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The prevalence of IGM antibodies to Zika virus in pregnant women in Northern Nigeria

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Introduction. Zika virus (ZIKV) infection during pregnancy can result in severe outcomes for both the pregnant woman and the developing fetus.
The **objective** of this study was to investigate the prevalence of Zika virus infection among pregnant women who sought healthcare services at Ahmadu Bello University Teaching Hospital.
Materials and methods. Serum samples were collected and analyzed using Enzyme Linked Immunoassay and RT-qPCR methods, while a structured questionnaire was used to gather relevant information about the participants.
Results. The results showed that 53 out of the 180 pregnant women tested positive for Anti-Zika IgM antibodies, which represents a 29.4% prevalence rate. Subsequent RT-qPCR analysis found that only 6 out of the 53 positive samples contained Zika virus RNA. Fever and headache were the most commonly reported symptoms related to the infection.
Conclusion. These findings indicate a potential outbreak of Zika fever in Northern Nigeria emphasizing the importance for pregnant women to take precautions to avoid getting infected.

Keywords: *Zika virus infection; Nigeria; examination of pregnant women; MAC-ELISA; RT-qPCR; Anti-Zika IgM antibodies*

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ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ
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Частота выявления IgM-антител к вирусу Зика у беременных женщин в Северной Нигерии

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Введение. Заражение вирусом Зика (ZIKV) во время беременности может приводить к тяжелым последствиям как для матери, так и для развивающегося плода.
Целью данного исследования являлось определение распространенности ZIKV-инфекции среди беременных женщин, обратившихся за медицинской помощью в Учебную больницу Университета Ахмаду Белло.
Материалы и методы. Собранные образцы сыворотки крови были протестированы методами иммуноферментного анализа и ОТ-ПЦР, для сбора соответствующей информации об участниках исследования использовалась структурированная анкета.
Результаты. Из 180 обследованных беременных женщин 53 были положительными по анти-ZIKV IgM, частота выявления составила 29,4%. Только 6 из 53 серопозитивных образцов были положительными по РНК ZIKV при последующем тестировании в ОТ-ПЦР. Лихорадка и головная боль были наиболее частыми симптомами, связанными с инфекцией.
Заключение. Полученные данные указывают на возможную вспышку лихорадки Зика в Северной Нигерии, что подчеркивает важность принятия беременными женщинами мер предосторожности во избежание заражения.

Ключевые слова: *заражение вирусом Зика; Нигерия; обследование беременных; MAC-ELISA; RT-qPCR; анти-ZIKV IgM антитела*

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Introduction

The incidence of Zika virus (ZIKV) infection in pregnant women is a growing global concern. Given the potential serious consequences of ZIKV infection during pregnancy for the developing fetus, measuring the prevalence of ZIKV antibodies among pregnant women is crucial. Nigeria, an African country, has a history of Zika infection dating back to 1975 [1]. The country's dense population and large number of economically disadvantaged people are said to be a significant facilitator of ZIKV spread [2]. Furthermore, due to the country's high burden of other mosquito-borne illnesses, such as dengue, malaria and numerous others, the clinical diagnosis of ZIKV infecrtion is difficult [3].

ZIKV infection in adults present as conjunctivitis, mild fever, headache, skin rash and diarrhoea [4]. However, in severe cases, arthralgia, Guillain–Barre syndrome and cardiovascular anomalies could occur [4]. ZIKVinfection, if contracted during pregnancy, can lead to severe outcomes for both the mother and the unborn child [5]. ZIKV manifestations in neonates often result in congenital microcephaly, optic neuropathy and congenital glaucoma, ventriculomegaly and lissencephaly [6, 7]. ZIKV infection during pregnancy is associated with a range of serious birth defects [7]. The highest risk of these birth defects occurs when a woman contracts ZIKV during the first trimester of pregnancy [6]. However, it should be noted that even if the infection occurs in later stages of pregnancy, there is still a risk of birth defects. Pregnancy infections are a known cause of low birth weight, small-for-gestational-age neonates and premature delivery [7]. Preterm and small-for-gestational-age infants are at a greater risk of morbidity and death in infancy and early childhood, as well as chronic illness, such as cardiovascular disease, later in life [7]. The Center for Disease Control and Prevention recommends that pregnant women avoid traveling to areas where the virus is actively spreading. Those who visit such areas should take precautions to avoid being bitten by mosquitoes. Furthermore, pregnant women residing in, or having visited areas with active ZIKV transmission, should undergo testing the antibodies specific for the virus or virus RNA, even if asymptomatic [6]. ZIKV infection has been linked to decreased birth weight, small-for-gestational-age neonates, and preterm delivery in recent studies. In a case series of 87 newborns with microcephaly and congenital ZIKV infection in Brazil, it was reported that 29% of the newborns were small-for-gestational-age, although no small-gestational-age neonates were detected in a cohort of 54 mothers with confirmed prenatal ZIKV infection [8, 9]. Preterm birth rates ranged from 7–15% in cohorts of pregnant women infected with ZIKV from Brazil and the United States [8–11]. In 2016, the overall frequency in the United States was 9.9% [12].

It is worth noting that the full impact of ZIKV on pregnancy is still unknown, and more research is needed to fully understand the dangers and implications of ZIKV infection during pregnancy [13]. Nevertheless, it is clear that infection with ZIKV during pregnancy can have serious consequences, and expectant mothers must take the necessary precautions to avoid contact with the virus [5]. In order to safeguard fetal health, it is important to measure the prevalence of anti-ZIKV antibodies in pregnant women. Information on the ZIKV prevalence can help health authorities better understand the spread and impact, as well as implement effective prevention and control strategies.

This study aimed to investigate the presence of anti-ZIKV IgM antibodies in the serum of pregnant women attending the Ahmadu Bello University Teaching Hospital.

Materials and methods

Ethical Consideration

The Health Research Ethics Committee of Ahmadu Bello University Teaching Hospital granted ethical approval (ABUTHZ/HREC/W16/2022). All participants provided informed consent in accordance with the Human Experimentation Standards and the Declaration of Helsinki of 1975, as revised in 2000. This was achieved by having all participants recruited for the study sign an informed consent form.

Study Design

This was a hospital-based cross-sectional study of pregnant women attending Ahmadu Bello University Teaching Hospital in Zaria, North-West Nigeria.

Sample Population and Size

The study involved pregnant women who visited the antenatal units of the hospital and provided their informed consent to participate. Recruitment of participants occurred during their routine hospital visits. The sample size was calculated using Fischer's formula for cross-sectional study design to be 59 but increased to 180, using the prevalence of 4% reported by Mathe et al. (2018) [14].

Data collection

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

Sample collection

Venous blood was drawn from study participants' antecubital veins by placing a tourniquet on the upper arm and tightening it enough to prevent venous return. The site of blood collection was sterilized with 70% alcohol and dried with sterile gauze. The vein was then punctured with a sterile needle attached to a syringe, and blood was obtained through gentle suction as the tourniquet was gradually removed. The needle was then removed, and 3 mL of blood was gently dispensed into a plain tube. After that, the tube was properly labelled with the participant's identification number. Sera were separated from blood samples by allowing the blood to clot at room temperature before centrifuging it at 2500 rpm for 10 minutes. It was then placed in cryovials and stored at –20 °C until laboratory analysis.

Sample analysis

VIRCELL Microbiologists Zika ELISA IgM (catalogue number: M1023) was used to screen the separated serum samples for the presence of anti-ZIKV IgM antibodies. Before use, the reagents were brought to room temperature for an hour. The plates were then removed, and four control wells were determined for the two cut-off controls, positive and negative. The serum dilutions were homogeneously mixed with the controls and samples in the microplate wells, followed by incubation, washing, and the addition of IgM conjugate, substrate, and stop solutions. Within an hour of stopping, the optical densities of the plates were determined using an ELISA plate reader at 450/620 nm. Following the manufacturer's instructions, the mean optical densities of the

cut-off serum were determined first, and the outcome of each of the remaining wells was calculated using the formula below:

(Sample optical density / mean optical density of cut-off serum) × 10 = Antibody Index.

Samples with an index greater than or equal to 11 were considered positive, while samples with an index less than or equal to 9 were considered negative, and samples with an index between 9 and 11 were retested.

Following the screening of serum samples obtained from pregnant women using the anti-ZIKV IgM ELISA procedure, PCR was used to detect Zika virus RNA in seropositive samples using primers targeting the membrane protein gene (forward primer: CCGCTGCCCAACACAAG; reverse primer: CCACTAACGTTCTTTTGCAGACAT).

Statistical Analysis

The questionnaire data and laboratory analysis results were entered into Microsoft Excel and analysed with GraphPad Prism 5. Graphs and tables were used to present and compare quantitative variables, and chi-square and p-values were calculated. The statistical significance level was set at P 0.05.

Results

This study included 180 pregnant women who were recruited from the Ahmadu Bello University Teaching Hospital, located in Zaria, which is situated in the North-West region of Nigeria. The age of participants ranged from 19 to 48 years old, with the majority (34.5%) being under the age of 30. The proportion of urban residents was 71.7% higher than the proportion of rural residents. Despite mostly having a tertiary education, 46.7% of the participants were unemployed. Medical records indicated that singleton pregnancy was common among participants, but the majority were in the second trimester and had given birth to two or more children. Enquiries about mosquito bites revealed that most participants had not been bitten by a mosquito in the past 30 days and the majority had no history of arboviral disease. Headache and fever were the most common symptoms among participants.

Anti-ZIKV immunoglobulin M antibodies (ZIKV IgM) were detected in serum samples obtained from recruited participants using an enzyme-linked immunoassay procedure. Among the 180 analyzed samples, 53 were seropositive, resulting in a prevalence of 29.4% (**table 1**). The age group 31–48 years had the highest seroprevalence (32.6%), while the age group 30 years or less had the lowest seroprevalence (26.6%). The prevalence was 34.1% among urban residents and 17.7% among rural residents. Seroprevalence by Education and Occupation was highest in participants with only primary school education (45.5%) and unemployed (35.7%), as shown in **table 2**.

The seroprevalence was recorded only in those with a singleton pregnancy based on pregnancy information. However, participants in the third trimester (33.3%) and primipara (39.2%) had the highest seroprevalence. The seroprevalence based on mosquito bite history was highest (35.7%) among those who were unaware of mosquito bite in the last 30 days. The seroprevalence of participants based on history of arboviral diseases showed a prevalence of 36.2% in those with a history of arboviral infections and a prevalence of 27.1% in those without a history of arboviral infections (**table 3**).

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

IgM	Samples Количество образцов	%
Positive / Положительные	53	29.4
Negative / Отрицательные	127	70.6
Total / Всего	180	100

Table 2. Seroprevalence of ZIKV IgM and sociodemographic variables

Variables	No tested (%) (out of a total of 180 examined) Число протестированных (%) (от общего числа, *n* = 180)	Seropositive (from 180) Число серопозитивных	Prevalence (from 53) Частота выявления	Significance (*p*-value) Достоверность отличий (значение *p*)
Age / Возраст				
≤ 30	94 (52%)	25	26.6%	0.5357
≥ 31–48	86 (48%)	28	32.6%	–
Residence / Место проживания				
Rural / Село	51 (28.3%)	9	17.7%	0.1097
Urban / Город	129 (71.7%)	44	34.1%	–
Education / Образование				
None / Отсутствует	6 (3.3%)	1	16.7%	0.8097
Primary / Начальное	11 (6.1%)	5	45.5%	–
Secondary / Среднее	39 (21.7%	11	28.2%	–
Tertiary / Высшее	124 (68.9%)	36	29.0%	–
Employment Status / Статус занятости				
Employed / Работают	96 (53.3%)	23	24.0%	0.2150
Unemployed / Безработные	84 (46.7%)	30	35.7%	–
Total / Всего	**180 (100%)**	**53**	–	–

Table 3. Seroprevalence of ZIKV IgM and Medical Variables

Variables Характеристики	No tested (%) Число протестированных (%)	Seropositive Число серопозитивных	Prevalence Частота выявления	Significance (*p*-value) Достоверность отличий (значение *p*)
Type of Pregnancy / Тип беременности				
Singleton / Одноплодная беременность	170 (94.4%)	53	31.2%	0.38
Twin / Двойня	5 (2.8%)	0	0%	–
Triple / Тройня	1 (0.6%)	0	0%	–
Other / Другое	4 (2.2%)	0	0%	–
Gestational Age / Гестационный возраст				
0–13	30 (16.7%)	5	16.7%	0.4211
14–26	84 (46.7%)	26	31.0%	–
27–40	66 (36.7%)	22	33.3%	–
Parity / Беременность				
Nulliparous / Нерожавшие	16 (8.9%)	3	18.8%	0.3743
Primiparous / Первородящие	51 (28.3%)	20	39.2%	–
Multiparous / Повторнородящие	113 (62.8%)	30	26.5%	–
History of Mosquito Bite / Наличие укусов москитов в анамнезе				
Yes / Да	67 (37.2%)	15	22.4%	0.4446
No / Нет	43 (23.9%)	13	30.2%	–
Unknown / Неизвестно	70 (38.9%)	25	35.7%	–
History of Arboviral Diseases / Наличие арбовирусных заболеваний в анамнезе				
Yes / Да	47 (26.2%)	17	36.2%	0.3875
No / Нет	133 (73.8%)	36	27.1%	–
Total / Всего	**180 (100%)**	**53**	–	–

Only six of the fifty-three samples that tested positive for anti-Zika IgM antibody were positive for ZIKV RNA, while the remaining forty-seven samples were negative, according to molecular analysis using RT-qPCR (**table 4**).

Discussion

Given the importance of preventing complications and hazards that arise during pregnancy, maternal and child health remains a public health priority. The current study, which screened pregnant women at Ahmadu Bello University Teaching Hospital, found an overall ZIKV seroprevalence of 29.4% among study participants. This finding was higher than those of Oderinde et al. (2020), who investigated the prevalence of locally undetected acute flavivirus infections in North-East Nigeria, and Sani et al. (2022), who conducted a serological study to detect ZIKV infection among HIV-infected pregnant women in a North-Western State of Nigeria [15, 16]. The prevalence rates of 22 and 4.5% were obtained in both studies, but this variation could be attributed to differences in study location and participants employed in the study.

When examining socio-demographic variables, the highest prevalence of 32.6% was found among participants over 30 years of age. Although this age group can still be referred to as childbearing age, a Puerto Rican study examining risk factors for ZIKV and Chikungunya infections found the highest prevalence in a similar older childbearing age group [17]. Nonetheless, the findings can be attributed to the age skewness of the study participants employed. The participants from urban areas had a higher prevalence of 34.1% than those from rural areas. This study's findings were consistent with those of Ticona et al. (2021) [18]. Although it is widely assumed that ZIKV infection is more prevalent in rural areas, rapid urbanisation, the presence of urban slums, and economically disadvantaged urban residents may all contribute to an increase in ZIKV infection prevalence. The participants' educational status revealed that those with only a primary education had the highest prevalence of 45.5%. In a seroprevalence study in peninsular Malaysia and Sabah, Khoo et al. (2022) also discovered higher prevalence rates among those with lower education status [19]. The striking similarities could be attributed to a lack of proper orientation and comprehension of the importance of arbovirus vectors in their environment. The unemployed participants were found to have a higher prevalence rate than the employed participants. The study's 35.7% prevalence rate contradicts the findings of Anejo-Okopi (2020) in a Nigerian study of ZIKV among HIV positive and HIV negative pregnant women [20]. This disparity could be attributed to differences in the study populations recruited for both studies.

According to the analysis of study participants' medical information, the prevalence was only recorded among participants with a singleton pregnancy but was highest among participants in their third trimester; this finding is consistent with those of Anejo-Okopi (2020), who observed a similar result among pregnant women in the study. Regardless of the trimester, pathogenesis of the virus is destructive. Primiparous participants had the highest prevalence of 39.2% based on parity. Previous studies, such as Cooper et al. (2019) and Shen et al. (2021), have linked primiparity to an increased risk of microcephalic cases in fetuses [7, 21]. This study al-

Table 4. Seroprevalence of ZIKV IgM and Symptomatic Characteristics

Symptoms Симптомы	No tested Число протести-рованных	Seropositive Число серопо-зитивных	Prevalence Частота выявления	Significance (*p*-value) Достоверность отличий (значение *p*)
None / Отсутствовали	36 (20%)	4	11.1%	0.1778
Fever / Лихорадка	30 (16.7%)	14	46.7%	–
Rash / Сыпь	2 (1.1%)	0	0.0%	–
Conjunctivitis / Конъюнктивит	2 (1.1%)	1	50.0%	–
Conjunctivitis + joint pain + headache + muscle pain / Конъюнктивит + боль в суставах + головная боль + мышечная боль	1 (0.6%)	1	100.0%	–
Joint pain / Боль в суставах	11 (6.1%)	4	36.4%	–
Headache / Головная боль	60 (33.3%)	16	26.7%	–
Muscle pain / Мышечная боль	18 (10%)	6	33.3%	–
Fever + joint pain + headache / Лихорадка + боль в суставах + головная боль	1 (0.6%)	0	0.0%	–
Joint pain + headache / Боль в суставах + головная боль	1 (0.6%)	0	0.0%	–
Joint pain + headache + muscle pain / Боль в суставах + головная боль + мышечная боль	2 (1.1%)	0	0.0%	–
Fever + headache / Лихорадка + головная боль	9 (5.0%)	2	22.2%	–
Fever + joint pain / Лихорадка + боль в суставах	2 (1.1%)	1	50.0%	–
Fever + joint + pain + muscle pain / Лихорадка + боль в суставах + боль в мышцах	1 (0.6%)	1	100.0%	–
Fever + headache + muscle pain / Лихорадка + головная боль + мышечная боль	1 (0.6%)	1	100.0%	–
Fever + joint pain +headache / Лихорадка + боль в суставах + головная боль	1 (0.6%)	1	100.0%	–
Fever + joint pain + headache + muscle pain / Лихорадка + боль в суставах + головная боль + мышечная боль	1 (0.6%)	1	100.0%	–
Headache + muscle pain / Головная боль + мышечная боль	1 (0.6%)	0	0.0%	–
Total / Всего	180 (100%)	53	–	–

so inquired about a history of mosquito bites and arboviral infections, both of which are known risk factors for Zika infection. The highest prevalence was found to be 35.7% and 36.2% among those who could not recall any recent mosquito bites and those who had previous arboviral infections, respectively. Despite the fact that most studies do not allow for the option of being unable to recall whether they have been bitten by a mosquito, Shaibu et al. (2021) found higher prevalence rates in pregnant women who were rarely bitten by mosquitoes, which appears to contradict the study's findings [22]. Although mosquito bites are still the main route of transmission, there are other routes of transmission that support the distribution of infection [22]. Based on history of arboviral infections, Mwayinka et al. (2021), who investigated the seroprevalence and associated risk factors of arboviral infections including Zika in Tanzania, found results similar to those obtained in this study [23]. Antibody Dependent Enhancement (ADE) is a common occurrence in people who have had previous arboviral infections. While fever and headache were the most common symptoms reported by study participants, participants with one or more ZIKV-related symptoms had a higher prevalence rate.

Molecular analysis detected ZIKV RNA in six out of fifty three samples positive for anti-Zika IgM, this equates to 11.3% sero-molecular prevalence. To the best of our knowledge, this is one of the recent studies to detect ZIKV RNA in Northern Nigeria since Mathe et al. (2016) who recorded a low prevalence from pregnant women in North Central Nigeria.

Conclusion

Infection with ZIKV during pregnancy can have severe implications for both the pregnant woman and the developing fetus. The current study conducted on pregnant women in Nigeria's northern region found a high prevalence of ZIKV infection, indicating a possible outbreak of the viral infection in the area. Even though the current disease burden in Nigeria is unknown, it is clear that ZIKV infection during pregnancy can have serious consequences, and pregnant women must take precautions to avoid infection.

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