgM antibodies in pregnant women in southern Nigeria.

The serum of the participants showed the presence of ZIKV IgG, indicating a previous Zika virus infection in the population. The seroprevalence obtained in this study was higher than that reported by Shaibu *et al.* (2021) in a similar study of pregnant women in the same geographic region in Nigeria (2.0%), suggesting moderately low herd immunity to Zika virus infection in the population due to low exposure (Shaibu *et al.*, 2021). The level of herd immunity in a population is greatly influenced by interdependencies of various factors. For instance, environmental factors, such as temperature, humidity, and the presence of vectors or reservoirs, can influence pathogen survival, replication, and transmission. At the same time, human activities, including urbanization, can alter the environment and affect the distribution and transmission of diseases. Despite that both studies were carried out in the same region, variations in environmental factors could affect the level of herd immunity among participants. The IgM seroprevalence, which indicates current infection, was only 8.7% lower than the 16% reported by Kolawole *et al.* (2020) in a similar study of pregnant women in Ilorin, North-central Nigeria (Kolawole *et al.*, 2020). The disparity in prevalence rates could be attributed to difference in socio-economic conditions in the regions of the participants. Generally, it is assumed that the socio-economic conditions in the southwest region of the country are better than that of the North central. However, even with this low prevalence rate, participants who tested positive for ZIKV IgM may still be at risk for the consequences of ZIKV infection.

A higher prevalence rate was observed for both ZIKV IgM and IgG among individuals over the age of 30 and those living in urban areas. Older individuals are more vulnerable to infection due to weakened immune systems, pregnancy, and residing in poor or newly developed urban areas. Although not statistically significant, these findings align with previous research on Zika virus infections (Adams *et al.*, 2022; Aguilar Ticona *et al.*, 2021). Unemployed participants had the highest prevalence for both IgM and IgG, whereas participants with tertiary education showed a higher prevalence rate of IgM, and those with primary education had the highest prevalence of IgG. While these results are consistent with prior studies, individuals in both groups may be at risk due to job engagement or outdoor activities (Khoo *et al.*, 2022).

Most of the participants in this study had a single pregnancy and were mostly positive for both IgG and IgM. However, most of these participants were multiparous and in their first trimester. It is well-known that Zika virus infection can have harmful effects on pregnant women, regardless of their pregnancy type or gestational age, due to its ability to cross the placental barrier and cause fetal and brain damage (Wang *et al.*, 2017). Pregnancies in the first trimester may

be at a higher risk, as studies have shown that ZIKV has tropism in the brains of 28-day-old organoids, causing reduced ventricular zones and neuronal damage resembling microcephaly (Qian *et al*., 2016). While mosquito bites remain the primary route of transmission, ZIKV antibodies were most prevalent among participants who claimed not to have been bitten by mosquitoes in recent days. Although these findings contradict those of Shaibu *et al.* (2021), it is worth noting that the virus can be transmitted through other routes, such as sexual intercourse or blood transfusion (Shaibu *et al*., 2021). The symptomology observed in this study was mostly characterized by headache, fever, muscle pain and joint pain, consistent with previous studies (Burger-Calderon *et al*., 2020; Guanche Garcell *et al*., 2020). Although these symptoms are non-pathognomonic, they have become established symptoms of ZIKV infection (Song *et al*., 2017).

CONCLUSION

This research offers evidence of ZIKV antibodies detected in pregnant women within the study area via serological testing. While the sample size is low, the findings of the study imply the presence of the ZIKV in the southwestern region of the country. ZIKV infections frequently present as asymptomatic; however, when symptoms do occur, fever, headache, muscle pain and joint pain were among the most commonly reported experiences, as indicated in the study. Infections during pregnancy can lead to severe complications, particularly among women in their first trimester, resulting in fetal abnormalities.

RECOMMENDATION

It is advisable to include Zika virus testing as part of routine diagnostics during antenatal check-ups.

Competing interests

The authors declare no competing interests.

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