Original Research Article

Molecular epidemiology of Zika virus and Rubella virus in pregnant women attending Sobi Specialist Hospital Ilorin, Nigeria

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ABSTRACT

Background: Zika virus and rubella virus are viruses of concern to public health owing to their independent ability to cross the placenta causing congenital defects and complications. This study aims to determine the molecular epidemiology of these viruses amongst pregnant women attending Sobi Specialist hospital in Ilorin, Kwara state.
Methods: After ethical approval and duly completed informed consent form, blood sample and respondent data were collected for Enzyme Linked Immuno Sorbent Assay after which the respective IgM positive samples were molecularly analyzed independently.
Results: The recorded immune status to the individual viruses were 32 (16.0%) and 14 (7.0%) for zika virus IgM and IgG while rubella virus IgM and IgG had 24 (12.0%) and 118 (59.0%) prevalence respectively. The molecular analysis of the rubella virus yielded a partial sequence of its E1 glycoprotein which was assigned accession number MT153585 after GenBank deposition while zika virus had no detectable molecular result. Further analysis of serostatus revealed coinfection (3.5% and 3.7%) and mono (1.0% and 36.0%) for IgM and IgG respectively and sero-conversion of 17.5%. The unexposed respondent was 38.0%. Amongst the evaluated demographic/risk factors, the viruses were statistically significant (p<0.05) for age, type of marriage, occupation, parity and frequency of contact with people while mosquito prevention strategy and its implementation were also significant for zika virus acquisition.
Conclusions: Awareness of respondent to the viruses was very low with 97.0% unaware prior to this study. Vaccination for the vaccine preventable rubella virus should be implemented while adherence to mosquito prevention strategies and discouragement of breeding site should be is encouraged. There is need for the enactment of surveillance route for these viruses to ascertain the extent of the silent burden on the health of the baby in Nigeria.

Keywords: Co-infection, Molecular, Prevalence, Rubella virus, Zika virus

INTRODUCTION

Congenital infections are caused by pathogens transmitted from mother to child trans-placentally or peripartum. The effect of this infection on fetal or neonatal health are detrimental most especially if infection occurs within the first trimester of pregnancy and this include deafness, inborn heart disease and mental retardation, ocular defects (cataracts, microphthalmia, glaucoma and retinitis or complete loss of sight) and cardiac system defects such as patent ductus arteriosus, ventricular septal defects and microcephaly.[1,2]

Rubella and Zika virus are example of viruses that have been globally documented and independently confirmed to cross placenta and cause congenital syndrome. Similar to other infectious diseases, rubella and zika virus are transmitted directly or indirectly from person to person or via intermediate host as noticed in zoonotic infections.[3] Presence of the virus's RNA in infected persons have

been partially confirmed in the blood, urine, semen, female genital secretions, saliva, breast milk, amniotic fluid and cerebrospinal fluid respectively.[4-6] Although they are of different family and Origin i.e. Zika virus-Flaviviridae and Rubella virus-Togaviridae, there symptomatic relatedness and ability to cause birth defects made them infectious viruses of global concern.[7]

The burden of congenital infection in most developing countries such as Nigeria is usually not well documented due to limited epidemiological data, under reporting, mild nature of infection and similarity with other common infection symptoms such as malaria or typhoid fever. Thus, this study aims to provide an epidemiological data via molecular and serological analysis which to the best of our knowledge, co-infection of rubella and zika virus has never been reported in a single study in the country.

METHODS

This is a cross sectional study involving two hundred (200) consenting pregnant women attending Sobi specialist Hospital in Ilorin, Kwara state, irrespective of pregnancy age. Ilorin is the state capital of Kwara in Western Nigeria. As of the 2006 census, it had a population of 777,667, making it the 6th largest city by population in Nigeria.[8] Sobi Specialist Hospital Ilorin was chosen as the sample collection Centre because of its special services for pregnant women and the geographical location which is characterized by the hill and tree. The period of study was from November, 2017 to November, 2018.

Inclusion and exclusion criteria

- The participant must be pregnant irrespective of age, give consent and be attending Sobi specialist hospital for their antenatal care while subject that didn't meet the criteria were excluded. Closeness of participant resident to Sobi hill was also considered.

Sample and data collection

Five (5) ml of blood was aseptically drawn from the consenting pregnant women and transferred in to sterile EDTA bottle, this was later transferred in to prelabelled plain bottle after serum separation via centrifugation for serological and molecular analysis respectively. Socio-demographic and risk factor data was obtained via a structured questionnaire administered after informed consent form was duly signed.

Assay

Zika virus and Rubella virus Enzyme Linked Immuno-Sorbent Assay for Immunoglobulin G and M (IgG and IgM) was carried out according to Abcam, UK and Melsin Co, China instructions respectively. After result interpretation and calculation in Abcam and Melsin Units

of the manufacturers manual, the IgM positive sera were selected for molecular analysis.

Molecular analysis

RNA extraction kit by Bio-Rad, US was used for the extraction process according to the manual. A cDNA was achieved by the use of BIONEER cycle script RTpremix (dT20) for more stability and easier storage. The PCR procedure for Zika virus was according to the protocol by Victor et al, using the 5'TGGAGATGAGTCATGTAT-G3' and 5'CTTCTTGACAACATCTACC3' forward and reverse primers respectively while Rubella virus analysis was achieved using Kolawole et al, protocol via GACAACTCGAGGTCCAGGTC and AGTCAGGGGA-ATGGCGTTG forward and reverse primers.[9,10] The method of dye termination technique was employed using the quick start kit procedure according to Bechman coulter dye terminator cycle sequencing and This involves the labeling of the chain terminator ddNTPs which permits sequencing in a single reaction rather than four reactions as in the labeled-primer method. Each of the four dideoxynucleotide chain terminator was labeled with fluorescent dyes emitting light at different wavelengths. The resulting sequence was subjected to live blast at National Center for Biotechnology Information (NCBI) and then deposited.

Data analysis

This was achieved via the use of the statistical package for the social science (SPSS) version 20 with statistical significance level at p<0.05. Presentation of result in table and charts was done using Microsoft package (Word and excel-version 2016). Analysis of genetic relatedness was achieved via MEGA 7.

Ethical consideration

Ethical approval for the study was obtained at Faculty of life science, University of Ilorin ethical board review and Kwara state ministry of health (UERC/ASN/2018/1324).

RESULTS

The prevalence of zika and rubella virus IgM and IgG seropositivity were 16.0%, 7.0% and 12.0%, 59.0% respectively (Figure 1) amongst the respondent in the study location while prevalence cross tabulation revealed 7 (29.2%) and 6 (25.0%) of the rubella virus IgM participants were Zika IgM and IgG positive respectively and 30 (25.4%) and 12 (10.2%) of the rubella IgG participants were also IgM and IgG sero positive for Zika virus at significant statistical correlation (p<0.05) (Table 1). Figure 2 the serological analysis of the viruses with respect to sero presence of each marker in the positive sera.

The highest recorded age population was amongst the 23 to 27 and 28 to 32 age group with 10 (31.3%) and 15

(46.9%)-Zika IgM, 6 (42.9%) and 6 (42.9%)-Zika IgG, 7 (29.2%) and 10 (41.7%)- Rubella IgM and 42 (35.6%) and 46 (39.0%) for Rubella IgG respectively (Table 2).
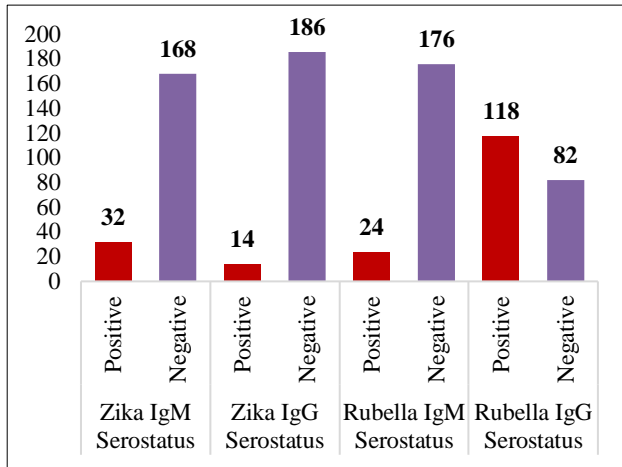

Figure 1: The prevalence of Zika and Rubella virus serostatus amongst the respondent.

Table 3 the epidemiological parameter of the respondent with respect to the immunological status to zika and rubella virus. The monogamous type of marriage had the most count of participants compared to the polygamous group which had 33 of 200 respondent. However, the seropositivity to zika IgM (p=0.000), Rubella IgM (p=0.232) and IgG (p=0.172) were highest amongst the polygamous marriage type. Most of the women reported to be in to business/trade (118 (59.0%)) and also had the highest seropositivity to the assayed viruses. This was followed by civil servants, housewives and student group. The statistical correlations for each immune-status were 0.360, 0.628, 0.134 and 0.000 for Zika IgM, IgG, Rubella IgM and IgG respectively. The participants mostly attained secondary (69) and tertiary (107) level of education while few (4) had no formal education. The prevalence of rubella IgG was noticed to be highest across the respective class compared to other immunoglobulin i.e. 50% for no education, 70% for primary, 65.2% for secondary and 53.3% for tertiary level of education (p=0.297). The respective p values for other immune-status are 0.153 (Zik-IgM), 0.542 (Zik-IgG) and 0.155 (Rub-IgM). The group with a child had 65 respondent, two and above children were 51, two children had 40 while no previous child were 38 of the total respondents. Rubella igG was most predominant amongst all in the increasing order of 53.4 (one child), 55.0 (2 children), 55.3 (None) and 66.7% (>2 children). The prevalence of rubella IgM was recorded to be 0.003.

Table 1: Zika virus and Rubella virus cross tabulation.

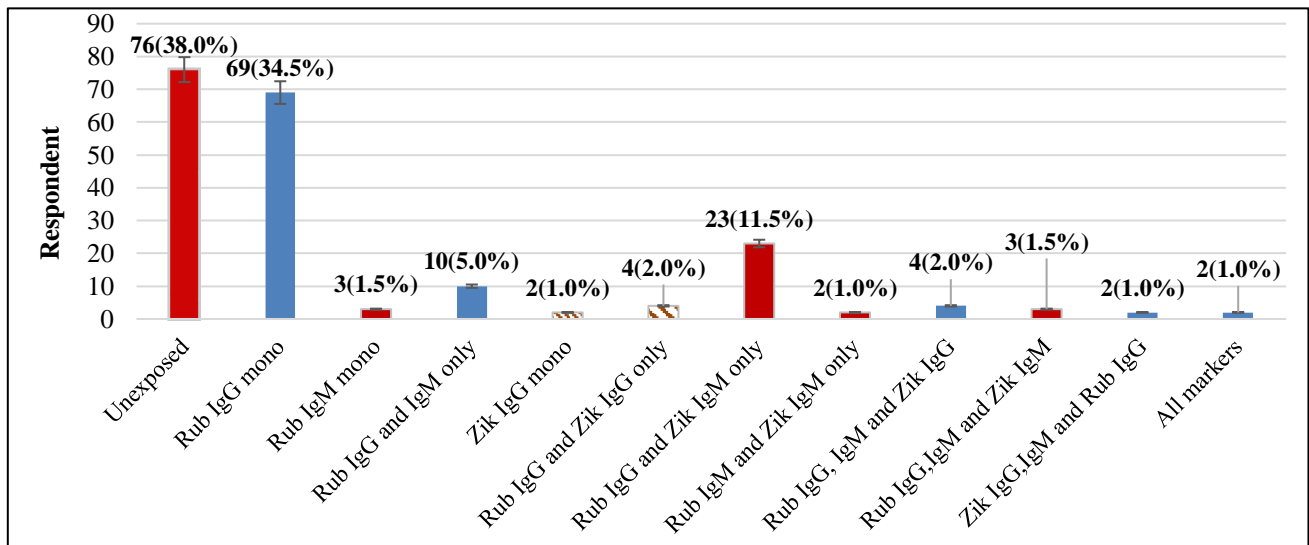|		Zika IgM Serostatus			Zika IgG Serostatus			
		Positive (%)	Negative (%)	p-value	Positive (%)	Negative (%)	Total (%)	p-value
Rubella IgM Serostatus	Positive	7 (29.2)	17 (70.8)	0.061	6 (25.0)	18 (75.0)	24 (100.0)	0.000
	Negative	25 (14.2)	151 (85.8)		8 (4.5)	168 (95.5)	176 (100.0)	
Rubella IgG Serostatus	Positive	30 (25.4)	88 (74.6)	0.000	12 (10.2)	106 (89.8)	118 (100.0)	0.035
	Negative	2 (2.4)	80 (97.6)		2 (2.4)	80 (97.6)	82 (100.0)	
	Total	32 (16.0)	168 (84.0)		14 (7.0)	186 (93.0)	200 (100.0)	

Figure 2: Serological analysis of Zika virus and Rubella virus.

Table 2: Serological status of respondent with respect to age.

Sero-status		Age					
		18-22	23-27	28-32	33-37	38-42	Total
Zika IgM (p=0.789)	Positive	2 (6.3%)	10 (31.3%)	15 (46.9%)	3 (9.4%)	2 (6.3%)	32 (100%)
	Negative	16 (9.5%)	64 (38.1%)	59 (35.1%)	17 (10.1%)	12 (7.1%)	168 (100%)
Zika IgG (p=0.564)	Positive	0 (0.0%)	6 (42.9%)	6 (42.9%)	2 (14.3%)	0 (0.0%)	14 (100%)
	Negative	18 (9.7%)	68 (36.6%)	68 (36.6%)	18 (9.7%)	14 (7.5%)	186 (100%)
Rubella IgM (p=0.005)	Positive	0 (0.0%)	7 (29.2%)	10 (41.7%)	7 (29.2%)	0 (0.0%)	24 (100%)
	Negative	18 (10.2%)	67 (38.1%)	64 (36.4%)	13 (7.4%)	14 (8.0%)	176 (100%)
Rubella IgG (p=0.681)	Positive	11 (9.3%)	42 (35.6%)	46 (39.0%)	13 (11.0%)	6 (5.1%)	118 (100%)
	Negative	7 (8.5%)	32 (39.0%)	28 (34.1%)	7 (8.5%)	8 (9.8%)	82 (100%)
	Total	18 (9.0%)	74 (37.0%)	74 (37.0%)	20 (10.0%)	14 (7.0%)	200 (100%)

Table 3: Zika and Rubella virus serostatus in relation marriage type, occupation, educational level and parity of the respondent.

	Zika IgM Serostatus		Zika IgG Serostatus		Rubella IgM Serostatus		Rubella IgG Serostatus		
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Total
Type of Marriage									
Monogamy	20*	147	12	155	18	149	95	72	167
Polygamy	12*	21	2	31	6	27	23	10	33
Total	32	168	14	186	24	176	118	82	200
Occupation									
Housewife	2	17	2	17	3	16	10*	9	19
Civil Servants	4	40	2	42	2	42	13*	31	44
Business Woman	24	94	10	108	19	99	84*	34	118
Student	0	3	0	3	0	3	0*	3	3
Others	2	14	0	16	0	16	11*	5	16
Total	32	168	14	186	24	176	118	82	200
Educational Level									
No education	2	2	0	4	0	4	2	2	4
Primary	4	16	0	20	0	20	14	6	20
Secondary	13	56	6	63	12	57	45	24	69
Tertiary	13	94	8	99	12	95	57	50	107
Total	32	168	14	186	24	176	118	82	200
Gravidy/ Parity									
No	2	36	0	38	0*	38	21	17	38
One child	13	52	6	59	7*	58	35	30	65
Two child	6	34	2	38	4*	36	22	18	40
>Two	9	42	4	47	13*	38	34	17	51
Total	30	164	12	182	24	170	112	82	194

NB: * signifies statistical correlation that is lower than 0.05

Table 4 presents the risk factors that have been associated with acquisition of zika virus infection and the respective statistical correlation for each group. The evaluated factors include participants proximity to drainage channel or stagnant water (estimated as very close, close, far and very far), mosquito bite frequency (very, minimal or less), prevention strategies (use of mosquito net, insecticides and other methods), implementation of the strategies and consumption of bushmeat. Based on statistical correlation of this factors to acquisition of infections, implementation of mosquito prevention strategy and the strategy were most significant to been exposed to zika virus while the frequency of bite, proximity to stagnant water or drainage channel and bushmeat consumption were less statistically significant. Furthermore, respondent's frequency of contact with people, blood transfusion history, and travel histories were evaluated and presented in Table 5.

The history of vaccination as reported by the participants revealed that about 60.93% had not taken vaccination to rubella virus or other flavivirus member, 31.16% had

been vaccinated for yellow fever while 0.93% had vaccination to rubella while over 6% did not respond. In addition, the awareness level for rubella and zika virus were 1 and 2% respectively while about 97% had no awareness for both viruses (Figure 3).

Figure 4 shows the molecular weight of the detected genetic material for rubella virus at 320bp. The sequence was derived from the RNA extracted from the clinical material (RVs) as a partial sequence and after deposition at the GenBank, an accession number (MT153585) was allocated for reference as indicated on the tree of relatedness (Figure 5) to the thirty-two (32) WHO reference standards.
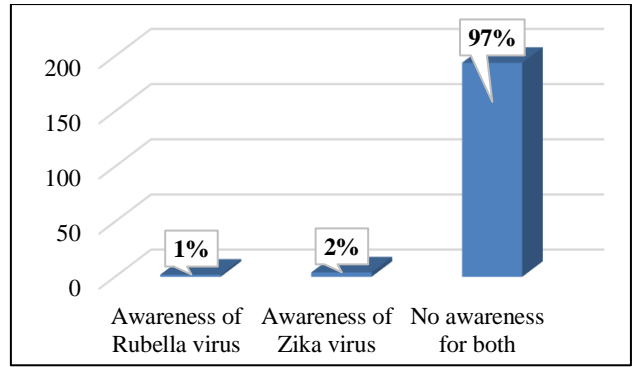

Figure 3: Awareness level for rubella and zika virus amongst respondent.

Table 4: Evaluation of risk factors to acquisition of Zika virus infection amongst respondent

		Zika IgM Serostatus				Zika IgG Serostatus			
		Positive (%)		Negative (%)	p-value	Positive (%)		Negative (%)	p-value
Proximity to stagnant water/drainage channel	Very close	5	(2.5%)	24	(12.0%)		4	(2.0%)	25	(12.5%)	
	Close	8	(4.0%)	20	(10.0%)	0.207	0	(0.0%)	28	(14.0%)	0.121
	Far	13	(6.5%)	74	(37.0%)		8	(4.0%)	79	(39.5%)	
	Very far	6	(3.0%)	50	(25.0%)		2	(1.0%)	54	(27.0%)	
Frequency of Mosquito Bite	Very	4	(2.0%)	14	(7.0%)		0	(0.0%)	18	(9.0%)	
	Minimal	12	(6.0%)	58	(29.0%)	0.660	2	(1.0%)	68	(34.0%)	0.062
	Less	16	(8.0%)	96	(48.0%)		12	(6.0%)	100	(50.0%)	
Prevention Strategy	Mosquito net	20	(10.0%)	134	(67.0%)		12	(6.0%)	142	(71.0%)	
	Insecticides	10	(5.0%)	30	(15.0%)	0.091	0	(0.0%)	40	(20.0%)	0.008
	Other	2	(1.0%)	4	(2.0%)		2	(1.0%)	4	(2.0%)	
Implementation of prevention strategy	Very	30	(15.0%)	152	(76.0%)		8	(4.0%)	174	(87.0%)	
	Minimal	0	(0.0%)	8	(4.0%)	0.433	4	(2.0%)	4	(2.0%)	0.000
	Less	2	(1.0%)	8	(4.0%)		2	(1.0%)	8	(4.0%)	
Consumption of Bushmeat	Yes	7	(3.5%)	33	(16.5%)	0.772	2	(1.0%)	38	(19.0%)	0.579
	No	25	(12.5%)	135	(67.5%)		12	(6.0%)	148	(74.0%)	

**Table 5: Prevalence of the Zika and Rubella status with respect to other associated risk factors**

		Zika IgM		Zika IgG		Rubella IgM		Rubella IgG		
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Total
Frequency of contact with people	Very	13.0%	69.5%	6.0%	76.5%	11.0%	71.5%	49.0%	33.5%	82.5%
	Minimal	3.0%	12.0%	1.0%	14.0%	1.0%	14.0%	9.0%	6.0%	15.0%
	Less	0.0%	2.5%	.0%	2.5%	.0%	2.5%	1.0%	1.5%	2.5%
Blood Transfusion	Yes	2.5%	5.5%	.0%	8.0%	1.5%	6.5%	6.5%	1.5%	8.0%
	No	13.5%	78.5%	7.0%	85.0%	10.5%	81.5%	52.5%	39.5%	92.0%
Travelled abroad	Yes	3.5%	4.5%	1.0%	7.0%	0.0%	8.0%	5.0%	3.0%	8.0%
	No	12.5%	79.5%	6.0%	86.0%	12.0%	80.0%	54.0%	38.0%	92.0%
	Total	16.0%	84.0%	7.0%	93.0%	12.0%	88.0%	59.0%	41.0%	100.0%

DISCUSSION

The highest serological prevalence recorded for the studied congenital virus was noticed with rubella virus

immunoglobulin G with prevalence of 59% (118). This posits that a reasonable number of the pregnant women that was recruited for this study had been exposed to rubella virus and thus the immunity against it. This

relates to Kolawole et al, 2015 that reported higher IgG serostatus. Contrary to the high immunity status recorded for rubella virus, the immunity to zika virus was low (7.0%; 14) which indicates that only few of the participants have been exposed to the virus. Primary exposure to zika virus which was determined by the Immunoglobulin M serostatus amongst the respondent had the second highest record in this study where 32 participants were positive indicating that 16% of the pregnant women are undergoing current zika virus infection which would have been contacted via both the established or unconfirmed risk factors for the virus such asmosquito bite, blood transfusion/blood product, other body fluids and sex.[11] Likewise, the prevalence status for rubella virus current infection also follows similar trend with a record of 12% (24) prevalence.
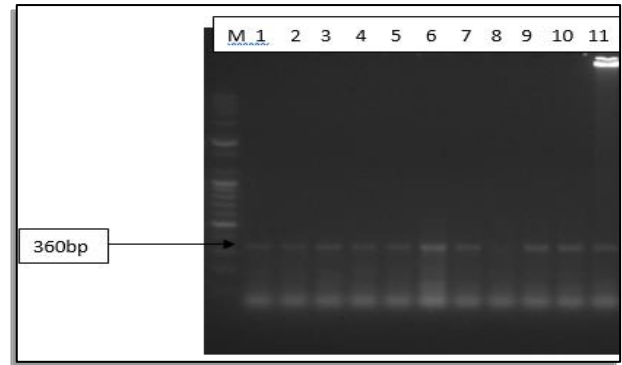
(M= Molecular marker; L1-11= Samples).

Figure 4: PCR amplicons for Rubella virus.
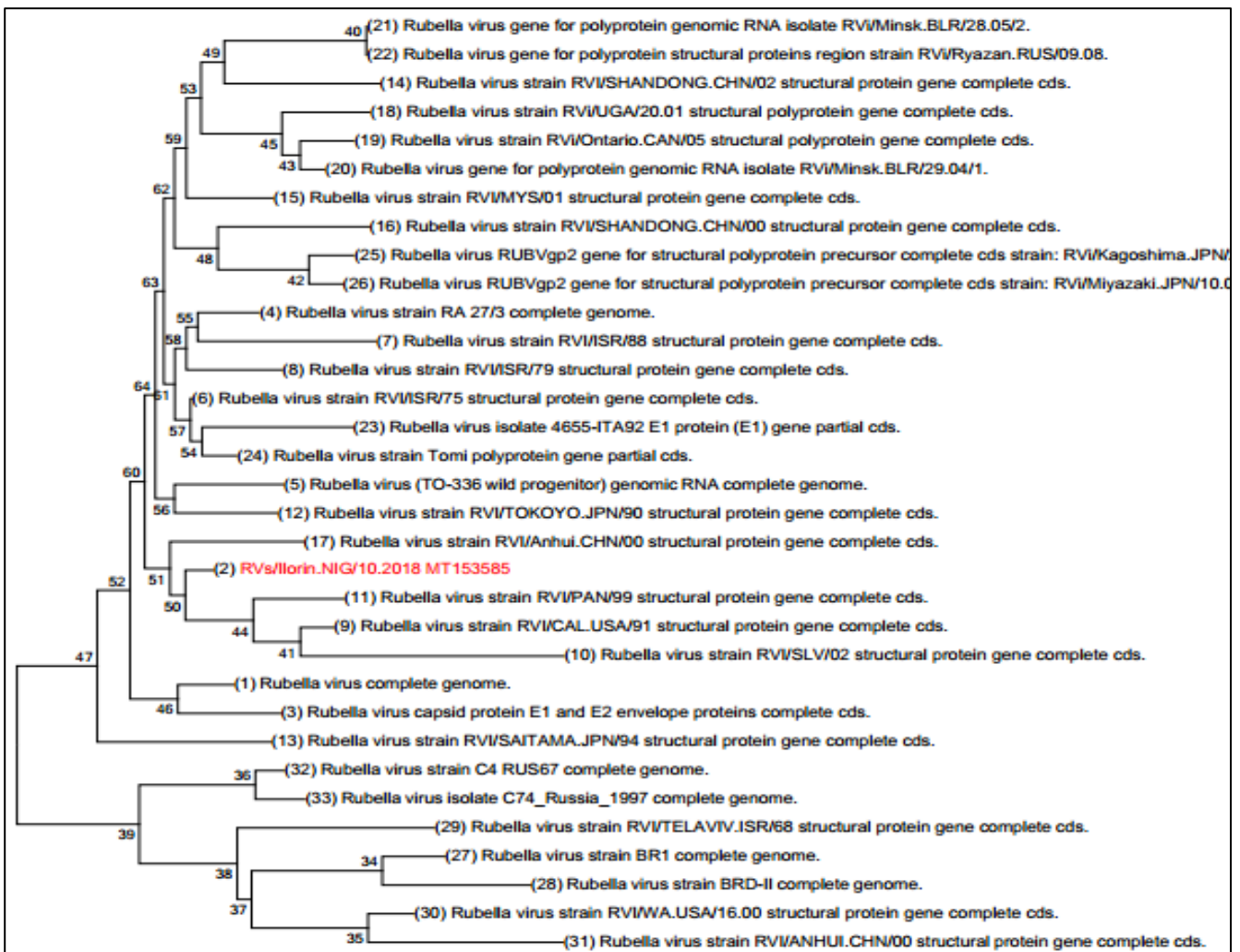
(NB: 2= deposited sequence: 1,3-33= WHO reference standard).

Figure 5: Evolutionary relationships of taxa.

The indication for this prevalence suggests possible risk to the fetus which depends on the stage of development. This corelates to other studies in Abuja, Abia, Kano, Ibadan, Lokoja, Ilorin and River state of Nigeria.[12-15] The consequence of the infection on the mother may not be noticeable because its mild and similarly for the fetus, the effects may not also be vivid at birth or at childhood because there are reports indicating complications

affecting the ear, eye or nervous related conditions later in childhood.[2]

The coinfection of the zika and rubella virus amongst pregnant women attending the hospital of the study location was evaluated through the serostatus co-presence in each blood samples that were analyzed. The statistical significance for each immune status was noticed to be significant in coinfection except for rubella and zika IgM which had 0.061. The increase of coinfection seropositivity amongst the respective positive samples was noticed to be from Rub-IgG/Zik-IgG-112 (10.2%), Rub-IgM/Zik-IgG-6 (25.0%), Rub-IgG/Zik-IgM-30 (25.4%), to Rub-IgM/Zik-IgM-7 (29.2%). This serological evidence for the presence of the two viruses which have been evidently implicated in most cases of congenital deformities posits a health challenge amongst pregnant women of this location which could significantly impact the child either on short on long term. This is further supported by the seven subjects noticed to have recently or currently been exposed to rubella and zika virus as presented in the table. Although the p-value was statistically insignificant, the transmissivity of the virus to unimmune persons which was noticed to be high (Figure 2) could result in to a more severe case. However, the intensity of complication and duration of manifestation would be greatly determined by the stages of pregnancy development. It has been recorded that significant short-term effect of infection with zika or rubella virus is most evident during the early stages of fetus development mostly within first trimester.[16,17] Further serostatus analysis of the viruses revealed that few of the fetus of the respondent are at higher risk than others as implicated by the detection of the immunoglobulin M in the sera of the mother as compared to others with IgG. The most protected group are the respondent with IgG for both viruses and those at convalescence stage of infection indicating recovery (Figure 2). The outcome on the fetus will be influenced by the pregnancy age because risk to fetus is measured at 80-100%, 20% and up to 60% across the first, second and third trimester respectively. About 38.0% of respondent was also noticed to have no detectable immunoglobulin to either Zika virus or Rubella virus and thus unexposed.

The demographic factors that could contribute to the transmission of the virus were examined to evaluate their relevance to the recorded respective prevalence of the studied viruses. Although there were differences in the serological status of the participants across difference age groups, the only prevalence that was of statistical significance in relation to age was the acquisition of rubella virus immunological marker M. since IgM has been implicated in the immunological response to new infection or primary infection that is recent, i.e. during or before the time of this study, it can be deduced that the risk of getting infected with rubella virus for the first time or as reinfection amongst the study group of the population is within the age of 33 to 37 years. This was revealed by the multiple comparison analysis of post hoc

test aimed to determine which of the age groups were significant. A critical evaluation of marital age in Nigeria suggests that most female marriages peaks around 25 to 30 years which also falls within the economic productive age. This indicates an age of constant exposure to several risk factors to the virus such as direct contact with infected individual and materials in working environment and hospital settings of antenatal clinics. The recorded increase of seropositivity in this study compares to other studies that reported higher IgG/IgM prevalence amongst the group within 21-32 age of respondent.[18] The age group with the highest participant amongst the studied population was 23-27 and 28-32 with a total of 148 (74%) while the age group with least participant is 38-42 years. This could be because most pregnancy conception occurs more within the former than the latter age group. Most of the participants are in Monogamous type of marriage and the seropositivity to the studied viruses were higher in the polygamous group of marriage across Zika IgM, and Rubella IgM and IgG respectively. However, only positivity to Zika IgM was statistically significant for its acquisition in polygamous marriage type. This could be due to the increase in contact and proximity to people because of the larger family size.

Furthermore, the recorded type of occupation was noticed to be in line with increase positivity amongst occupation with higher risk of contact with crowd such as the business/trade and civil servant group at a statistically significant level for Rubella IgG. All attended highest formal educational level was accounted for in this study with the highest in tertiary group but the trend in seropositivity was not specific as a risk factor. However, higher seropositivity across all assayed immunological markers was noticed within the secondary to tertiary class of education of respondent where rubella virus IgM and Zika IgG had 100%, rubella IgG had 86% and Zika IgM had 81% of the total seropositivity respectively. The possible risk of infection or acquisition of immunity to rubella and or Zika virus in correlation to the parity or gravidity of the women was evaluated and it was revealed that protection against the two congenital viruses of study increases within the participants with one child and above. While the respondents without previous child prior to this study showed sparse seropositivity to rubella and zika virus immunoglobulins in the assayed serum samples. The reason that could be adduced to this variance cannot vividly be related to the number of birth or pregnancy but posits the direct contacts with infected persons, formites/infected droplets or insect host as possible route. This is evidence in the noticeable crowded environs at the hospital which promotes closer contacts amongst the pregnant women and thus spread of contagious infection is enhanced.

Additional predisposing factors to zika virus infection includes proximity to breeding site of mosquitoes, frequency of mosquito bite, mosquito prevention strategies and its implementation and consumption of bush meats. Although, this study has recorded varying

responses to the stated associated risk factors, frequency of bite, prevention strategy against mosquito bite and the adherence to the prevention strategy was found to be most statistically significant to zika virus infection. Reports about adequate usage of mosquito prevention strategy in Ilorin revealed inappropriate net maintenance practices and usage and this affirms the need of proper usage and adherence to good practices of mosquito net usage. The use of insecticides has been reported to offer certain controls over the breed and presence of mosquito in the environment but some has also been reported to have no significant benefits such as the larvicide of larval source management.[19] Frequency of contact with people has been suspected to be amongst the risk factors facilitating the transmission of rubella virus however, no report has been made about zika virus. This study reveals that the participants with higher level of contact with people had the highest prevalence of previous exposure to rubella virus as detected by Rub IgG, followed by the group with Zika IgM positivity. The noticeable increase of prevalence amongst the respondent with most contact with people posits that the route of transmission for zika could also encompass direct transmission as implicated in rubella virus transmission. Furthermore, transmission of zika virus can also be enhanced within this group by the presence of the known vector i.e. mosquito which is most prevalent in this location.

Blood transfusion amongst respondent in respect to seropositivity was recorded to be lower across the assayed immunoglobulins. This could be adduced to the lower number of participants that has had transfusion of blood prior to this study which accounted for 8.0%. Although, there were lower respondent that had travelled or visited other countries, the positivity count amongst them was significant to support visitation to other countries as a possible risk factor to acquisition of infection for zika and rubella virus.

Vaccination against flavivirus which included Zika, Dengue, Chikunguyaetc is not available but yellow fever vaccine has been reported to be available and response from participants also confirms its availability. This was deduced from the number of respondents that stated to have been immunized against yellow fever virus. Although rubella virus vaccine is in circulation, the number of people that has access to it is however scanty. This could have resulted from the low awareness level amongst respondent or the populace in general which was also indicated in the 97% recorded amongst the participant that had no prior knowledge about rubella virus or zika virus. Although, the molecular confirmation of Zika virus was not achieved in this study which could be attributed to low viral loads, timing of acute phase, short periods of positivity in common specimens and its asymptomatic nature during primary infections, blast of the sequence from the Rubella virus amplicons revealed similarity to sequence deposited by Curti et al, from a molecular epidemiological study of rubella virus in congenital infections in Sao Paulo, Brazil (AGO20648.1)

which corelates to reported global distribution of the virus.[20-22] The analysis of the sequence phylogeny to the standard sequence provided by World Health Organization for epidemiological studies revealed close relatedness to reference strain 1F than 1C which shared same ancestor.[23] Genotype 1F is amongst the four (4) genotypes namely 1D, 1F, 1I and 2A that are less reported in recent times and thus almost considered inactive and probably extinct.[23] In contrary, this study provides the most recent epidemiological data of the genotype indicating its presence and thus not extinct.

CONCLUSION

Conclusively, this study has provided molecular evidence of rubella virus and serological evidence of current mono and co-infection of rubella virus and zika virus in this location. Rubella virus infection is still recognized as an important disease in global context, while knowledge of its geographical distribution in this location is still scanty, advocacy for vaccine implementation and appropriate surveillance channels is recommended to prevent congenital defects since it's a vaccine preventable disease. Adequate prevention strategies such as proper hygienic practices, discouragement of mosquito breeding site, proper usage of mosquito net, proper ventilation in crowded homes amongst other is highly encouraged to forestall outbreak of congenital anomaly due to Zika virus infection of pregnant women.

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