



SEROLOGICAL ASSESSMENT OF RUBELLA INFECTIONS AMONG PREGNANT WOMEN IN A TERTIARY INSTITUTION IN RIVERS STATE, NIGERIA

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ABSTRACT: Background: Rubella is an acute contagious viral infection that can cause devastating effects on a developing foetus resulting in miscarriage, foetal death, or the birth of an infant with congenital rubella syndrome (CRS). **Materials and Method:** Using ELISA kits, a cross-sectional study was performed to determine anti-Rubella IgG and IgM antibodies in blood samples obtained from 90 pregnant women attending an antenatal clinic. **Results:** An overall seroprevalence of 96.7% for rubella-IgG antibodies and 15.6% for rubella-IgM antibodies were obtained, while 3 (3.33%) and 74 (84.4%) tested seronegative for rubella-IgG and -IgM respectively. However, none (0%) participants were Rubella IgG seronegative and rubella-IgM seropositive. While most factors studied were found not to influence rubella ($p>0.05$) significantly, marital status ($p=0.035$) and history of abortion ($p=0.000$) were found to affect IgG seropositivity significantly, and STD history significantly affected IgM seropositivity ($p=0.006$). **Conclusion:** This study found a large proportion (96.7%) of pregnant women to be immune to rubella and 15.6% with evidence of recent infection. However, 3.3% remain susceptible to acquiring rubella. Thus, to keep this susceptible group at the bare minimum, women of childbearing age should be continually screened before conception, and adequate vaccination programs and a continuous surveillance system should be implemented.

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1. INTRODUCTION

Rubella, commonly known as German measles or three-day measles, is an acute, usually mild, contagious disease caused by the Rubella virus (RubV) (Wondimeneh et al., 2018) that frequently occurs in humans as the only natural host and reservoir of rubella virus (Mounerou et al., 2015). It belongs to the *Togaviridae* family, which also includes the chikungunya virus. With an incubation period of 2-3 weeks, the virus is transmitted mainly through the respiratory route postnatally and transplacental during pregnancy (Tamirat et al., 2016).

Rubella is of high public health importance due to its teratogenic effect on women. It causes devastating effects on a developing foetus (Adam et al., 2013). Infection with the virus during pregnancy may lead to miscarriage, foetal death, or congenital rubella syndrome (CRS) (Olajide et al., 2015; Praveen et al., 2016). There is no exact treatment for the virus (Olajide et al., 2015), but it can be prevented by using the live attenuated rubella vaccine (Alleman et al., 2016; Demicheli et al., 2012; WHO, 2014), which has dramatically reduced its occurrence to low levels in most industrialised regions.

Varying reports amid pregnant women have demonstrated a detectable amount of IgG antibodies

conferring immunity against RubV (Kolawale et al., 2014; Adewumi et al., 2015; Olajide et al., 2015; Wondimeneh et al., 2018). However, outbreaks still arise, especially in evolving countries where the vaccine is still not readily accessible (Njeru et al., 2015). There is a paucity of studies on the seroprevalence of RubV amid pregnant women in Rivers State, Nigeria. Thus, this study aims to detect the presence of anti-rubella antibodies (IgG and IgM) in pregnant women undergoing routine antenatal check-ups in the antenatal clinic of the University of Port Teaching Hospital. This study assessed the immune status and level of susceptibility to RubV of these pregnant women.

2. MATERIALS AND METHOD

2.1. Study Area and Design: This study adopted a hospital-based cross-sectional design. It surveyed the prevalence of RubV- IgG and IgM antibodies in pregnant women presenting at the antenatal clinic, University of Port Harcourt Teaching Hospital, Rivers State, Nigeria.

2.2. Study Population: We randomly selected pregnant women attending the antenatal clinic for a routine check-up at UPTH until a total of 90 participants was attained. Other relevant information

from all participants was obtained. Their consent was duly obtained.

2.3. Ethical Approval: The Hospital Ethical committee of UPTH were consulted for the approval of the study, and the request was granted to carry out the research.

2.4. Sample Collection and Preparation: Specimen of 5ml venous was aseptically drawn by venepuncture from the enrolled subjects into sterile, properly labelled EDTA tubes. Then specimen was transported on ice packs to the Virus Research Unit of the Department of Microbiology, the University of Port Harcourt, for analysis and processing using standard laboratory procedures.

2.5. Serological Analysis: Sera were analysed for anti-Rubella IgG and IgM antibodies using DIA's commercially diagnostic ELISA kits. PRO Diagnostic Bioprobes Srl Via G. Carducci n° 27 20099 San Giovanni (Milano)-Italy. The manufacturer's instructions were strictly adhered to in performing the analyses. The microplates were washed in 5 cycles with an automated washer (Biotek ELx 50, USA), and the coloured reaction product was read using a microplate reader (Biotek ELx808i, USA) at 450-630nm. Samples were considered positive for anti-Rubella virus IgG antibody when the concentration was higher than 10 WHO IU/ml and negative when lower than 10 WHO IU/ml. For the anti-Rubella virus IgM antibody, samples were regarded as positive when the IgM index is equal to or greater than 1.0 and negative when equal to or less than 0.90.

2.6. Data Analysis: The data were analysed using the Statistical Package for the Social Sciences (SPSS), IBM version 22. The seroprevalence was calculated as the proportion of seropositive samples divided by the total samples tested. The Chi-square test was employed to determine associations between seropositivity and socio-demographic factors. The level of statistical significance was set at $P \leq 0.05$

3. RESULTS

3.1. Overall Prevalence: From the 90 study participants screened for rubella antibodies, 87(96.7%) tested seropositive for RubV-IgG antibodies, while 3 (3.3%) were seronegative. Also, 14(15.6%) were seropositive for RubV-IgM antibodies, while 74 (84.4%) tested seronegative. Furthermore, 15.6% of them were both RubV-IgG/IgM seropositive. However, none (0%) of participants were RubV-IgG seronegative and RubV-IgM seropositive.

3.2. Rubella IgG antibodies with the Socio-demographics of participants: Table 1 shows the socio-demographic characteristics of the pregnant women concerning rubella IgG. Analysis by age showed the highest (100.0%) IgG seropositivity to occur in age groups 20-29 years and 40-49 years compared to the 95% observed in those within 30-39 years. Only single pregnant woman tested positive for RubV-IgG (100.0%), while the rest that was married were seropositive (96.6%). Pregnant women in polygamous families had the highest seroprevalence (100.0%), while the others in monogamous families had a lower prevalence of 96.0% ($n=72$). The distribution of rubella in pregnant women according to their educational level showed the highest (100.0%) Rubella IgG seropositivity to occur in the women who had secondary education and no formal education compared to the prevalence (96.1%) obtained for tertiary education.

Occupation-related RubV-IgG antibody shows a 100.0% prevalence rate among businessmen/women, traders, artisans, students and the unemployed. The lowest prevalence was obtained in civil servants, with 89.0%. Pregnant women with no recognised religion had the highest prevalence (100.0%) of RubV IgG antibody, followed by Christian pregnant women (96.3%). No ($p > 0.05$) significant statistical association existed with the socio-demographic factors except marital status ($p = 0.035$).

Table 1: Rubella IgG antibodies concerning the Socio-demographics of participants

Variables	Groups	No. Tested	Rubella IgG		Chi-Square Analysis
			No. Positive	%	
Age groups	20-29	24	24	100.0	1.552
	30-39	60	57	95.0	
	40-49	6	6	100.0	
Marital status	Married	89	86	96.6	0.035
	Single	1	1	100.0	
Education	Secondary	12	12	100.0	0.524
	Tertiary	77	74	96.1	
	None	1	1	100.0	
Occupation	Civil servant	29	26	89.6	6.528
	Business	5	5	100.0	
	Student	9	9	100.0	
	Trading	31	31	100.0	
	Artisan	4	4	100.0	
	Unemployed	12	12	100.0	
Religion	Christian	81	78	96.2	0.345
	Islam	0	0	0.0	
	None	9	9	100.0	
Family type	Polygamous	15	15	100.0	0.621
	Monogamous	75	72	96.0	
Total		90	87	96.7	

3.3. Rubella IgM antibodies concerning the Socio-demographics of participants: Higher Rubella IgM seropositivities were obtained for the age bracket of 30-39 years (18.3%), followed by 20-29 years (12.5%), that were married (15.7%), without a formal education

(100.0%), that were business executives (20.0%), traders (12.9%), unemployed (16.6%) and Christians (16.0%). These associations were not statistically significant ($p > 0.05$) as in Table 2.

Table 2: Rubella IgM antibodies concerning the Socio-demographics of participants

Variables	Groups	No. Tested	Rubella IgM		Chi-Square Analysis
			No. Positive	%	
Age groups	20-29	24	3	12.5	1.628
	30-39	60	11	18.3	
	40-49	6	0	0.0	
Marital status	Married	89	14	15.7	0.186
	Single	1	0	0.0	
Education	Secondary	12	1	8.3	5.905
	Tertiary	77	12	15.6	
	None	1	1	100	
Occupation	Civil servant	29	7	24.1	4.273
	Business	5	1	20.0	
	Student	9	0	0.0	
	Trading	31	4	12.9	
	Artisan	4	0	0.0	
	Unemployed	12	2	16.6	
Religion	Christian	81	13	16.0	0.15
	Islam	0	0	0.0	
	None	9	1	11.1	
Family type	Polygamous	15	2	13.3	0.068
	Monogamous	75	12	16.0	
Total		90	14	15.6	

3.4. Rubella IgG antibodies concerning the obstetric characteristics: Table 3 shows the obstetric characteristics concerning rubella IgG. The highest IgG seropositivity was found in first trimester (100.0%), followed by those in their second trimester (97.7%) and third trimester (94.7%). Women with no children had the highest (100.0%) prevalence rate as opposed to those with 1-2 children (94.7%) and 3-4 children (94.4%). Among the 87 positive cases of IgG

antibody, there was an equal prevalence of 96.6% in participants with a history of abortion and those without. HIV seropositive expectant mothers also had the highest (100.0%) seroprevalence, while HIV seronegative women had a lower prevalence of 96.5%. Participants with no history of STD had a RubV IgG seropositive rate of 97.6%, higher than 83.3% of those with the history.

Table 3: Rubella IgG antibodies concerning the obstetrics characteristics

Variables	Groups	No. Tested	Rubella IgG		Chi-Square Analysis
			No. Positive	%	
Gestation	First	8	8	100.0	0.869
	Second	44	43	97.7	
	Third	38	36	94.7	
Parity	0	34	34	100.0	1.887
	1-2	38	36	94.7	
	3-4	18	17	94.4	
History of abortion	Yes	30	29	96.6	0.000
	No	60	58	96.6	
HIV status	Yes	3	3	100.0	0.107
	No	87	84	96.5	
STD History	Yes	6	5	83.3	3.547
	No	84	82	97.6	
Total		90	87	96.7	

3.5. Rubella IgM antibodies concerning the obstetric characteristics: An analysis of RubV IgM seropositivity with obstetric characteristics showed that only STD history statistically related to RubV IgM seropositivity significantly ($p=0.006$), while others showed no association. Higher IgM seropositivity rates were obtained for second (15.9%) and third (15.8%) trimesters than first trimester. RubV

IgM seroprevalence was found to increase with an increase in parity, with the highest rate (22.2%) observed in those with 3-4 children. Also, higher IgM seroprevalence rates occurred in monogamous family type (16.0%), had a history of abortion (23.3%) and STD (16.6%), and were HIV seropositive (33.3%).

Table 4: Rubella IgM antibodies concerning the obstetrics characteristics

Variables	Groups	No. Tested	Rubella IgM		Chi-Square Analysis
			No. Positive	%	
Gestation	First	8	1	12.5	0.063
	Second	44	7	15.9	
	Third	38	6	15.8	
Parity	0	34	3	8.8	2.02
	1-2	38	7	18.4	
	3-4	18	4	22.2	
History of abortion	Yes	30	0	0.0	8.289
	No	60	14	23.3	
HIV status	Yes	3	1	33.3	0.747
	No	87	13	14.9	
STD History	Yes	6	1	16.6	0.006
	No	84	13	15.4	
Total		90	14	15.6	

4. DISCUSSION

In investigating rubella virus (RubV) prevalence in a given area, requires two critical immunoglobulins (IgG and IgM) (Olajide et al., 2015). When both IgM and IgG or only IgM occur at the same time, an acute or recent infection is indicated, but the occurrence of IgG antibody alone in the absence of IgM is a seromarker of immunity against RubV (Taneja & Sharma, 2012; Wondimeneh et al., 2018). However, there is susceptibility to getting rubella infection when both IgM and IgG antibodies are absent. This study aimed at ascertaining the presence of RubV antibodies amid pregnant women in Rivers State, Nigeria.

It was observed that 96.7% were positive for anti-Rubella IgG antibodies in this study, suggesting a prior exposure of these women to the Rubella virus. This probably occurred in early childhood, accounting for their high immunity (Gieles et al., 2020). This 96.7% compares with high seroprevalence rates of 97.9% in Zaria (Mohammed et al., 2010), 97.8% in Soweto (Gieles et al., 2020), 96.5% in Kaduna (Mangga et al., 2014) and 95.1% in Ibadan (Adewumi et al., 2015).

Gieles et al. (2020) suggested that higher population mobility and contact exposure in these areas may contribute to the high seroprevalence rates.

The overall seroprevalence recorded in this study differs from 89.3% in Port Harcourt (Adam et al., 2020a), 86.1% in Port Harcourt (Okonko et al., 2020), 53.0% in Benin (Onakewhor & Chiwuzie, 2011), 68.5% in Ibadan (Bamgboye et al., 2004), 73.8% in Lagos (Olajumoke et al., 2014). This difference could be variations in the size of samples, methods of assays used and their cut-off points, and differences in the endemicity of the virus (Wondimeneh et al., 2018).

According to Olajide et al. (2015), reinfection, which is rare (Mendelson et al., 2006), or a primary infection that was resolving may have been present at the time of sample collection. Furthermore, 15.6% was obtained for anti-RubV-IgM antibodies, and all were seropositive for anti-Rubella IgG antibodies. These women were mostly in their second and third trimesters, perhaps exposed to rubella virus in first trimester or just before falling pregnant. They

subsequently developed IgG antibodies within 30 days of infection (Wondimeneh et al., 2018). This IgG antibody infers that they might not have been immune before becoming pregnant but was still in the recovery stage. As a result, their fetuses may be at risk of rubella-associated congenital disabilities. This risk is especially elevated if exposure of a susceptible pregnant woman occurs in the early gestational weeks. Thus, screening women of childbearing age for rubella before conception or during pregnancy is imperative to dampen the consequences of congenital rubella infection.

The IgM seroprevalence of 15.6% is higher than other rates obtained from other studies (Onakewhor & Chiwuzie, 2011; Tamirat et al., 2017; Okonko et al., 2019) and lower than 38.8% reported by Olajide et al. (2015). The 15.6% reported here is comparable to the 16.3% reported by Adam et al. (2020b) and higher than the 7.8% by Okonko et al. (2019) in similar studies in Rivers State. As pointed out earlier, the differences in prevalence rates can be attributed to variations in the endemicity of RubV and continuous transmission in susceptible groups, population density differences, variations in temperature/humidity, and the presence/absence of RubV vaccination. However, none (0.0%) participants were Rubella IgG seronegative and rubella IgM seropositive.

The lack of connotation between rubella exposure and some of the socio-demographic and obstetric characteristics in the study has also been reported in various African studies (Tamirat et al., 2017; Pennap & Egwa, 2016; Jonas et al., 2016). Tamirat et al. (2017) suggest this may be because most people in endemic settings are exposed to rubella virus infection early. Nevertheless, significant association existed between IgM seropositivity and STD history, marital status and history of abortion.

From the study, the rubella seroprevalence rate did not increase with an advance in age, as opposed to other studies reported in Nigeria (Olajide et al., 2015). The highest rubella IgG seroprevalence rates occurred between 20-29 and 40-49 years. High seroprevalence within 20-29 years of observation suggests a prior infection with rubella early in life before attaining childbearing age. In addition, high seropositivity rates also observed among age group ≥ 40 years support the earlier suggestion that more extended periods and probably higher frequency of childbearing and nursing experience in a such age group may predispose them to risk of exposure and infection (Adewumi et al., 2013). This finding can also explain why IgM antibodies were absent (0.0%) for the older age group,

40-49 years, as most of them would have gained immunity from prior exposures.

Only single expectant mother was positive for RubV IgG antibody and negative for Rubella IgM antibody, while 96.6% of the married pregnant women tested positive. This variance can be ascribed to the smaller sample size of single pregnant women. However, the high RubV prevalence observed in the married might be due to their prior contact to the RubV as stated by Praveen et al. (2016). In addition, pregnant women from the polygamous family had the highest prevalence (100.0%).

Pregnant women with no education and secondary education recorded the highest seropositivity rates compared to those with tertiary education. This observation is possibly due to their lower education and low socio-economic status. This observation agrees with the report by Junaid et al. (2011) that secondary school graduates had the highest seropositivity. The present observation disagrees with previous reports by Adim et al. (2020a), Ekuma et al. (2018), and Mohammed et al. (2010) which reported higher prevalence amongst pregnant women with tertiary education. Our present finding is conflicting to Kolawole et al. (2014) who reported that primary school graduates had the highest prevalence of IgG. The present results indicated that the educational status was an insignificant ($p > 0.05$) risk factor. This deviated from Okonko et al. (2020) who reported significant association ($p = 0.023$) between RubV IgG antibodies and educational status. This observation is consistent with reports by Ekuma et al. (2018); Kuta et al. (2017) and Adim et al. (2020a). The present observation may be credited to prolonged contact to the virus from various environments and lifestyle pattern as students are generally more mobile (Onakewhor & Chiwuzie, 2011; Adim et al., 2020a). Artisans, business executives, traders, and those unemployed had a 100.0% prevalence compared to civil servants who had an 89.7% prevalence. This observation may be due to the predisposition of these women to factors that enhance RubV spread (Mohammed et al., 2010; Adim et al., 2020a). This aligned with Adim et al. (2020a) observation that higher prevalence (93.5%) occurred among traders. Prevalence regarding occupation cannot be explicitly ascertained as it might not have been a predisposing factor to RubV IgG antibody. This deviated from Okonko et al. (2020) who reported significant association ($p = 0.040$) with occupational status. This deviated from Adim et al. (2020a) who also reported significant association ($p = 0.000$) with occupational status. The 100.0% prevalence obtained in artisans and

traders may involve crowded conditions related to occupation, which may heighten the likelihood of exposure.

Rubella seroprevalence declined as the gestational age increased. Those in their first trimester had the highest prevalence of 100.0%. This observation agrees with the findings of Kolawole et al. (2014) and Fokunang et al. (2010) but contradicts Agbede et al. (2011), who reported the higher RubV prevalence in second trimester. Present observation shows that the women in their first trimester and many in the other stages are immune serologically and as such, their babies are less expected to be at risk of CRS.

Relating to parity, 100.0% prevalence occurred with zero parity. This observation agrees with Praveen et al. (2016) and Onakewhor and Chiwuzie (2011), who observed a higher prevalence with zero parity than others. In contrast, Adewumi et al. (2013) inferred that rubella antibody comes with age and parity. IgM seropositivity was found to increase with increasing parity. This observation suggests that the experience of a higher frequency of childbearing and nursing may predispose them to risk of exposure and infection (Adewumi et al., 2013).

Based on religion, participants were classified into three categories, Christian, Muslim and those belonging to no religion. The category belonging to none had the highest prevalence (100.0%).

A higher prevalence occurred with abortion history (though comparable to those without history), those with HIV-positive status and those without an STD history. This aligned with Jonas et al. (2016) in a similar study in Namibia. However, these factors did not associate significantly with RubV IgG antibodies, except for abortion history ($p < 0.05$). In contrast, Olajide et al. (2015) observed that abortion history did not associate significantly with RubV IgG antibodies.

While the 96.7% rubella IgG seroprevalence obtained in the study signifies a large proportion of pregnant women with immunity against RubV and 3.3% remains seronegative. These women are vulnerable to acquiring rubella. According to Gavin et al. (2015), developing countries have about 10-25% seronegative women, and countries with high rates of susceptibility to rubella virus among women of childbearing age might be at risk of CRS (Lambert et al., 2015). According to WHO (2011), outbreaks of CRS could occur when a susceptibility rate of 10.0% occurred among adult women. Therefore, measures should be

implemented to ensure that the susceptible group remains at the bare minimum.

5. CONCLUSION

This study confirmed a high RubV IgG antibodies (96.7%) amongst pregnant women in Rivers State, Nigeria, and also found many (15.6%) of them to have had acute RubV, suggesting the virus is endemic. A small percentage of these women also possessed no immunity. They are vulnerable to RubV infection and were likely to transmit the virus to their unborn child. Thus, to keep this susceptible group at the bare minimum, women of childbearing age should be screened before conception, and adequate vaccination programs and a continuous surveillance system should be implemented.

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