Brief Report

Serologic evidence of Zika virus, hepatitis B virus and rubella virus in pregnant women attending a south-west based Nigerian tertiary care centre
An enzyme-linked immunosorbent assay based study

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Abstract

This study investigated serological evidence for Zika, hepatitis B and rubella virus infection in 92 pregnant women attending a south-west based Nigerian tertiary care centre from January to May 2023. Of the 92 samples, 31 (33.7%), 14 (15.2%), and 90 (97.8%) exhibited seropositivity for Zika, hepatitis B, and rubella virus respectively. Cases of co-positives were also reported, with 28.3% seropositive for both Zika and rubella virus, 10.9% for hepatitis B and rubella virus, and 4.3% for all three viruses. The findings of this study underscore that a considerable portion of participants are susceptible to Zika and hepatitis B virus. Conversely, the majority of the participants appeared to be protected against rubella virus. This study strongly advocates initiation of public awareness campaigns and formulation of vaccination policies targeting women of reproductive age. Such measures are crucial for countering viral infections which could harm the developing foetus and give rise to pregnancy-related complications.

Keywords: viral infection, pregnancy complication; Zika, rubella, hepatitis B

Introduction

Pregnant women are particularly vulnerable to viral diseases due to physiological and immunological changes during pregnancy. These changes can increase the risk of infection and worsen the severity of viral illnesses.[1] The immune system undergoes modifications to ensure the well-being of the foetus, resulting in a weakened immune response and increased susceptibility to viral infections.[2] Zika virus (ZIKV), rubella virus (RUBV), and hepatitis B (HBV) virus are three viral infections of particular concern as they can be transmitted from mother to child during pregnancy.[3,4] Seroprevalence of viral infections among pregnant women varies, based on location,

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population characteristics, and exposure risks. Vaccines for hepatitis B and rubella infections have been encouraged but are not monitored or widely accessible in Nigeria. Recent studies have reported the possible endemicity of these viruses, particularly in southwest Nigeria.[5–7] The objective of this study was to investigate serological evidence of Zika virus, hepatitis B virus and rubella virus infection in pregnant women attending Olabisi Onabanjo University Teaching Care Centre, Nigeria.

Methods

Study design

A cross sectional study was conducted on pregnant women receiving care at Olabisi Onabanjo University Teaching Hospital in Sagamu, Southwest, Nigeria. from January to May 2023.

Sample population and size

This study encompassed pregnant women who attended the antenatal units at the hospital. The participants were recruited for the study during their visits. Based on the prevalence rate of 4% obtained by Mathé et al., (2018)[8] in a study investigating Zika virus infection, an initial sample size of 59 was determined using Fischer's formula for a cross-sectional study design. However, it was later adjusted to 92 due to attrition and to determine statistical significance.

Data collection

Structured questionnaires were used to gather sociodemographic details of the participants, including gender, age, educational level, occupation, and residential area. Additionally, medical information such as gravidae and gestational age were also collected. Face-to-face interviews with the participants were conducted for this purpose.

Sample collection and analysis

Three ml of blood was collected aseptically from study participants and carefully transferred into a plain tube labelled with the participant's identification number. Serum was separated, transferred to cryovials and stored at -20 °C until further laboratory analysis.

Zika virus IgG ELISA

VIRCELL Zika ELISA IgG (VIRCELL Microbiologists Spain) was used to test for ZIKV IgG antibodies following manufacturer’s instructions. Sensitivity and specificity of the ELISA kit is 96% and 97% respectively.

Rubella virus IgG ELISA

The presence of rubella IgG was determined using the BIO-INTECO Rubella ELISA IgG test (Inteco Diagnostics UK. Ltd), following manufacturer’s instructions, Specificity and sensitivity are 100%

Hepatitis B Surface antigen ELISA

The BIO-INTECO Microbiologists (Inteco Diagnostics UK. Ltd) Hepatitis B Surface Antigen ELISA test was used to detect hepatitis B surface antigen (HBsAg) following manufacturer’s instructions. Specificity and sensitivity are 100%.

Statistical Analysis

The questionnaire data and laboratory analysis results were entered into Microsoft Excel and analysed using GraphPad Prism 5. Graphs and tables were used to present and compare

quantitative variables, and the chi-square and p-values were calculated. The statistical significance level was set at $P< 0.05$.

Results

The study involved the assessment of serum samples from 92 pregnant women recruited from Olabisi Onabanjo University Teaching Hospital for serological evidence of ZIKV, HBsAg and RUBV. Among the 92 participants, 31 (33.7%) were positive for ZIKV, 14 (15.2%) for HBsAg and 90 (97.8%) for RUBV. Analysis of the co-positives showed 26 (28.3%) were positive for both ZIKV and RUBV. No participant was positive for ZIKV and HBsAg and 10 (10.9%) were positive for HBsAg and RUBV. Only 4 (4.3%) were seropositive for all three viruses. Overall, participants with only RUBV IgG had the highest seropositivity.

For analysis of the sociodemographic variables in women with a single positive and those who were co-positive, the participants were grouped into two age categories, those less than 30 years of age and those 30 years and older. The highest seropositivity for all the viruses was noted with women 30 years and older. The highest co-positive rates were also in those 30 years and older. Those positive for all the three viruses had the same number of cases in both categories (Table 1).

Higher single and co-positives were noted in women residing in urban areas when compared to those residing in rural areas (Table 1). Seropositivity to ZIKV and RUBV was highest among those who had tertiary education while HBsAg seropositivity was highest among those with secondary school education. ZIKV and RUBV co-positives were also highest among participants with tertiary education while HBsAg and RUBV co-positive was higher among participants with secondary education. Based on their employment status, participants were grouped into employed, self-employed and unemployed. Seropositivity (single and co-positive) was highest in self-employed participants except for those positive for all the three viruses which was highest among the employed participants (Table 1).

Table 1: seropositivity and sociodemographic variables

Variables	**No tested (%)**	**Seropositive cases**			**Co-positive cases**		
		ZIKV	**HBV**	**RUBV**	**ZIKV & RUBV**	**HBsAg & RUBV**	**ZIKV, HBsAg, & RUBV**
Age							
< 30	44 (47.8%)	11 (25%)	3 (6.8%)	44 (100%)	8 (18.1%)	4 (9.1%)	2 (4.6%)
≥30-44	48 (52.2%)	20 (41.7%)	11 (22.9%)	46 (95.8%)	18 (37.5%)	6 (12.5%)	2 (4.2%)
Residence							
Rural	25 (28.3%)	8 (32%)	3 (12%)	25 (100%)	7 (28%)	2 (8%)	1 (4%)
Urban	67 (71.7%)	23 (34.3%)	11 (16.4%)	65 (97%)	19 (28.4%)	8 (11.9%)	3 (4.5%)
Education							
Primary	5 (6.1%)	3 (60%)	0 (0%)	5 (100%)	3 (60%)	0 (0%)	0 (0%)
Secondary	33 (21.7%)	11 (33.3%)	8 (24.2%)	33 (100%)	9 (27.2%)	6 (18.1%)	2 (6.1%)
Tertiary	54 (68.9%)	17 (31.5%)	6 (11.1%)	52 (96.3%)	14 (25.9%)	4 (7.4%)	2 (3.7%)
Employment Status							
Employed	30 (32.6%)	9 (30%)	3 (10%)	29 (96.7%)	8 (26.7%)	2 (6.7%)	3 (10%)
Self Employed	59 (64.1%)	21 (35.6%)	11 (18.6%)	59 (100%)	17 (28.8%)	8 (13.6%)	1 (1.7%)
Unemployed	3 (3.3%)	1 (33.3%)	0 (0%)	2 (66.7%)	1 (33.3%)	0 (0%)	0 (0%)
Total (100%)	**92**	**31**	**14**	**90**	**26**	**10**	**4**

The analysis of medical variables was based on the type of pregnancy of participants, gestational age, and parity. Most of the participants with single and co-positive seropositivity had a single pregnancy. For the gestational age, single seropositivity for ZIKV and HBsAg was the highest for participants in their third trimester while RUBV seropositivity was the highest for participants in the first two trimesters (Table 2)

Table 2: seropositivity and medical variables

Variables	No tested (%)	Seropositive (Prevalence)			Significance (p-value)
		ZIKV	HBsAg	RUBV	
Type of Pregnancy					
Single	89 (96.7%%)	31 (34.8%)	13 (14.6%)	87 (97.8%)	.5972
Twin	2 (2.2%)	0 (0%)	1 (50%)	2 (100%)	
Do not know	1 (1.1%)	0 (0%)	0 (0%)	1 (100%)	
Gestational Age					
0-13	16 (17.4%)	6 (37.5%)	1 (6.3%)	16 (100%)	.7272
14-26	42 (45.7%)	12 (28.6%)	6 (14.3%)	42 (100%)	
27-40	34 (36.9%)	13 (38.2%)	7 (20.6%)	32 (94.1%)	
Parity					
Nulliparous	29 (31.5%)	7 (24.1%)	5 (17.2%)	29 (100%)	.8451
Primiparous	30 (32.6%)	9 (30%)	3 (10%)	28 (93.3%)	
Multiparous	38 (41.3%)	15 (39.5%)	6 (15.8%)	38 (100%)	
Total (100%)	92	31	14	90	

Discussion

In this study, it was found that the prevalence of ZIKV stood at 33.7%, a notably higher figure compared to the 14.4% recorded by Anejo-Okopi et al., in 2020.[9] Cross-reactivity is a typical occurrence and could affect the outcome of the analysis, particularly when utilising serological techniques to detect antibodies to closely related viruses of the Flavivirus genus. There has been little research on the current incidence of flavivirus infection like dengue in the study area with possible dengue virus circulation due to its endemicity in various parts of the country. Furthermore, vaccination against yellow fever is recommended in Nigeria, but specific information on the yellow fever vaccination status of study participants was not collected. While the kit's specificity for Zika virus IgG detection was established, we did not specifically test cross-reactivity with other flaviviruses because it was outside the scope of the study.

The current study found the prevalence of HBsAg seropositivity to be 15.2%. which is notably higher than the 7.9% prevalence noted by Tanga et al., in 2019.[10] Although generally recommended, hepatitis B vaccination has been reportedly low in the study area despite its prevalence, which could be due to lack of awareness and vaccine hesitancy among the population. This study's observation of a high prevalence of RUBV of 97.8% shows adherence to the country's vaccination policy in the study area and aligns with earlier investigations by Akele et al (2020).[11] There is no routine surveillance or vaccination policy for rubella virus infection in Nigeria. Administration of rubella vaccine is done for children at a very early age with the measles mumps and rubella (MMR) vaccine. IgG seroprevalence could therefore be attributed to vaccination coverage in the study area.[12] The prevalence of HBsAg -RUBV co-positivity was low. Given the

importance of vaccination in preventing viral infections, this could be due to vaccine unavailability, low public awareness, and limited healthcare access in the studied region.

Examining sociodemographic factors, a greater prevalence rate of ZIKV IgG was noted among individuals aged 30 and above, and those living in urban areas. The susceptibility of older people to infection is attributed to compromised immune systems, pregnancy, and living in underprivileged or recently developed urban regions. Although not statistically significant, these findings align with earlier research on Zika virus infections.[9]

The study found a higher prevalence of HBsAg in individuals >30 years, consistent with Kolawole et al. (2012)[13], and a prevalence of 16.4% among urban residents was also reported. RUBV antibodies were significantly present in those aged 30 and older, aligning with Akele et al. (2020) [11], who reported a 100% prevalence in participants aged 35 years and above. Prevalence of seropositivity to RUBV, HBsAg, and ZIKV was higher in individuals who were self-employed or had secondary education. Although the vaccination history was not obtained, participants, mostly in their third trimester showed a high prevalence of HBsAg (20.6%), aligning with Kolawole et al. (2012). Parity also influenced HBSAG prevalence, contrasting with Mustapha et al. (2020).[14] Despite extensive vaccination campaigns for hepatitis B and rubella in Nigeria, coverage gaps remain, particularly in rural and underserved areas.[15] Zika virus circulation continues to be a concern, necessitating enhanced public health measures and surveillance to mitigate its impact on vulnerable populations.[9]

Conclusion

The study provides valuable insights into the seroprevalence of IgG antibodies against Zika virus (ZIKV), rubella virus (RUBV), and hepatitis B surface antigen (HbsAg). The findings also revealed the presence of co-positivity for ZIKV and RUBV, indicating prior contact to these viruses in the community either through infection or vaccination. Furthermore, the identification of HbsAg seropositivity emphasises the necessity of ongoing vaccination efforts to avoid vertical transmission of the hepatitis B virus to newborns. These findings underline the importance of comprehensive screening and vaccination programmes for pregnant women in preventing adverse pregnancy outcomes caused by congenital infections.

Declaration

Conflicts of Interest: None declared by the authors.
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References

1. Rychlik KA, Sillé FCM. Environmental exposures during pregnancy: Mechanistic effects on immunity. *Birth Defects Res*. 2019; 111(4):178-196. *doi:10.1002/bdr2.1469*
2. Phoswa WN, Khaliq OP. Is pregnancy a risk factor of COVID-19? *Eur J Obstet Gynecol Reprod Biol*. 2020; 252:605-609. *doi:10.1016/j.ejogrb.2020.06.058*

3. Rasmussen SA, Jamieson DJ. Teratogen update: Zika virus and pregnancy. *Birth Defects Res*. 2020; 112(15):1139-1149. *doi:10.1002/bdr2.1781*
4. Cheung KW, Lao TTH. Hepatitis B – Vertical transmission and the prevention of mother-to-child transmission. *Best Pract Res Clin Obstet Gynaecol*. 2020; 68:78-88. *doi:10.1016/j.bpobgyn.2020.02.014*
5. Otu AA, Udoh UA, Ita OI, et al. Prevalence of Zika and malaria in patients with fever in secondary healthcare facilities in south-eastern Nigeria. *Trop Doct.* 2019; 50(1):22-30. *doi:10.1177/0049475519872580*
6. Atilola G, Tomisin O, Randle M, et al. Epidemiology of HBV in Pregnant Women, South West Nigeria. *J Epidemiol Glob Health*. 2018; 8(3):115-123. *doi:10.2991/j.jegh.2018.09.002*
7. Fatiregun AA, Fagbamigbe AF, Adebowale AS. Epidemiology of rubella disease in south-west nigeria: trends and projection from measles case-based surveillance data. *South African J Infect Dis*. 2014; 29(2):60-64. *doi:10.1080/23120053.2014.11441571*
8. Mathé P, Egah DZ, Müller JA, et al. Low Zika virus seroprevalence among pregnant women in North Central Nigeria, 2016. *J Clin Virol*. 2018; 105:35-40. *doi:10.1016/j.jcv.2018.05.011*
9. Anejo-Okopi J, Gotom DY, Chiehiura NA, et al. The seroprevalence of Zika Virus Infection among HIV positive and HIV negative pregnant women in Jos, Nigeria. *Hosts and Viruses*. 2020; 7(6):129-136 *doi:10.17582/journal.hv/2020/7.6.129.136*
10. Tanga AT, Teshome MA, Hiko D, et al. Sero-prevalence of hepatitis B virus and associated factors among pregnant women in Gambella hospital, South Western Ethiopia: facility based cross-sectional study. *BMC Infect Dis*. 2019; 19(1):602. *doi:10.1186/s12879-019-4220-z*
11. Akele RY, Pam BN, Oluboyo BO, et al. Seroprevalence of Rubella IgG and IgM Antibodies among Pregnant Women Attending Antenatal Clinic in Federal Teaching Hospital Ido-Ekiti, Nigeria. *Asian J Immunol*. 2020; 4(1):27-35. *doi: https://dx.doi.org/10.21010/ajid_v15i2.3*
12. Durowade K, Musa O, Jimoh M, et al. Burden, epidemiological pattern, and surveillance gap of rubella in Nigeria: A call for routine vaccination policy. *Indian J Heal Sci Biomed Res*. 2021; 14(1):31. *doi:10.4103/kleuhsj.kleuhsj_54_20*
13. Kolawole OM, Wahab AA, Adekanle DA, et al. Seroprevalence of hepatitis B surface antigenemia and its effects on hematological parameters in pregnant women in Osogbo, Nigeria. *Virol J.* 2012; 9:1-6. *doi:10.1186/1743-422X-9-317*
14. Magaji FA, Okolo MO, Yiltok ES, et al. Prevalence of hepatitis B virus infection in pregnant women with and without HIV in Jos, Nigeria. *Int J Infect Dis*. 2021; 104:276-281. *doi:10.1016/j.ijid.2020.12.058*
15. Mahachi K, Kessels J, Boateng K, Baptiste AE, Mitula P, Ekeman E, Lochlainn LN, Rosewell A, Sodha SV, Abela-Ridder B, Gabrielli AF. Zero-or missed-dose children in Nigeria: contributing factors and interventions to overcome immunization service delivery challenges. Vaccine. 2022 Sep 2;40(37):5433-44. *doi.org/10.1016/j.vaccine.2022.07.058.*