



Serum Proteogenomic Investigation of C-X-C Motif Chemokine 10 (CXCL10) and Zika Virus RNA in Pregnant Women of Nigerian Tertiary Teaching Hospitals

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Abstract

Zika virus (ZIKV) is a type of mosquito-borne virus belonging to the Flavivirus genus. It has been associated with microcephaly and neurological disorders in newborns. During Zika virus infection in pregnant women, the chemoattractant C-X-C motif chemokine ligand 10 (CXCL10) shows a significant increase, up to 200-fold and has been linked to the pathogenesis of ZIKV. This study investigated the relationship between Zika virus infection and CXCL10 overexpression. Sixty-two samples were positive for ELISA while Seven out of sixty-two seropositive samples were confirmed by PCR for the presence of Zika virus RNA, indicating an 11.1% prevalence. The number of ZIKV RNA detected in the seven serum samples ranged from 1.0×10^2 to 11.6×10^3 copies/ml. Among samples positive for ZIKV RNA, gene expression analysis revealed regulation values ranging from 1.0 to 126.2. CXCL10 was overexpressed in four of the seven samples, with 4-, 24-, 27-, and 126-fold increase. This study linked Zika viremia with CXCL10 overexpression in pregnant women and identified age, gestational age, and ZIKV-related symptoms as risk factors for CXCL10 overexpression in pregnant women infected with ZIKV

Keywords: ZIKV; CXCL10; Disease Epidemiology; Infection Diagnosis; Pregnancy Complications; Serology

1.0 Introduction

The re-emergence of infectious diseases is projected to increase global health risks in the next two decades (van Doorn, 2021). However, the success of national and international efforts to establish effective public health infrastructure with robust surveillance and response systems will significantly influence this future trajectory (Fauci, 2016). Zika virus, a mosquito-borne flavivirus in the Flaviviridae family, has gained attention in recent years due to its association with microcephaly and neurological disorders (Noorbakhsh et al., 2019; Tan et al., 2022). Phylogenetically, Zika virus is closely related to other medically important flaviviruses such as Dengue, West Nile, Japanese encephalitis, and Yellow fever viruses (Guarner & Hale, 2019; Song et al., 2017). While the virus remained largely unknown for several decades, isolated cases of Zika virus-related illnesses were reported in African and Asian countries (Yudhaputri et al., 2017).

Aedes mosquito species are the primary transmitters of Zika virus, although other modes of transmission have been confirmed (Kazmi et al., 2020; Marcellin et al., 2022; Rabaan et al., 2017). The Zika forest was known to harbor mosquito species of the *Aedes* and *Culex* genera (Dasti, 2016). Previously, the virus primarily infected monkeys, but mosquitoes have played a central role in transmitting the virus to humans (Depoux et al., 2018). Human transmission can occur through mosquito bites, blood transfusions, sexual contact, mother-to-child transmission during pregnancy and breastfeeding, posing challenges in developing effective control measures for the virus (Centeno-Tablante et al., 2021; Yarrington et al., 2019).

Zika virus was first discovered in the Zika forest of Uganda during yellow fever investigations, with the first human isolation occurring in Nigeria in 1954 (Asebe et al., 2021; Gubler et al., 2017). The virus did not cause epidemics until 2007 when an outbreak occurred on Yap Island in Oceania (Boyer et al., 2018; Gubler et al., 2017). Since then, Zika virus has become widespread in Africa, Asia, the South Pacific, and South America, with recent outbreaks reported in these regions (Akhtar et al., 2022; Custer et al., 2022; Kama et al., 2019; Mathé et al., 2018b; Parra et al., 2022; Rainey et al., 2022; Silva et al., 2019; Tinto et al., 2022; Zhou et al., 2020).

Proper immune clearance of viral pathogens relies on precise chemokine and cytokine signaling, as they coordinate the localization, activation, and polarization of innate and adaptive immune cell subclasses (Antonia et al., 2019). The C-X-C motif chemokine ligand 10 (CXCL10) is a small 8.7 kDa protein that is well characterized as a chemoattractant for immune cells such as T-lymphocytes and monocytes (Jin et al., 2017; McKimmie & Michlmayr, 2014). The CXCL10 which is also known as interferon-gamma-induced-protein 10, signals via binding to its receptor CXCR3 (Lee et al., 2017). During inflammatory conditions, CXCL10 is secreted from a variety of cells such as monocytes, keratinocytes, fibroblasts, and endothelial cells in response to the interferon-gamma (Jin et al., 2017; van den Borne et al., 2014). CXCL10 plays a significant role in NK cell recruitment and limitation of viral replication, it appears more during acute infection and increased levels of CXCL10 have been significantly associated with decreased levels of viral titer (Loux et al., 2010; Manickam et al., 2020).

The immune response during the acute phase of Zika virus is broadly inflammatory and polyfunctional (Naveca et al., 2018). There is a robust proinflammatory cytokine response during this acute phase, so there is a polyfunctional immune activation associated with increased levels of chemokines such as CXCL10 (Kam et al., 2017; Ornelas et al., 2017). However, high levels of pro-inflammatory biomarkers, including CXCL10, can increase the permeability of the blood-brain barrier, potentially facilitating viral transmission to the central nervous system during virus clearance (Manickam et al., 2020).

The overexpression of CXCL10, induced by the Zika virus NS5 protein via the interferon-gamma signaling pathway, has been linked to viral pathogenesis (Chaudhary et al., 2017; Coish et al., 2022). Pathway analysis during Zika virus infection has also shown that the majority of cytokines induced were involved in the recruitment of monocytes and NK cells, whereas excessive infiltration of these cells to the maternal-foetal interface has been associated with pregnancy complications such as preeclampsia and preterm birth (Maucourant et al., 2021; Roth et al., 2022). Therefore viral pathogenesis of the Zika virus may be a result of the infiltration of leukocytes, particularly monocytes and NK cells during the inducement of cytokines in pregnant women (Foo et al., 2018).

The Zika virus disease, although usually a mild condition, has been more associated with microcephaly and other congenital abnormalities (Oeser & Ladhani, 2019; Rasmussen & Jamieson, 2020). The Zika virus infects human dermal fibroblasts, epidermal keratinocytes, and immature dendritic cells, the virus is also capable of bypassing the placental barrier and infecting placental macrophages and cytotrophoblasts. This results in infection of the placenta and foetal brain damage (Gabriel et al., 2017; Souza et al., 2022).

In Africa, Zika fever has largely been under-reported, physicians have misdiagnosed or underdiagnosed the disease due to its overlapping symptoms with other arbovirus diseases such as dengue and chikungunya fever (Asebe et al., 2021; Paixão et al., 2018). Since the discovery of the Zika virus in Africa, the virus has been classified into two genetically distinct lineages: the Asian and the African Lineage (Nutt & Adams, 2017). The African Zika virus circulated quietly until recently in which the disease has been reported in African counties such as Guinea Bissau, Angola, Central African Republic, and Nigeria (IAMAT, 2020; NCDC, 2016; Nutt & Adams, 2017; Reuters, 2018). Recent reports have suggested an African epidemic of Zika fever could be overlooked and undetected, the growing suspicious concern of the virus linking to birth defects in some parts of the continents has been reported (Nutt & Adams, 2017). Although Reports of congenital defects such as microcephaly and Central Nervous System damage have been made from

countries like Guinea Bissau and the Central African Republic, there is a paucity of data to follow up on these reports (Nutt & Adams, 2017).

The challenge of Zika virus infection in resource-limited settings such as Nigeria remains enormous, infections could be misdiagnosed or underdiagnosed as malaria, typhoid or other forms of pyrexia in pregnant women (Okunade, 2018). In recent years, only a few isolated studies have reported significant prevalence among pregnant women in various regions of the country, but most of these studies were based on the detection of Zika Virus antibodies (Anejo-Okopi et al., 2020; Kolawole et al., 2020; Mathé et al., 2018a; Oluwole et al., 2022; Shaibu et al., 2021). Considering the non-pathognomonic symptoms and cross antigenicity with flaviviruses with genetic and vector relatedness such as Dengue, Yellow fever, Chikungunya and West Nile viruses which are relatively common in Nigeria, there is a need for deeper investigation of Zika virus in Nigeria using molecular methods and immunological biomarkers. This study aims to determine the prevalence of Zika virus among pregnant women in northern and southern Nigeria as well as investigate the relationship between Zika virus infection and overexpression of CXCL10.

2.0 Materials and Methods

Study Area

The research was conducted at two different sites, namely Ahmadu Bello University Teaching Hospital (ABUTH) in Zaria, which represents the Northern Region of Nigeria, and Olabisi Onabanjo University Teaching Hospital (OOUTH), which represents the Southern Region of Nigeria.

Ethical Approval

Ethical approval for this study was granted by the Health Research Ethics Committees of both hospitals. In accordance with established human experimentation standards and the Helsinki Declaration of 1975 (revised in 2000), informed consent was obtained from all participants. This was achieved by ensuring that all recruited participants completed informed consent forms, thus affirming their willingness to participate in the study.

Study Design

This study was conducted in Northern (Ahmadu Bello University Teaching Hospital, Zaria) and Southern (Olabisi Onabanjo University Teaching Hospital, Sagamu) Nigeria, focusing on pregnant women who attended antenatal clinics at tertiary hospitals. It was designed as a cross-sectional study, taking place within the hospital setting.

Study Population

The study included consecutively consenting pregnant women who visited the Antenatal Units of tertiary hospitals. The participants were enrolled in the study as they arrived at the hospital.

Sample Size Determination

The sample size was calculated using Fischer's formula for a cross-sectional study design. Using the 20% prevalence reported by Otu et al (2019)

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

Where n = sample size of subjects required for the study

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

P = Prevalence rate = 0.20 (Otu, et al., 2019)

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

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

$$n = \frac{1.96 \times 1.96 \times 0.8 \times 0.2}{0.0025} = 245$$

The sample size considered in this study was increased to 360 for equal distribution in both study areas.

Questionnaires

Structured questionnaires were used to collect sociodemographic information (gender, age, educational level, occupation, and residential area) and medical information from the participants (gestational age, Zika virus infection-related symptoms, history of mosquito bites, and history of arboviral infection). Data were obtained through face-to-face interviews.

Sample Collection, Preparation and Storage

Venous blood was drawn from the antecubital veins of the study participants by placing a tourniquet on the upper arm and tightening it sufficiently to prevent venous return. The blood collection site was sterilised with 70% alcohol and dried using 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 mls of blood was carefully dispensed into a plain tube. Subsequently, the tube was labelled with the participant's identification number. Sera were extracted from blood samples collected from the participants by allowing the blood to clot at room temperature before centrifugation at 2500rpm for 10 min. It was then placed in cryovials and stored at -80 °C until laboratory analysis.

Laboratory Analytical Procedure

Anti-Zika IgM Enzyme-Linked Immunosorbent Assay for pregnant women

VIRCELL Microbiologists Zika ELISA IgM (catalogue number M1023) was used to screen serum samples for anti-ZIKV IgM antibodies. According to the instructions included in the kit, positive, negative, and cut-off controls were included in each test. This allowed for the validation of the assay and kit. The optical density must also be within the control parameters. Otherwise, the test is null and void, and must be redone. The results were interpreted by first calculating the mean optical densities for cut-off control and then calculating the outcome of each well using the formula below:

$$\text{Antibody index} = \left(\frac{\text{sample optical density}}{\text{mean optical density of cut off serum}} \right) \times 10$$

Samples with equivocal results were to be retested using the same or new samples; samples with indexes less than 9 were considered negative for IgM-specific antibodies against Zika, and samples with indexes greater than 11 were considered positive for IgM-specific antibodies against Zika (i.e., positive). Subsequently, the positive samples were chosen for Molecular Analysis.

Detection of Zika Virus RNA from Anti-ZIKV IgM seropositive samples

RNA was extracted from anti-ZIKV IgM-positive samples recruited for molecular analysis using the Zymo Research Quick-RNA Viral Kit (catalogue number: R1034/1035) according to the manufacturer's instructions. For easier storage and stability of samples, cDNA was synthesised using the New England BioLabs One Taq RT-PCR Kit (catalogue number: E5310). The Zika virus RNA was amplified using primers containing the viral membrane protein sequence (forward primer: CCGCTGCCCAACACAAG; reverse primer: CCACTAACGTTCTTTTGCAGACAT), as described by Hcini et al. (2021). The molecular procedure involved the use of both conventional and RT qPCR protocols, and gel image confirmation was performed after each protocol. For conventional PCR, a 25µl PCR reaction containing 2µl of the cDNA synthesized from the extracted RNA sample was mixed with 12.5µl of One Taq Hot Start 2x Master Mix, 0.5µl each of the forward and reverse primers, and 9.5µl of nuclease-free water to make a total volume of 25µl in each microfuge tube. After mixing the reaction cocktail with the microfuge, the microfuge tubes were placed in a thermal cycler to amplify the targeted Zika virus sequences. After approximately 2 h 30 min of amplification, the tubes were removed and subjected to gel electrophoresis. For RT qPCR, the volume per 20 µl reaction for each PCR was composed of 6 µL of SYBR Green Master Mix (2X), 1 µL of ZIKV primers, and 5 µL of the synthesised cDNA sample. Samples were run in replicates to increase accuracy. The cDNA amplification was carried out with a Roche Light Cycler 480 real-time cycler, using the following cycling program: 55°C for 10 min followed by 40 cycles of 95°C for 1 min and another 10 secs, 60°C for 30 secs, which was then followed by a melting curve (65 °C to 95 °C) acquisition step. The resulting threshold cycle values for the plate were then exported to a blank Excel worksheet. An

automatic datasheet for analysis was then downloaded. For gel electrophoresis, 5 µl each of the loading dye and the PCR products were first added together in a 600 µl Eppendorf tube, the entire volume of the mixture was then aliquoted into each well of the gel. The DNA ladder was as well loaded into one of the wells. The condition for electrophoresis was then set at 110V and 70A for 40 minutes. The gel was then placed in an EBOX manufactured by VILBER for Gel Imaging and documentation with the aid of UV light.

CXCL10 Expression Analysis

Using CXCL10 specific primers (forward-CAAGGCTTCCCAATTCTC and reverse-ACCTGGACTGCATTTGA) were used as described by Cui et al. (2014). The data obtained were analysed using fold-changes. The PCR components and CXCL10 RT-qPCR primers were mixed to form a cocktail. Samples were run in replicates to increase accuracy. The expression of CXCL10 (fold induction) was quantified by calculating the $2^{-\Delta CT}$ value using human beta-actin (ACTB) as an internal control. The fold changes in gene expression after PCR amplification were calculated in comparison to the values for the controls as follows: fold change = $2^{-(\Delta CT_{\text{experiment}} - \Delta CT_{\text{control}})}$, where $\Delta CT = CT(\text{gene of interest}) - CT(\text{housekeeping gene})$. The average of the reverse transcription control values and the positive PCR control CT values were used to normalise gene expression and determine the fold changes between groups. To evaluate gene expression, a fold change threshold of at least 2-fold up- or down-regulation was selected and compared with the level in the analysed samples. Gene regulation was considered statistically significant at the 95% confidence level ($p < 0.05$). Statistical analysis was performed using RT2 profiler RT-PCR array data analysis software version 3.5.

Statistical Analysis

The data obtained from the questionnaire and the results of the laboratory analysis were entered into Microsoft Excel and analysed using GraphPad Prism 5. The quantitative variables were presented and compared using graphs and tables, and chi-square and p-values were calculated. Statistical significance was set at $p < 0.05$.

3.0 Results

All participants in this study were pregnant women who presented at Ahmadu Bello University Teaching Hospital, Zaria (North), and Olabisi Onabanjo University Teaching Hospital, Sagamu (South). Equal numbers of serum samples (180 each) from both study areas were initially tested for anti-ZIKV IgM seropositivity. Of the 360 samples collected, only 62 tested positive for anti-ZIKV IgM, 53 (29.4%) tested positive for the northern samples, and 9 (5%) tested positive for the southern samples (Table 1). The age range of the seropositive northern participants was between 20 and 45 years (median age = 30 years), while that of the southern participants was between 23 and 41 years (median age = 30 years). Northern participants had the highest seropositivity (26) among those in their second trimester, while those in their first trimester had the lowest (5). The southern participants had the highest seropositivity in both the second and third trimesters (4),

Table 1: Distribution of Anti-ZIKV IgM Seropositivity across the Study population

	North		South		Overall	
	Samples	Percentage	Samples	Percentage	Samples	Percentage
Anti-ZIKV IgM						
Positive	53	29.4%	9	5.3%	62	17.2%
Negative	127	70.6%	171	94.7%	298	82.8%
Total	180	100%	180	100%	360	100%

while only one seropositive participant was recorded in the first trimester. Overall, participants in their second trimester had the highest number of seropositive samples (30), while those in their first trimester had the lowest seropositive samples (6) (Table 2). Although the majority of the seropositive participants (71%) experienced only one symptom related to ZIKV infection, most participants did not experience fever (75%) and did not admit being bitten by a mosquito in the last 30 days (41.9%). It was also observed that the majority had not experienced a previous arboviral infection (Table 2).

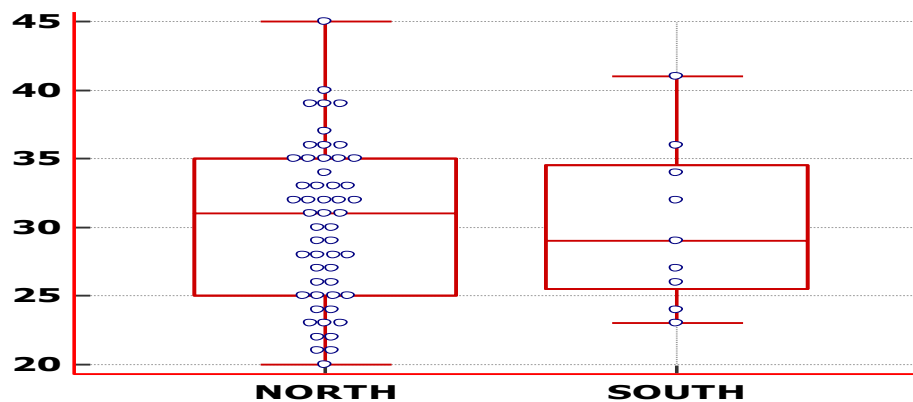


Figure 1: A box and whisker plot showing the age distribution of seropositive participants in each study group

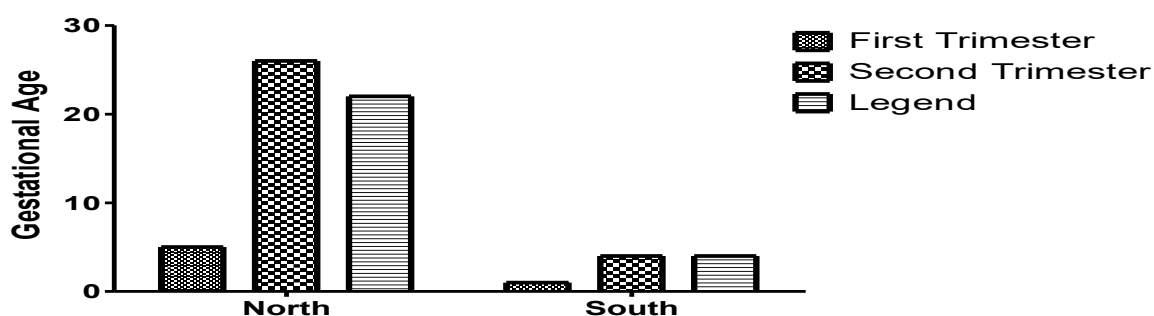


Figure 2: Clustered column showing seropositivity of participants in relation to gestational age.

Table 2: Anti-ZIKV IgM Seropositivity and Medical Information of Seropositive Participants

VARIABLES	NORTH N (%)	SOUTH N (%)	TOTAL
Frequency of Symptoms			
0	4 (7.6%)	4 (44.5%)	8 (12.9%)
1	41 (77.4%)	3 (33.3%)	44 (71.0%)
2	3 (5.6%)	2 (22.2%)	5 (8.1%)
3	3 (5.6%)	0 (0%)	3 (4.8%)
>3	2 (3.8%)	0 (0%)	2 (3.2%)
History of Fever			
Yes	14 (26.4%)	1 (11.1%)	15 (24.2%)
No	39 (73.6%)	8 (88.9%)	47 (75.8%)
History of Mosquito bites			
Yes	15 (28.3%)	1 (11.1%)	16 (25.8%)
No	13 (24.5%)	7 (77.8%)	20 (32.3%)
Don't know	25 (47.2%)	1 (11.1%)	26 (41.9%)
History of Arboviral infection			
Yes	16 (30.2%)	0 (0%)	16 (25.8%)
No	37 (69.8%)	9(100%)	46 (74.2%)
TOTAL	53 (100%)	9 (100%)	62 (100%)

Viral load Quantification and CXCL10 Expression

Following the screening of serum samples obtained from both northern and southern participants using the anti-ZIKV IgM ELISA procedure, the seropositive samples from both groups were subjected to PCR. An initial assessment was performed using the conventional PCR method to detect and eliminate false-positive samples. Only seven of 62 serum samples from participants initially suspected of Zika virus infection were found to be positive for the presence of Zika virus RNA using primers targeting the membrane protein gene, while the remaining 55 were found to be negative by the assay. Six of the seven samples (11.3%) belonged to the northern group, whereas only one belonged to the southern group (11.1%).

RT-qPCR was used to quantify viral copies in seven samples that tested positive for Zika virus RNA. After RT-qPCR analysis, all seven samples were positive for ZIKV RNA and were subjected to Gel Electrophoresis a second time. Amplification was not observed in the negative control reaction, which used nuclease-free water instead of the targeted templates. The quantification cycle (C_q) values were plotted, and the copy numbers of ZIKV genomes were calculated per millilitre using DNA Copy Count (Table 3). The melt-curve analysis of the RT-qPCR showed a T_m of 77.0 °C – 82.2 °C whenever ZIKV RNA was present in the samples. The number of ZIKV RNA detected in the 7 serum samples ranged from 1.0×10^2 to 11.6×10^3 copies/ml, as shown in Table 4. The table shows the participants' ages and gestational ages, their history of mosquito bites, and the number of symptoms they experienced.

CXCL10 overexpression was investigated using gene expression analysis in samples positive for ZIKV RNA. Gene expression analysis of seven samples tested in duplicate revealed that four samples positive for ZIKV RNA showed overexpression of CXCL10, while the other three showed normalized expression. All four samples with overexpression came from northern participants. From the relative normalized expression values obtained from gene expression analysis, the quantification cycle (C_q) values were plotted, and the regulation threshold was determined (Table 4). The regulation values ranged from 1.0 to 126.2, as shown in table 4.18. The table also included chi-square and p-values for significance.

Table 3: RT-qPCR End point Data for Samples Positive ZIKV RNA

S/N	Fluor	Sample	C_q^*	T_m (°C)*	End RFU*	Call
1	SYBR	N-11	33.17	80	64.4	(+) Positive
2	SYBR	N-27	32.42	81.5	75	(+) Positive
3	SYBR	N-42	35.31	78.5	53.4	(+) Positive
4	SYBR	N-54	34.56	80.3	66.1	(+) Positive
5	SYBR	N-76	34.15	82.2	68	(+) Positive
6	SYBR	N-80	35.79	78.5	46	(+) Positive
7	SYBR	S-134	37.26	80.3	28.7	(+) Positive

*- mean values were reported, N-North, S-South

Table 4: Quantification of ZIKV RNA Copies in Positive Samples

S/n	Sample	Copies	Age	Gestational age	History of mosquito bite	No of symptoms	Chi-Square (x^2)	Significance (p-value)
1	N-11	6×10^3	26	28	Don't know	3	0.0447	0.8325
2	N-27	11.6×10^3	29	21	Yes	1		
3	N-42	5×10^2	23	26	No	2		
4	N-54	4.3×10^3	25	26	Don't know	1		
5	N-76	4.3×10^3	21	29	No	3		
6	N-80	1.7×10^3	39	49	Don't know	1		
7	S-134	1×10^2	24	28	No	1		

N-North, S-South

Table 5: Gene expression analysis of Samples Positive for ZIKV RNA**Table 6: Overexpression of CXCL10 in Samples Positive for ZIKV RNA**

S/n	Sample	Cq	Normalized expression	Relative expression	normalized	Regulation	Comparison to regulation threshold
1	N-11	38.85	0.04240	4.28872		4.28872	Up-regulated
2	N-27	35.74	0.27375	27.69034		27.69034	Up-regulated
3	N-42	36.97	1.24798	126.23446		126.23446	Up-regulated
4	N-54	36.27	0.24062	24.33936		24.33936	Up-regulated
5	N-76	39.81	0.02852	2.88501		2.88501	No changes
6	N-80	38.35	0.00989	1.00000		1.00000	No changes
7	S-134	39.94	0.03914	3.95895		3.95895	No changes

S/n	Sample	Regulation	CXCL10 Overexpression	Chi-Square (x ²)	Significance (p-value)
1	N-11	4.288716	(+) Positive	54.365	<0.0001
2	N-27	27.69034	(+) Positive		
3	N-42	126.2345	(+) Positive		
4	N-54	24.33936	(+) Positive		
5	N-76	2.885009	(-) Negative		
6	N-80	1.00000	(-) Negative		
7	S-134	3.958953	(-) Negative		

4.0 Discussion

There is currently no Zika virus pandemic in the world, but reports of prevalence have been recorded in nearly all countries in the tropics and subtropics that aid in the breeding of their vectors (Meltzer et al., 2019). India, the United States, Indonesia, Brazil, Bangladesh, and Nigeria are the leading countries with current or past transmission cases of Zika infection, with the possibility of a Zika outbreak as of 2022 (World Population Review, 2022). In Africa, seromolecular prevalence has been reported in more than 15 countries since the first Zika virus case in the continent. However, studies carried out in the post-epidemic era (after the 2015 epidemic) have reported its prevalence in Guinea-Bissau, Cape Verde, Kenya, Angola, and Nigeria (Asebe et al., 2021; Faye et al., 2020; Gobillot et al., 2020; Hill et al., 2019; Rosenstierne et al., 2018). Zika infections in Angola and Cape Verde are significant outbreaks (Adam & Jassoy, 2021).

In this study, a seromolecular prevalence of 11.3% was obtained after seven samples were confirmed by RT-qPCR for the presence of ZIKV RNA. This prevalence is low when compared with a similar molecular study by Mota et al. (2021)(Mota et al., 2021), which reported a prevalence of 19.23%. Mota et al. (2021) (Mota et al., 2021), who aimed to determine the serological and molecular epidemiology of Dengue, Chikungunya and Zika viruses in a risk area in Brazil, initially tested for the presence of IgM antibodies of these viruses in plasma samples obtained from participants with symptomatic features of arboviral infections before confirmation using RT-qPCR. Nonetheless, Sanchez-Carbonel et al. (2018)(Sánchez-Carbonel et al., 2018), in a study that identified infection by Chikungunya, Zika and Dengue in an area of the Peruvian coast, reported a lower prevalence of 7.9% when compared to the findings of this study. In two recent studies conducted in Nigeria, Mathe et al (2018) (Mathé et al., 2018b) in a seroprevalence study among pregnant women in North Central Nigeria reported a very low prevalence of 0.2% among seropositive samples while Shaibu et al., (2021) (Shaibu et al., 2021) in a sero-molecular prevalence study

among pregnant women in Lagos, Nigeria; detected no ZIKV RNA in the seropositive samples subjected to RT-qPCR. The apparent varying differences between this current study and other compared studies could be due to the study area, sample participants, or other factors, such as socio-demographics.

According to previous studies, high viral loads in the blood are associated with an imbalanced immune response (Santiago et al., 2019). Weak neutralisation or failure of antibodies to neutralise the infecting virus results in more efficient virus uptake and replication, and ultimately, higher viral loads in serum samples (Santiago et al., 2019). Another significant factor affecting Zika blood viral load is antibody-dependent enhancement. Secondary flavivirus infection results in a significantly higher peak viral load and increased disease severity after an initial primary flavivirus infection (Tang et al., 2020). Tang et al. (2020) investigated the impact of antibody-dependent enhancement on Zika virus and dengue virus sequential and co-infection disease severities using a model approach. Analysis revealed that antibody-dependent enhancement could significantly determine a sharp increase or decrease in the viral load during secondary Zika infection (Tang et al., 2020). On the other hand, pre-existing immunity to ZIKV is the determinant of the high-level effect of antibody-dependent enhancement. Although a possible antibody-dependent enhancement effect results in a higher peak viral load, earlier peak time, and greater disease severity, secondary infection is beneficial for the virus (Tang et al., 2020). ZIKV infections in pregnancy potentially affect the foetus, causing spontaneous abortion, intrauterine growth retardation, and foetal death, or affect the newborn, causing congenital anomalies and organ diseases with varying degrees of severity (Auriti et al., 2021). Increased viral load may cause placental damage, foetal growth restriction, and foetal resorption (Brown et al., 2019). Vazzeile et al (2019) revealed the minimum viral load set for transmission during bloodmeal by mosquito vectors is 12,000 viral copies (Vazeille et al., 2019). In pregnant women, transmitted virions eventually infect trophoblast and endothelial cells, resulting in higher viral loads exposed to foetal cells downstream of the barrier (Arumugasaamy et al., 2018). Despite that blood viral loads of Zika virus in pregnant women are expected to be higher due to decreased immunological status, there is paucity of *invitro* human studies to determine the threshold for the severity of the viral infection (Camacho-Zavala et al., 2021). Nonetheless, animal studies using primates and mice have revealed that viral load can reach 100,000 copies per ml or higher (Crooks et al., 2021; Duggal et al., 2018; Seferovic et al., 2018). In this study, only two of the seven samples were established to have a blood viral load of more than 5000 copies per ml. Although a study carried out by Crooks et al (2021) set the lowest limit of quantification to 100 copies per ml, indicating that none of the samples tested fell below this limit. While persistent viremia has been linked to an increased risk of congenital Zika syndrome, there has been little research into the significance of high maternal viral load as a risk factor for adverse foetal outcomes (Pomar et al., 2019). Several factors, including infection stage, days after exposure, and host immune response activity, may influence viral loads during infection.

CXCL10 has been reported as a potential serum biomarker for acute ZIKV infection in pregnant women (Lima et al., 2019). Lima et al. (2019) observed increased CXCL10 levels in pregnant women infected with Zika virus infection (Lima et al., 2019). It was also found that CXCL10 elicits apoptosis in foetal neurones, thus acting synergistically in the pathogenesis of microcephaly induced by Zika virus infection (Lima et al., 2019). In this study, CXCL10 overexpression was investigated using gene expression analysis in samples that tested positive for ZIKV RNA. Gene expression analysis of seven samples tested in replicates revealed that four samples positive for ZIKV RNA showed overexpression of CXCL10, whereas the other three showed normalised expression. In four out of the seven samples, 4-, 24-, 27-, and 126-fold increases were observed in the analysed samples. These findings were consistent with the immunological profiling studies conducted by Camacho-Zavala et al., (2021) where pregnant women infected with Zika virus showed immunoregulatory cytokines profile with CXCL10 increase; and Naveca et al., (2018) that analysed immunological biomarker profile during acute Zika virus infection. In both studies, in which overexpression of CXCL10 was detected. CXCL10 overexpression has also been linked to neuronal damage in ZIKV-infected pregnant women, but the pro-inflammatory systemic immune activation profile varies depending on the viral load and endemicity of the viral infection in the area of the participants (Barros et al., 2018;

Maucourant et al., 2019). Thus, differences in environmental factors in the study area may influence the level of CXCL10 overexpression.

5.0 Conclusion

The epidemiology of Zika virus (ZIKV) infection in Nigeria and Africa as a whole remains a mystery that has yet to be unravelled. Nevertheless, this study stands out as one of the first to concurrently investigate ZIKV infection in two distinct regions of Nigeria. The findings from this study provide further confirmation of Zika virus infection among pregnant women in Nigeria. The identification of Zika virus circulation in both the northern and southern regions of the country, which were examined in this study, suggests that the virus is widely distributed across Nigeria, possibly co-circulating with other arboviruses such as Dengue and Chikungunya viruses that share similar characteristics. Given the climatic and environmental conditions that favor the breeding of arboviral vectors, pregnant women in Nigeria continue to face a significant risk of ZIKV and other arboviral infections. Since there are currently no approved vaccines to prevent the consequences of ZIKV infection, there is an urgent need for reliable, rapid, and affordable diagnostic tools to detect ZIKV infection, particularly due to the high incidence of asymptomatic cases, especially in endemic areas. Notably, the investigation of CXCL10, a critical chemokine involved in the immune response of ZIKV-infected pregnant women, revealed a substantial increase in CXCL10 production, indicating overexpression. This overexpression of CXCL10 has been associated with placental barrier permeability and fetal damage in ZIKV-infected pregnant women, particularly in cases with no apparent symptoms. Thus, this feature holds potential as a diagnostic tool for identifying ZIKV infection in pregnant women.

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