

Seroprevalence of Zika Virus IgM Antibodies in Pregnant Women in Nigeria

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ABSTRACT

Backgrounds: In developing countries like Nigeria, screening of Zika virus (ZIKV) infection in pregnant women remains limited due to a lack of diagnostic facilities and non-specific symptoms, leading to potential misdiagnosis of the disease as other febrile illnesses such as malaria or typhoid.

Materials & Methods: To address this issue, this study aimed to investigate the prevalence of anti-ZIKV IgM antibodies in pregnant women using enzyme-linked immunoassay. Additionally, the quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay targeted a specific region of the membrane protein (prM) gene to detect Zika virus presence in the collected serum samples. For a period of four months from December 2021 to March 2022, a total of 360 serum samples were collected from pregnant women attending antenatal care units in two tertiary hospitals located in different regions of Nigeria.

Findings: The results of this study revealed a prevalence of 17.2% (62 samples) for anti-ZIKV IgM antibodies among pregnant women. Further analysis using the RT-qPCR method detected Zika virus (prM gene) in 1.9% (7/62) of the serum samples. In addition to these virological results, the statistical analysis of sociodemographic data, clinical characteristics, and risk factors for ZIKV infection demonstrated a significant correlation between seropositivity and various factors including ethnicity, residence, occupation, and history of arboviral diseases ($p < .005$).

Conclusion: Given the potential consequences of ZIKV infection in pregnant women, early diagnosis and intervention could improve maternal outcomes and prevent fetal abnormalities.

Keywords: ZIKV, IgM, Pregnancy, Arboviruses.

CITATION LINKS

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Introduction

Zika virus (ZIKV), as a flavivirus that is transmitted to humans primarily through the bite of an infected *Aedes* mosquito, has sparked widespread concerns due to its potentially severe consequences, particularly in pregnant women [1]. Microcephaly and other brain malformations in developing fetuses have been linked to the virus [2]. In 2016, the World Health Organization (WHO) declared the Zika virus as a public health emergency of international concern, emphasizing the need for more research on the prevalence of this virus among at-risk populations such as pregnant women [3]. Nigeria is an African country affected by Zika virus, with reported cases dating back to 1975 [4]. The country has a high population density and a large number of poor people, which may contribute to the spread of the virus [5]. Furthermore, the burden of other mosquito-borne diseases such as dengue and malaria is high in this country, making clinical diagnosis of Zika virus infection difficult [6].

One of the most difficult challenges in determining the prevalence of Zika virus in Nigeria is the detection of the virus in infected individuals. The symptoms of Zika virus infection could be similar to those of other viral infections such as dengue or chikungunya, making diagnosis solely based on clinical presentations difficult. Furthermore, many ZIKV-infected individuals may be asymptomatic and exhibit no symptoms [7].

Enzyme-linked immunoassay (ELISA) is one of the most widely used methods for Zika virus detection [8]. This test detects the presence of viral antibodies in a person's serum sample. The immune system produces these antibodies in response to the virus, and their presence in the serum indicates that the person is infected with the virus [8]. Given the potentially severe consequences

of Zika infection in pregnant women, it is critical to determine its prevalence among the Nigerian population. The presence of anti-Zika IgM antibodies in pregnant women could provide information about the prevalence of the virus in this population. This study results are expected to contribute to public health efforts to control the spread of the virus and protect the most vulnerable population in Nigeria.

Objectives: This study aimed to determine the prevalence rate of Zika virus infection among pregnant women in Nigeria using both ELISA and quantitative reverse transcription polymerase chain reaction (RT-qPCR) to detect anti-Zika virus IgM antibodies and confirm the presence of Zika virus RNA in serum samples, respectively.

Materials and Methods

Study design: This research was a cross-sectional hospital-based study conducted on 360 pregnant women attending two tertiary hospitals in northwest (Ahmadu Bello University Teaching Hospital, Zaria) and southwest (Olabisi Onabanjo University Teaching Hospital, Sagamu) Nigeria from November 2021 to August 2022.

Sample population and size: The study was conducted on consenting pregnant women who visited antenatal care units of the selected tertiary hospitals. Participants were recruited for the study when they visited the hospital. Using Fischer's formula for a cross-sectional study design, the sample size was calculated to be 245 people, which was then increased to 360 considering the prevalence of 20% reported by Otu et al. (2019) [4].

Inclusion criteria: This study enrolled pregnant women with or without the symptoms indicating Zika virus infection. **Data collection:** Structured questionnaires were used to collect sociodemographic information (sex, age, education level, occupation, and residential area) and

medical information of the study participants (gravidae, gestational age, ZIKV infection-related symptoms, history of mosquito bites, and history of arboviral infections). The required data were obtained through face-to-face interviews.

Sample collection: Blood samples were taken aseptically from the participants and poured in to sterile tubes. The sera obtained from blood samples were stored in cryovials at -20 °C until laboratory analysis.

Sample analysis: The separated serum samples were screened for the presence of anti-ZIKV IgM antibody using Zika IgM ELISA kit (Viracell Microbiologists, Spain). The reagents used in this study were kept at room temperature for one hour before use. The separated serum samples were poured into multi-well plates, and four wells were considered for controls: cut-off (two wells), positive, and negative controls. Serum diluents were homogenously mixed with the controls and the samples in the respective wells of the plates, followed by incubation, washing, and addition of IgM conjugate, substrate, and stop solutions. The optical densities of the plates were determined using an ELISA plate reader at 450/620 nm one hour after stopping. To interpret the results, following the manufacturer's instructions, the mean optical density of the cut-off sera was first determined, and then the result of each of the remaining wells was determined using the following formula: Antibody index = (Sample optical density / mean optical density of cut-off sera) x10. Samples with antibody indices above 11 were considered as positive, while those with index values below 9 were considered as negative. Samples with indices between 9 and 11 were retested.

After screening the serum samples obtained from the participating pregnant women using the anti-ZIKV IgM ELISA procedure, further analysis was carried out by the

RT-qPCR to detect Zika virus RNA in the serum samples using primers targeting the membrane protein (prM) gene (forward primer: CCGCTGCCCAACACAAG; reverse primer: CCACTAACGTTCTTTTGCAGACAT). In order to obtain the complementary DNA (cDNA), a mixture was prepared by combining the eluted RNA sample (1 µl) with dd(T)23VN (50 µM) (2 µl) in a sterile RNase-free microfuge tube. The mixture was then supplemented with the M-MuLV Reaction Mix (2x) (10 µl) and M-MuLV Enzyme mix (2 µl), followed by the addition of nuclease-free water to reach a total volume of 20 µl. The resulting mixture was incubated at 42°C for 1 hour. For the RT-qPCR procedure, a cocktail was prepared by mixing the SYBR Green Master Mix, the synthesized cDNA, and the RT-qPCR primers for Zika. Each PCR reaction (20 µl) consisted of 6 µl of SYBR Green Master Mix (2X), 1 µl of Zika primers, and 5 µl of the synthesized cDNA sample. Replicates of the samples were run for increased accuracy. The cDNA amplification was performed using a Roche Light Cycler 480 real-time cycler with the following cycling program: 55°C for 10 minutes, followed by 40 cycles of 95°C for 1 minute and 10 seconds, and 60°C for 30 seconds. A melting curve acquisition step was then carried out by increasing the temperature from 65°C to 95°C.

Statistical analysis: The data obtained through the questionnaires and laboratory analysis results were entered into Microsoft Excel and analysed using GraphPad Prism 5. Quantitative variables were presented and compared using graphs and tables, and Chi-square and p values were calculated. Statistical significance was set at $p < .05$.

Findings

This study was conducted on 360 pregnant women attending Ahmadu Bello Teaching Hospital in Zaria (northern participants) and Olabisi Onabanjo University Teaching

Table 1) Distribution of seropositivity based on socio-demographic variables

Variables	No. of Tested (%)	Seropositive	Prevalence	Chi-Square (x²)	Significance (P-Value)
Age					
16-22	34 (9.4%)	5	14.7%	0.6319	.9595
23-28	118 (32.8%)	21	17.8%		
29-34	124 (34.4%)	20	16.1%		
35-40	69 (19.2%)	14	20.3%		
41-46	15 (4.2%)	2	13.3%		
Total	360 (100%)	62			
Residence					
Rural	114 (31.7%)	9	7.9%	6.726	.0095
Urban	246 (68.3%)	53	21.5%		
Total	360 (100%)	62			
Ethnicity					
Yoruba	168 (46.7%)	15	8.9%	11.581	.009
Hausa	130 (36.1%)	33	25.4%		
Igbo	14 (3.9%)	2	14.3%		
Others	48 (13.3%)	12	25%		
Total	360 (100%)	62			
Education					
None	6 (1.7%)	1	16.7%	1.543	.6724
Primary	18 (5.0%)	5	27.8%		
Secondary	101 (28.1%)	14	13.9%		
Tertiary	235 (65.3%)	42	17.9%		
Total	360 (100%)	62			
Occupation					
Financial	8 (2.2%)	2	25.0%	16.063	.0134
Education	50 (13.9%)	4	8.0%		
Health	33 (9.2%)	2	6.1%		
Public	35 (9.7%)	7	20.0%		
Religious	1 (0.3%)	0	0.0%		
Self-employed	135 (37.5%)	16	11.9%		
Unemployed	98 (27.2%)	31	31.6%		
Total	360 (100%)	62			

Table 2) Distribution of seropositivity based on clinical characteristics

Variables	No. of tested (%)	Seropositive	Prevalence	Chi-Square (x ²)	Significance (P-Value)
Type of pregnancy					
Single	343 (95.3%)	62	18.1%	3.051	.5494
Twin	11 (3.1%)	0	0%		
Triple	1 (0.3%)	0	0%		
Others	4 (1.1%)	0	0%		
Don't know	1 (0.3%)	0	0%		
Total	360 (100%)	62			
Gestational age					
0-13	57 (15.8%)	6	10.5%	1.63	.4426
14-26	152 (42.2%)	29	19.1%		
27-40	151 (41.9%)	27	17.9%		
Total	360 (100%)	62			
Parity					
Nulliparous	63 (17.5%)	6	9.5%	2.993	.2239
Primiparous	106 (29.4%)	23	21.7%		
Multiparous	191 (53.1%)	33	17.3%		
Total	360 (100%)	62			
Symptoms					
Fever		15	44.1%	7.726	.1720
Headache		18	19.1%		
Rash		0	0%		
Conjunctivitis		1	50%		
Muscle Pain		6	30%		
Joint Pain		4	15.4%		
History of mosquito bites					
Yes	131 (36.4%)	16	12.2%	5.642	.0595
No	130 (36.1%)	20	15.4%		
Don't know	99 (27.5%)	26	26.3%		
History of arboviral diseases					
Yellow fever	45(12.5%)	16	35.6%	15.34	.0015
Dengue	1 (0.3%)	1	100.0%		
Chikungunya	1 (0.3%)	0	0.0%		
None	313(86.9%)	45	11.5%		
Total	360 (100%)	62			

Table 3) ZIKV RNA Quantitative Reverse Transcription PCR -positive samples

Sample	Age	Gestational Age	History of Mosquito Bite	No. of Symptoms
ABUZ11	26	28	Don't know	3
ABUZ27	29	21	Yes	1
ABUZ42	23	26	No	2
ABUZ54	25	26	Don't know	1
ABUZ76	21	29	No	3
ABUZ80	39	40	Don't know	1
OOUS134	24	28	No	1

Hospital in Sagamu (southern participants). Enzyme-linked immunoassay was used to detect anti-ZIKV immunoglobulin M antibody (ZIKV IgM) in the serum samples of 360 participants, evenly divided into northern and southern participants. According to the results, 53 out of 180 northern participants' serum samples were seropositive for anti-ZIKV IgM antibody, whereas only nine out of 180 southern participants' serum samples were seropositive. The overall prevalence rate of anti-ZIKV IgM antibody among pregnant women was 17.2%. A total of 62 out of 360 serum samples were seropositive for anti-ZIKV IgM antibody.

The age of the participants ranged from 16 to 46 years. The highest prevalence was observed among the age group of 35-40 years (20.3%), while the lowest prevalence was observed among the age group of 41-46 years (13.33%). The prevalence rate among the participants living in urban and rural areas was 21.5 and 7.9%, respectively. Based on ethnicity, the highest prevalence was observed in the Hausa tribe (25.4%), whereas the lowest prevalence was observed in the Yoruba tribe (8.9%). The highest seroprevalence based on education and occupation was observed among those with primary education (27.8%) and unemployed

participants (31.6%), respectively (Table 1). Based on pregnancy information, seroprevalence was observed only among those with single pregnancies. Nonetheless, the highest seroprevalence was observed among those in the second trimester of their pregnancy (19.1%) and primiparous participants (21.7%). The prevalence among the participants experiencing fever was higher than among those experiencing other symptoms, the highest prevalence was observed among those who experienced only one symptom related to ZIKV infection (Fig. 1). Most participants had no history of arboviral diseases. Inquiries about the history of mosquito bites within the past 30 days revealed that the highest prevalence was among those who had no memory of being bitten by a mosquito in the past 30 days (26.3%) (Table 2).

Figure 1 shows that the frequency of symptoms among 360 participants enrolled in this study was statistically significant. RT-qPCR analysis results revealed that out of the 62 samples that tested positive, only seven (1.9%) samples contained Zika virus RNA. The assay was repeated for ZIKV RNA-positive samples for confirmation. It was found that 86% (6 of 7) of the women who tested positive for Zika virus RNA were less

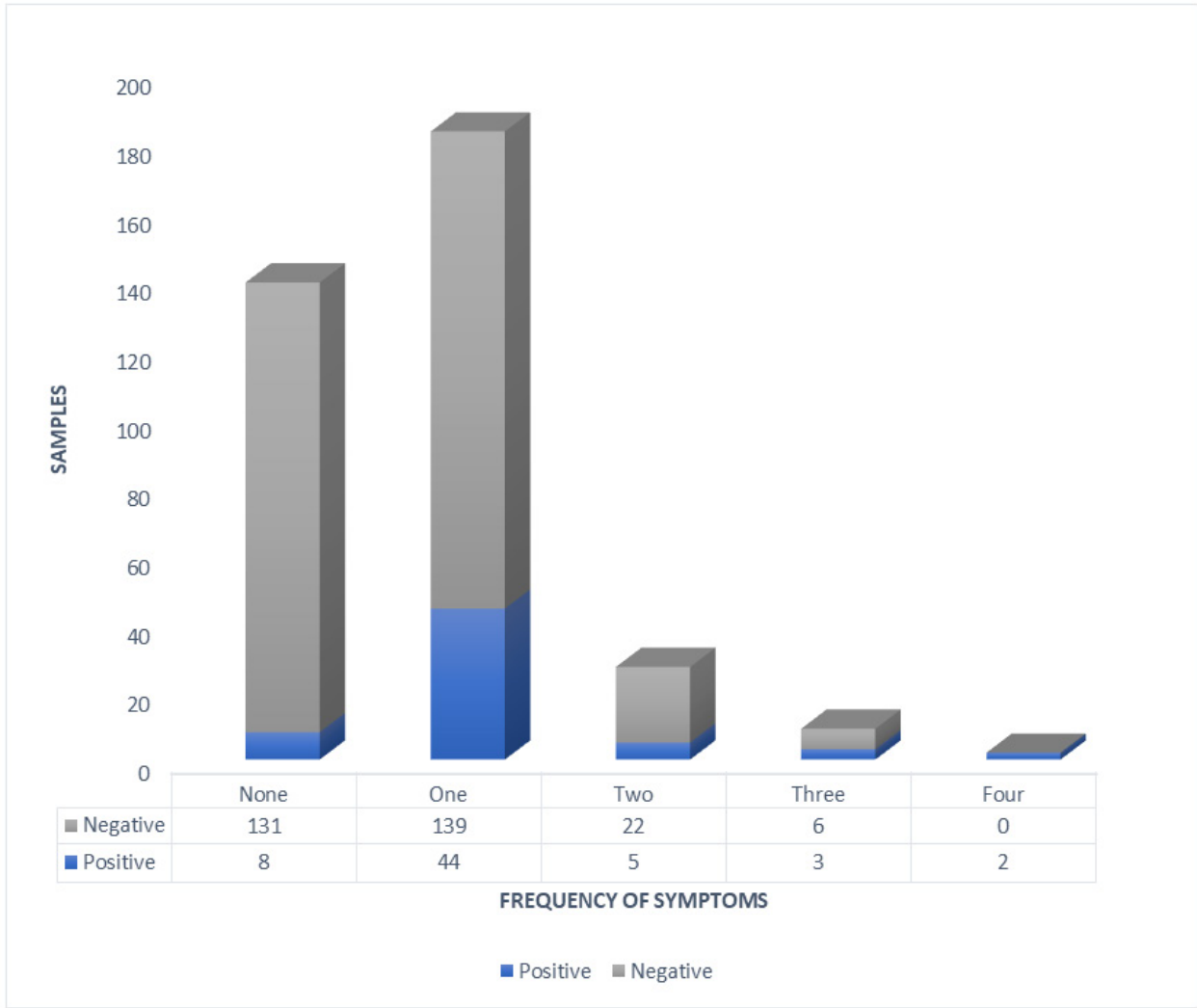


Figure 1) Frequency of symptoms and seroprevalence of ZIKV ($\chi^2=19.039$, $p\text{-value}= .0008$)

than 40 years old. All the participants had at least one symptom of the viral infection and were either in the second or third trimester of pregnancy (Table 3). Table 1 shows the relationship between seropositivity and socio-demographic variables of the participants. Residence, occupation, and ethnicity were significantly associated with seropositivity. Table 2 shows the relationship between seropositivity and clinical characteristics of the participants. Only the history of arboviral diseases was significantly correlated with seropositivity. Table 3 displays the key information about the participants who were positive for the presence of Zika virus RNA using

Quantitative Reverse Transcription PCR.

Discussion

Zika virus is a flavivirus belonging to the family Flaviviridae, which includes several other clinically important mosquito-borne viruses (e.g., Dengue virus, West Nile virus, and yellow fever virus). The virus is transmitted to humans primarily through the bite of an infected Aedes mosquito [1]. Previous studies investigating ZIKV infection in pregnant women in Nigeria have focused their efforts on one region of the country [9-14]. In this study, pregnant women attending two tertiary hospitals located in two regions of Nigeria (northwest and southwest) were assessed for ZIKV antibody seroprevalence.

This is possibly the first study conducted to investigate the prevalence of ZIKV infection among pregnant women in two different regions of the country. In this study, the overall seroprevalence in both regions was 17.2%. This result is lower than the result of another study conducted by Oluwole et al. (2022) to investigate the seroprevalence of some arboviruses among pregnant women in Ibadan, southwestern Nigeria (55.6%). A possible reason for this variation could be that Oluwole et al. (2022) used a small sample size of 36 participants and found a combined prevalence of ZIKV IgM and IgG antibodies among pregnant women ^[10]. The age distribution of ZIKV IgM seroprevalence showed the highest overall prevalence of 20.3% among the age group of 35-40 years, which is consistent with the finding of Rosenberg et al. (2019) reporting a prevalence of 26.3% among the age group of ≤ 40 years while investigating the prevalence of ZIKV infection among household contacts of patients with ZIKV infection in Puerto Rico ^[15]. The slight difference in the prevalence rates could be attributed to the difference in sample sizes of these two studies. Nonetheless, in another study investigating the risk factors for infection with Chikungunya and Zika viruses in southern Puerto Rico, Adams et al. (2022) reported the highest prevalence of 19% among the age group of 41-50 years, which contradicts the present study finding ^[16]. This disparity could be attributed to the skewness of the age groups of the study participants. Regarding residential areas, the highest overall prevalence of 21.5% was observed among those living in urban areas. In line with this study result, Anejo-Okopi et al. (2020) investigating the seroprevalence of Zika virus infection among HIV-positive and HIV-negative pregnant women also reported the highest prevalence of 20.5% among urban residents of Jos, Nigeria ^[9].

According to reports, increasing unplanned urbanization in developing countries has turned urban residences into breeding grounds for arbovirus vectors. Nonetheless, in previous studies conducted by Soghaier et al. (2018) and in 2016, the World Health Assembly declared it a public health emergency of international concern. The discovery of Zika virus circulation in Sudan came as a secondary finding in a 2012 country-wide yellow fever prevalence study, when laboratory tests were done to exclude cross-reactions between flaviviruses. The study was cross-sectional community-based, with randomly selected participants through multi-stage cluster sampling. A subset of samples were tested for the Zika virus using ELISA, and the ones that demonstrated reactive results were subsequently tested by PRNT. Results: The prevalence of Zika IgG antibodies among ELISA-tested samples was 62.7% (59.4 to 66.1, 95% CI to explore evidence of pre-existing active Zika virus circulation in Sudan and Souza et al. (2018) to explore the relationship between the ZIKV-related microcephaly epidemic and living conditions in Recife, Brazil, the prevalence of Zika virus infection was higher among rural residents, contradicting this study finding ^[17, 18]. Poor living conditions are another significant factor that could predispose residents to Zika virus infection through blood-feeding vectors. The overall prevalence of 25.4% in the Hausa tribe represents the majority of the population living in the northern region of the country. In the studies conducted by Oderinde et al. (2020) to investigate the prevalence of locally undetected acute infections caused by flaviviruses in north-eastern Nigeria and Mathe et al. (2018) to investigate Zika virus seroprevalence among pregnant women in north-central Nigeria, the prevalence of Zika virus IgM antibodies in these populations was 22 and 4%, respectively ^[13, 14]. The

difference in the prevalence rate between the present and the aforementioned studies could be attributed to differences in the study area, sample size, and study design used in these studies. Regarding education level, ZIKV seroprevalence was higher among the participants with primary education (27.8%). This finding is consistent with the finding of another study conducted by Nery Jr et al. (2021) to evaluate the social determinants of Zika virus infection in pregnant women ^[19]. In their study, there was a significant correlation between ZIKV prevalence and lower education levels, which corroborates this study finding. This could be attributed to a lack of health education about arboviral diseases (e.g., Zika virus disease, dengue fever, and West Nile virus disease) and their impact on pregnancy and fetal development. Ignoring arboviruses reduces the possibility of implementing preventive measures against arbovirus vectors, thereby increasing the chance of exposure to arboviruses. In this study, the prevalence among unemployed participants was higher (31.6%) compared to employed participants, this finding contrasts with the results of other studies reporting higher prevalence rates among participants with different occupations, including Mwanyika et al. (2021) dengue (DENV) ^[20] who investigated the seroprevalence and associated risk factors of selected arboviruses including Zika in several districts in Tanzania and Anejo-Okopi et al. (2020) ^[9] who assessed seroprevalence among HIV-positive and HIV-negative pregnant women. This study finding could be attributed to the large number of unemployed participants recruited. Nonetheless, unemployed participants, including housewives and students, may engage in outdoor activities that expose them to mosquito bites.

The prevalence among pregnant women with a single fetus was 18.1%, while no

prevalence was recorded for other types of pregnancy. However, the prevalence was higher among those in the second trimester of their pregnancy (19.1%). This finding is consistent with the finding of another study conducted by Phatihattakorn et al. (2021) especially during the first trimester, often results in congenital anomalies. However, the pathogenic mechanism is unknown and one-third of ZKV infected pregnancies are asymptomatic. Neutralizing antibodies against ZKV has been reported in 70% of Thai adults, but the prevalence among pregnant women is unknown. Currently, vaccines and specific treatments for ZKV are under development. A better understanding of the immune status of pregnant women will increase the success of effective prevention guidelines. The prevalence of ZKV infection in pregnant women in antenatal care clinics was investigated during the rainy season from May to October 2019 at Siriraj Hospital, Bangkok, Thailand. Who recruited 650 pregnant women (39.42% first, 52.26% second and 7.36% third trimester to evaluate the seroprevalence of Zika virus among pregnant women in central Thailand ^[21]. However, Anejo-Okopi et al. (2020) found the highest prevalence in the first trimester of pregnancy among HIV-positive and HIV-negative pregnant women in Jos, contradicting this study finding ^[9]. Nonetheless, ZIKV infection during the first and second trimesters poses a great risk to pregnant women. In a study reporting the first local cases of Zika virus infection in Europe, Brady et al. (2019) high rates of microcephaly were reported in Northeast Brazil following the first South American Zika virus (ZIKV demonstrated a direct link between infection in the first two trimesters and microcephaly in newborns ^[22]. In the current study, the prevalence was higher among primiparous participants compared to the other two parity groups, which is

consistent with the finding of Anejo-Okopi et al. (2020) who reported the highest prevalence among participants with a parity of ≤ 3 [9]. However, the methods used in these two studies to classify participants' parity levels were different. Inquiries about the history of mosquito bites within the past 30 days revealed that the highest prevalence (26.3%) was among those who had no memory of being bitten. In contrast, in a study investigating the seroprevalence and risk factors of arboviral infections in several Tanzania districts, Mwanyika et al. (2021) dengue (DENV reported the highest prevalence among those not bitten by mosquito vectors [20]. Also, in another study investigating the seroprevalence of arboviruses including Zika virus at a community level in south Ethiopia, Asebe et al. (2021) showed the highest prevalence among those admitted to being bitten by mosquitoes [23]. It should be noted that unlike the present study which provided participants with three options in this section, both Mwanyika's and Asebe's studies provided participants with only two options (yes and no).

Fever with a frequency of 44.1% was the most common symptom among the participants, which is consistent with the findings of previous studies by Mathe et al. (2018) [14], Phatihattakorn et al. (2021) [21], and Sirinam et al. (2022) Zika virus (ZIKV [24]. This study finding confirms previous evidence showing fever as a primary symptom of Zika virus infection. Although four ZIKV-related symptoms were more common among seropositive participants, most seropositive participants had only one ZIKV-related symptom. Inquiries about the history of arboviral infections revealed that most seropositive participants had no history of arboviral infections. However, the prevalence was higher among the participants with a history of dengue

fever and yellow fever. These findings are consistent with those of previous studies by Marban-Castro et al. (2020) chikungunya (CHIKV [25] and Asebe et al. (2021) [23], in which participants revealed their history of dengue and yellow fever infections, respectively, using an immunoglobulin G antibody assay.

The RT-qPCR analysis results revealed that out of the 62 samples that tested positive, only seven samples contained Zika virus RNA, which equates to a sero-molecular prevalence of 1.9%, this finding is similar to that of another study by Mathe et al (2018) in north-central Nigeria, recording a low prevalence of 0.2% [14]. Although these two studies have been conducted in different regions of the country, there is a possibility of matching sociodemographic profile due to the similarity of sample participants. The disparity in the serological and molecular prevalence rates could be attributed to the possibility of cross-reactivity with other arboviruses with similar genetic makeup. Due to the structural and genetic similarities between Zika virus and other genetically related arboviruses such as Dengue, West Nile, and yellow fever viruses, serological assays that detect antibodies against one of these viruses could often cross-react with antibodies against the other viruses, leading to false-positive results [26]. This cross-reactivity could be a significant challenge in the diagnosis of arboviral infections, particularly in areas where multiple arboviruses are co-circulating. Therefore, it is important to consider the potential for cross-reactivity when interpreting serological assay results and to confirm any positive results by performing additional tests in order to ensure an accurate diagnosis and appropriately manage patients.

Conclusion

ZIKV remains as a threat to pregnant women in tropical and subtropical regions of the

world owing to the climate and environment that favour the proliferation of its mosquito vectors. This study revealed a high seroprevalence of anti-ZIKV IgM antibodies in pregnant women, indicating current infection. This study provided critical information about the current status of the virus in Nigeria and aided in identifying potential risk factors for infection in pregnant women. Furthermore, this study results are expected to contribute to public health efforts to control the spread of the virus and protect the most vulnerable population in Nigeria. Given that this viral infection is mostly asymptomatic and subclinical, as well as considering the potential complications that may occur in infants as a result of this infection during pregnancy, there is an urgent need for continuous effective epidemiological surveillance and prevention of the spread of arboviral diseases, particularly among vulnerable populations such as pregnant women.

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