



Rubella Antibody Avidity Among Rubella Seropositive Women Attending a Tertiary Care Facility in Nigeria

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Abstract

Objectives: The aim of this study was to determine the seroprevalence of rubella virus antibodies among women of reproductive age group and assess risk factors of rubella infection.

Materials and Methods: A cross-sectional study was carried out among 285 women aged between 15 and 49 years. Enzyme-linked immunosorbent assay (ELISA) method was used to detect and quantify human immunoglobulin G (IgG) antibodies with avidity for rubella virus in sera of participants. Socio-demographic characteristics of the participants, along with recent and previous history of fever and rash among others were obtained using a questionnaire. Statistical analysis was carried out using SPSS version 16.

Results: Seroprevalence of rubella IgG among women of reproductive age in Ilorin was 92.3% (95% CI: 89.2%-95.4%). Thus susceptibility rate to rubella infection was 7.7%. Majority (87.1%) of the IgG seropositive participants had high rubella IgG avidity, implying past rubella infection or reinfections. Seroprevalence was significantly higher among participants from lower educational and socio-economic classes compared with other participants ($P = .035$ and $P = .023$, respectively). There was a negative correlation between age and rubella specific IgG titer ($P = .000$). Thus rubella specific IgG titer decreases with increasing age.

Conclusion: There is need for vaccination of susceptible women of reproductive age in Ilorin as the rubella susceptibility rate was higher than the 5.0% target set by the World Health Organization (WHO) for prevention of congenital rubella syndrome (CRS).

Keywords: Antibody avidity, Nigeria, Rubella, Seroepidemiologic studies

Introduction

Rubella is a vaccine-preventable disease caused by rubella virus. It is usually mild, self-limiting and may be subclinical in more than 50% of cases (1). However, when acquired during pregnancy, particularly in the first and early second trimester can infect the placenta and foetus resulting in either spontaneous abortion; stillbirth/foetal death, neonate born with congenital rubella syndrome (CRS) or congenital rubella infection (CRI) without congenital defects (2).

CRS may manifest as serious foetal anomalies affecting virtually all organs, especially the eyes, ears, heart and neurological system, making it the leading cause of preventable congenital defects (3). The highest risk of CRS is found in regions with high susceptibility (low seroprevalence) rates among women of childbearing age, as presence of high circulating maternal antibody (minimum serum rubella IgG level of 10-15 IUml⁻¹) which indicates immunity to rubella and virtually excludes the possibility of rubella transmission to the foetus (1).

IgG antibody avidity assay (ie, the strength of IgG binding to a multivalent antigen of the virus) is an adjunct test used to distinguish recent from past infections in indi-

viduals with IgG antibodies (4). Seroprevalence of rubella varies widely between countries, different parts of the same country and overtime within a particular region of a country (5). The seroprevalence among women of reproductive age is reported as greater than 90% in most developed countries (6). Rubella seroprevalence in African region range from 71% to 99% and varied by age group (range from 84% to 94% among women of reproductive age) (6).

Rubella is endemic in Nigeria (7). Studies among women of child bearing age in Nigeria put seroprevalence at 66.6% in Imo, 77% in Lagos and 93.5% in Oyo (8-10). Although data on CRS in Africa are very limited and the true burden is largely unknown, the burden of the chronic disabilities from CRS is high as these disabilities incur high treatment costs thus increasing the public health importance of Rubella (7).

It has been estimated that to prevent CRS the percentage of susceptible women of child-bearing age must not exceed 5.0% (11). A recent study in this environment however put the seroprevalence of rubella among pregnant women at 16.3% (12). Thus as part of the control measure, rubella serosusceptibility especially among women

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of reproductive age needs to be assessed with a view to ascertain the risk of CRS. The result of this study would be a useful tool to influence policy on routine immunization among susceptible female groups. This would in turn reduce the menace and the public health impact of CRS since part of preventive measures is screening women of childbearing age for rubella antibodies and immunizing those susceptible (9). This study therefore aims to determine the seroprevalence of rubella among women of reproductive age in Ilorin.

Materials and Methods

Study Design

A cross-sectional descriptive study was carried out among of reproductive age attending general outpatient's clinic at University of Ilorin Teaching Hospital (UITH). Collected blood specimens were analyzed in the same hospital. The inclusion criterion was basically to be consenting females aged 15-49 years. The exclusion criteria included the refusal to participate in the study and previous history of rubella vaccination.

Sampling Method

Consecutive sampling was used. Subjects who satisfied the inclusion criteria during the study period were included in the study.

Data and Blood Specimen Collection

Each patient was made to sit comfortably. After completion of consent form, the bio data was recorded in a standard questionnaire. Then 4 ml of venous blood was drawn via venepuncture and sample placed in a sterile dry tube. Centrifugation was done at 2000 rotations per minute (rpm) for 5 minutes. Serum was collected in cryotubes and stored at -20°C.

Laboratory Analysis of Specimens

Rubella IgG were assayed using RUB IgG (quantitative) kits (Dia Pro. Diagnostic Bioprobes Srl Columella Milano, Italy) according to the manufacturer's instructions (13).

Data Presentation and Analysis

All data were entered into computer and analyzed using SPSS version 16. The prevalence of rubella virus among women of reproductive age was calculated as: $P = \frac{N1}{N2} \times 100\%$. Where P is prevalence; N1, the total number of women presenting antibodies to the rubella virus; N2, the total number of women tested for antibodies.

Socio-economic index scores to which the participants are entitled to were assigned according to their occupations and educational classes using the Oyediji socio-economic classification scheme (Appendix I) (14).

In Oyediji classification scheme, scores ranging I to V are assigned based on the highest level of education and occupation of participants. The mean of 2 scores (one for the highest level of education and one for occupation) approximated to the nearest whole number was the socio-economic classification (SEC) assigned to the partic-

ipants (14). For example, if the participant was an artisan (score = 3) and the educational attainment was primary 6 (score = 4), the SEC of this participant was $4 + 3/2 =$ to the nearest whole number 4. Socio-economic index scores range from I to V, where socio-economic class I is the highest SEC, while SEC V is the lowest SEC.

Comparison of categorical data was done by chi-square and Fisher exact tests (Fisher exact test was used when the value of any variable is less than 5). Also, rubella-specific IgG antibody levels in the different stratified age groups were compared using one-way analysis of variance (ANOVA). A *P*-value < .05 was considered statistically significant.

Results

A total of 285 women of reproductive age group (15-49 years) resident in Ilorin attending general outpatient clinic at UITH were recruited for the study. The study period was between August 2012 and November 2012.

The mean age of the 285 participants was 28 ± 7 years. The demographic characteristic of participants is presented in Table 1. Majority, 223 (78.3%) participants, were in age group 15-34 years, 144 (50.5%) single, 189 (66.3%) practicing Islam and minorities (2.1%) were in SEC I.

Out of the 285 participants recruited into this study, 263 (92.3%) were seropositive for rubella specific IgG antibodies while the remaining 22 (7.7%) participants were seronegative. Thus, the overall seroprevalence of rubella IgG antibodies was 92.3% (95% CI: 89.2%-95.4%).

Table 2 shows the distribution of participants according to

Table 1. Demographic Distribution of Participants

Variable	No.	%
Age range (years)		
15-24	106	37.2
25-34	117	41.1
35-44	56	19.6
>44	6	2.1
Religion		
Christianity	96	33.7
Islam	189	66.3
Marital status		
Single	144	50.5
Married	140	49.1
Widow	1	0.4
Resident		
Ilorin-east	103	36.1 ^a
Ilorin-south	83	29.1
Ilorin-west	99	34.7
Socioeconomic class		
I	5	1.8 ^a
II	43	15.1
III	97	34.0
IV	121	42.5
V	19	6.7

^aPercentages do not add to 100.0% due to rounding error.

the stratified age seroprevalence of rubella. The age-specific rubella seroprevalence increased with increasing age. The age-specific rubella seroprevalence was lowest (90.6%) among participants in the 15-24 years age group and highest (100%) among those above 44 years. However, the observed increase in rubella seroprevalence with increasing age is not statistically significant ($P=.493$). A noticeable variability in the values of antibodies was noted in the quantitative analysis for rubella IgG antibodies. The rubella-specific IgG titer ranged between 0.5 IUml⁻¹ and values above than 250 IUml⁻¹. The lowest rubella-specific IgG titer obtained among the seropositive participants was 22 IUml⁻¹, while 155 (58.9%) had rubella IgG titer above 249 IUml⁻¹.

The mean rubella IgG titer of participants in various age groups is presented in Table 3. The highest mean (200.8 IUml⁻¹) was found in the age group 15-24 years, while the lowest mean (82.5 IUml⁻¹) was among those above 44 years of age.

Rubella IgG titer in the different stratified age groups were compared using one way ANOVA. With an F-ratio of 4.411, it was concluded that a significant difference exists among different age groups in respect to their rubella specific IgG titer at the 0.005 significance level.

The Pearson correlation between age and rubella IgG titer was negative at r^2 value of -0.225. Thus, the decrease in rubella IgG titer with increasing age is significant ($P=.000$). Majority (87.1%) of the IgG seropositive participants had high rubella IgG avidity as shown in Table 4. The high rubella IgG avidity was found more in older age group than lower age groups. The observed difference was found to be statistically significance ($P=.001$).

The seroprevalence of rubella in various SEC is presented in Table 5. There was a significant association between rubella IgG status and SEC of participant. Rubella IgG seroprevalence was highest (100.0%) among participants in SEC V and lowest (60.0%) among those in SEC I. There was a significant correlation between rubella IgG seropositivity and SEC ($r = 0.150$; $P = .011$). Thus, as SEC decreases the rubella seroprevalence increases.

In participants from low SEC, rubella seropositivity was 2.0 times higher than in those from high SEC (OR = 2.0; 95% CI: 1.3-17.2). Thus, seroprevalence was significantly higher among lower SEC than higher SEC ($P = .022$).

The seroprevalence of rubella IgG among participants according to past history of fever and rash is presented in Table 6. Sixty-six (23.2%) participants had a past history of fever and rash. Of the 219 participants with no past

Table 2. Stratified-Age Distribution of Rubella IgG Antibodies

Age (y)	Rubella IgG		χ^2	P Value
	Positive (%) ^a	Negative (%) ^a		
15-24	96 (90.6)	10 (9.4)		
25-34	107 (91.4)	10 (8.6)		
35-44	54 (96.4)	2 (3.6)		
>44	6 (100.0)	0 (0.0)	2.404	.493

^aRow percentage.

Table 3. Age Group Related Mean Rubella IgG Titre

Age (y)	Mean IgG Titre \pm SD (IUml ⁻¹)	r^a	P Value
15-24	200.8 \pm 78.8		
25-34	193.6 \pm 80.7		
35-44	183.5 \pm 74.2		
>44	82.5 \pm 55.8	-0.225	<.001

^aPearson correlation coefficient.

Table 4. Rubella IgG Avidity by Age

Age (y)	Rubella IgG Avidity		r^a	P Value
	High (%) ^b	Low (%) ^b		
15-24	73 (76.0)	23 (24.0)		
25-34	98 (91.6)	9 (8.4)		
35-44	52 (96.3)	2 (3.7)		
>44	6 (100.0)	0 (0.0)		
Total	229 (87.1)	(12.9) 34	-0.277	<.001

^aPearson correlation coefficient.

^bRow percentage.

Table 5. Rubella-Specific IgG Antibodies by Socio-Economic Class

SEC	Rubella IgG status		r^a	P Value
	Positive (%) ^b	Negative (%) ^b		
I	3 (60.0)	2 (40.0)		
II	38 (88.4)	5 (11.6)		
III	88 (90.7)	9 (9.3)		
IV	115 (95.0)	6 (5.0)		
V	19 (100.0)	0 (0.0)	0.150	.011

Abbreviation: SEC, socio-economic classification.

^aPearson correlation coefficient.

^bRow percentage.

Table 6. Past History of Fever and Rash by Rubella-Specific IgG Antibodies

History of fever & rash	Rubella Specific IgG		OR (95% CI)	P Value ^a
	Positive (%)	Negative (%)		
Yes	63 (95.5)	3 (4.5)		
No	200 (91.3)	19 (8.7)		
Total	263 (92.3)	22 (7.7)	0.50 (0.14-1.75)	.270

Abbreviation: OR, Odd ratio.

^aFisher exact test.

history of fever and rash, 200 (91.3%) had rubella specific IgG antibodies. The rubella IgG seroprevalence increased to 95.5% for participants with history of fever and rash. However, the increase is not statistically significant ($P = .205$).

Discussion

Rubella is a potentially eradicable, vaccine-preventable disease. CRS, a serious consequence of maternal rubella infection during early pregnancy, results in devastating chronic disabilities in foetus. Incidence of CRS depends on the percentage of seronegative (susceptible) women in their reproductive age (11). Seroprevalence of rubella in women of reproductive age in Ilorin according to IgG ru-

rubella assay was found to be 92.3% (95% CI: 89.2%-95.4%). Thus, the percentage of seronegative women of reproductive age was 7.7% which is higher than the 5.0% estimated value for elimination of CRS (11). This is an important finding for susceptible women who might acquire rubella infection during pregnancy leading to CRI in their babies. The high seroprevalence could be due to the fact that rubella virus is wide spread, primarily infecting young children and by the age of 15 years most will have immunity from natural infection (6). Rubella vaccine is not included in Nigeria's Immunization schedule; this further suggests that the presence of antibodies is related to natural infection.

Across the globe, there is considerable variation in the seroprevalence of rubella among women of reproductive age. In a review of rubella epidemiology in Africa by Goodson et al (6), rubella seropositivity among women of reproductive age ranged between 84% and 94%. The rubella seropositivity obtained in this study is within this range. It is also similar to 93.5% obtained in a previous study among women of child bearing age in Oyo state by Adesina et al (8) and 90.1% obtained among women 15-45 years of age in Senegal by Dromigny et al (15). Conversely, it is at variance from a previous study by Agbede et al (12) among pregnant women at UITH, Ilorin where a value of 16.3% was obtained. It is also higher than 77.0% reported among nonvaccinated women by Onyenekwe et al (10) in Lagos, Nigeria and 88.6% among women of reproductive age in Cameroon (6). The observed differences might be because of the differences in study population, sample size and study period. In addition, the observed variation might be due to a recent outbreak. The risk of rubella and subsequently CRS is highest in countries with high susceptibility rate among women of childbearing age, which might be the case in Ilorin as earlier reported by Agbede et al (12) 3 year ago when the susceptibility among pregnant women was 83.7%, thus a recent epidemic might have occurred.

In places where susceptibility rate of rubella virus in women of reproductive age is low, this may reflect low socio-economic status, recent outbreak of rubella or intense circulation of virus in younger age group (16). If a recent outbreak has occurred, then a high number of cases of CRS may have occurred, as the risk of CRS may increase by tenfold following epidemic (17). Thus, it may be useful to assess the burden of CRS in this environment.

Rubella IgG seropositivity rate increased with increasing age, but it was not statistically significant. Studies by Dayan et al (18) and Palihawadana et al (19) found significant increasing seroprevalence of rubella with increasing age. A study by Pehlivan et al (20) among Turkish women however, reported no significant relationship between age and rubella seroprevalence.

Conversely, a significant difference exists among different age groups in respect to their rubella-specific IgG titer ($P = .005$). Rubella-specific IgG antibodies titer significantly decrease with increasing age ($P = .000$) which might be due to natural waning of antibodies with age. Although

rubella IgG antibodies persist for at least 20 years, and probably for life, they may decline to very low levels in old age. The mean antibody titer was higher in younger age group than in older participants. Similar findings were reported by other studies, among vaccinated and nonvaccinated populations (21,22).

To distinguish participants with reinfection from those with primary infection, since there were no prescreening sera available, the rubella IgG avidity were determined. The IgG antibody avidity (ie, the strength of IgG binding to a multivalent antigen of the virus) is an adjunct test used to distinguish recent from past infections in individuals with significant IgG antibodies (14). In the acute phase of an immune response, the IgG antibody generated by the antigen has low avidity (below 50%). This is because the antibody binds with less strength to the antigen. The strength of the antibody-antigen bonds increases within 2-4 months after infection, reflected in an avidity reading of greater than 50%.

Sera from cases of recent rubella primary infection have low IgG avidity, while sera taken from participants with past infection (including cases of rubella reinfection) have higher avidity (4). Thirty-four (11.9%) participants had a low avidity IgG among which 23 (67.6%) were aged 15-24 years. Low avidity rubella IgG antibodies were significantly more in younger age group than older age group ($P = .001$). The group with low avidity IgG are at risk of a reinfection which carries a low (8%) risk for having children with CRS (18). It may also indicate recent rubella infections in 11.9% participants which are in their reproductive age. This particular proportion of women in their reproductive age had a chance of having a child with CRS if they were to be in early stage of pregnancy. Five percent of all reported rubella infection in Africa occur in women of reproductive age (6).

Rubella seroprevalence were compared within various SECs. Significantly higher rubella seroprevalence was observed among participant from low SEC compared with those from high SEC. This could be due to association between close contact, overcrowding or poor environment and hygiene and acquisition of rubella infection in participants from low SEC. Similar findings have also been reported by Raveendran et al (23).

Rubella is a common cause of maculopapular rash with fever. However, no significant differences were observed in the rubella antibody seropositivity of participants according to past history of fever and rash. This can be explained by the fact that rubella infections could be asymptomatic or symptomatic (1). Moreover some with previous history of fever and rash may not remember. Previous studies have presented no significant association between rubella seroprevalence and history of fever and rash.

Conclusion

The seroprevalence of rubella in women of reproductive age in Ilorin was 92.3%. There is a significant risk of rubella infection among women of reproductive age in Ilorin as rubella susceptibility rate in this study was 7.7%. A few

(8.7%) of the participants had a low rubella IgG avidity which connotes a recent rubella infection. There is therefore a need for rubella vaccination among susceptible women. Thus, this study recommends routine preconception rubella screening for women of reproductive age in Nigeria and rubella vaccination should be provided for those susceptible.

Considerations should also be made on the necessity to introduce rubella vaccination into the current National Programme on Immunization in Nigeria as soon as greater than 80% measles coverage is achieved as suggested by World Health Organization (WHO). Finally, effective rubella/CRS surveillance should be put in place in this country.

Ethical Issues

Ethical clearance was obtained from the Ethics and research committee of UIITH Ilorin. Informed, written and signed consent was obtained from participants.

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Conflict of Interests

The authors declare that they have no competing interests.

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Appendix 1. Socio-Economic Classification Scheme by Oyedeji (14)

For Occupation	
Class	Occupation
I	Senior public servants, professionals, managers, large scale traders, businessmen and contractors.
II	Intermediate grade public servants and senior school teachers.
III	Junior school teachers, drivers, artisans.
IV	Petty traders, labourers, messengers.
V	Unemployed, full-time housewife, students and subsistence farmers.
Educational status	
Class	Educational attainment
I	University graduates or equivalents
II	School certificate holders, ordinary level (GCE) who also had teaching or other professional training.
III	School certificate or grade II teacher's certificate holders.
IV	Modern three and primary six certificate holders.
V	Those who could either just read and write or were illiterate.

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