

Original Research Article

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Prevalence of Rubella Virus Infection among Pregnant Women Accessing Antenatal Clinic at Federal Medical Centre, Keffi, Nigeria

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ABSTRACT

Rubella virus infection is a global public health problem especially in pregnant women leading to congenital defects. There is dearth of information on its prevalence in Keffi, thus this baseline study as a prelude for the requirement for rubella vaccination policy. Blood samples from 220 consenting pregnant women were screened for rubella IgG antibody using an ELISA test kit (Cortez Diagnostic, Inc, USA). Chi-square test was used to determine possible risk factors associated with the viral seropositivity. The overall seroprevalence of the viral infection was 11.4%. Participants aged ≤ 19 years recorded the highest prevalence (25.0%) while there was no infection recorded among those aged ≥ 40 years ($p > 0.05$). There was a statistically significant association between the seroprevalence of infection and gestational period. Participants in their 2nd trimester had the highest prevalence (23.5%) while women in their 1st trimester were seronegative to IgG ($p < 0.05$). Other probable risk factors studied were educational level, occupation, parity and locality but none of these had a statistically significant association with rubella virus infection ($p > 0.05$). A significant number (88.6%) of the pregnant women were found to be susceptible to rubella virus infection. The initiation of a vaccination policy for all women of child bearing age is advocated for this area.

Keywords

Seroprevalence, Rubella virus, IgG, pregnant women.

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Introduction

Rubella (which means “little red”) commonly known as “German Measles” was originally thought to be a variant of measles (Fokunang, 2010; Mounerou *et al.*, 2015). It is caused by Rubella virus which is the sole member of the genus Rubivirus in the Togaviridae family (Olajide *et al.*, 2015) and also have humans as their only reservoir (Mounerou *et al.*, 2015).

The virus which has an incubation period of 2 – 3 weeks is transmitted through the

respiratory route (WHO, 2011; Kolawole *et al.*, 2014; Mounerou *et al.*, 2015).The disease is characterized by a rash and lymphadenopathy that affects children and young adults. It is the mildest of common viral exanthema (Al-Rubaiet *al.*, 2010).Rubella has symptoms are similar to that flu, however, the primary symptom of rubella virus infection is the appearance of rash (exanthema) on the face which spreads to the trunk and usually fades after three days. Other symptoms include low-grade fever, swollen glands (sub occipital and

posterior cervical lymphadenopathy), joint pains, headache and conjunctivitis (Lezan, 2015). The clinical diagnosis is not easy to establish because of the transient symptoms (Mounerou *et al.*, 2015).

When a woman is infected with the virus during the first 20 weeks of pregnancy, it might result in a miscarriage, stillbirth, or the baby born with Congenital Rubella Syndrome (CRS). This syndrome results in cardiac, cerebral, ophthalmic and auditory defects (Al Rubaa *et al.*, 2010; WHO 2011, WHO 2013a; Kolawole *et al.*, 2014). Studies have shown that if maternal infection occurs before 9 weeks of gestation, the risk of fetal manifestation is 85%, 52% if between 9 – 12 weeks and rarer if after 16 weeks of gestation (Chopra and Mahajan, 2015).

Rubella vaccines are live attenuated vaccines and a single dose of the vaccine confers long-lasting immunity in more than 95% of the vaccine recipients. Immunity is also naturally induced after rubella infection. Thus immunoglobulin G (IgG) antibody in the serum is a seromarker of rubella immunity and IgG antibodies ≥ 10 IU/ml is generally considered protective (WHO, 2011; WHO, 2013b).

Since the infection is vaccine preventable, active immunization with live vaccine combined with measles and mumps vaccine have been used in some countries (Adewumi *et al.*, 2013; Mounerou *et al.*, 2015). Although the infection has declined with the implementation of rubella vaccination over the years, it is still considered as an important public health problem around the world especially in the third world countries. In their effort to eliminate rubella virus infection and its attendant sequelae, Brazil 16 years ago vaccinated 70 million adolescents and adults (Onakwe and

Chiwuize, 2011). Despite a vaccine against rubella virus being available, most African countries do not include it in their national public health immunization programs (Mamvura *et al.*, 2015). The rubella virus is therefore circulating freely in many African regions. Data of the seroprevalence of the virus in most African population is also very limited (Mamvura *et al.*, 2015) although the World Health Organization had earlier advised that countries should key into the accelerated measles control and elimination programs so as to introduce rubella containing vaccines also (WHO, 2011).

Materials and Methods

Study Area

This research work was carried out in Keffi. It is approximately 68km away from Abuja, the Federal Capital Territory and 128km from Lafia the capital city of Nasarawa State. Keffi is located between Latitude $8^{\circ}5'N$ of the Equator and Longitude $7^{\circ}8'E$ and situated on an altitude of 850m above sea level (Akwa *et al.*, 2007).

Study Population and Design

The study was a cross-sectional study carried out among pregnant women attending antenatal clinic of Federal Medical Centre, Keffi. A total of 220 pregnant women were recruited for the study. The socio-demographic information of the clients was obtained by oral interview.

Sample Collection

Five ml blood sample was aseptically collected by venipuncture, transferred into a plain tube and allowed to clot at room temperature. The samples were transported in a cold box to Innovative Biotech Limited Laboratory, Keffi. The blood was centrifuge

at 3000rpm for 5 minutes. Each supernatant serum was carefully collected into a labelled eppendorf tube using a Pasteur pipette.

Ethical Approval

Approval for this study was obtained from the Ethical Review Committee on Human Research of the Federal Medical Centre, Keffi, Nasarawa State.

Laboratory Investigations

The rubella virus specific IgG ELISA kit (Cortez Diagnostic Inc. USA) was used to detect the rubella virus IgG antibody in the sera according to the manufacturer's instructions.

Purified rubella antigen is coated on the surface of microwells. Diluted patient serum is added to the wells. The rubella IgG specific antibody binds to the antigen, all unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody antigen complex. Excess enzyme conjugate is washed off and chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell reader and compared in a parallel manner with calibrator and controls.

ELISA Procedure

The desired number of coated strips were placed into the holder and 1:40 dilution of each test serum was prepared by adding 5 μ l to 200 μ l of the sample diluent and mixed well. 100 μ l of the diluted sera, calibrator and controls were dispensed into appropriate wells. For the reagent blank, 100 μ l sample diluent were dispensed into the well in position A1. The holder was tapped gently

to remove air bubbles from the liquid and also to mix the contents of each well. The test strips were incubated for 30minutes at room temperature. The liquid content was removed from all the wells and a proper washing of the wells was done using the wash buffer. This step was repeated three times and then 100 μ l of enzyme conjugate was dispensed into each well. The test was further incubated for 30 minutes at room temperature after which the enzyme conjugate was discarded and the wells washed three times with the washing buffer. After this step, 100 μ l of Tetra methyl benzidine (TMB) chromogenic substrate was dispensed into each well and incubated for 30minutes at room temperature after which 100 μ l of 2N HCL was added to stop the reaction. The absorbance of the contents of each micro plate was read at 450nm with a micro well reader.

Negative Result

Rubella IgG Index 0.90 or less was seronegative for IgG antibody to rubella

Equivocal Result

Rubella IgG Index of 0.91-0.99 was equivocal, and the sample should be retested.

Positive Result

Rubella IgG Index of 1.00 or greater was positive.

Statistical Analysis

Data obtained from the study were analyzed using Chi-Square test to determine the association of the viral prevalence of infection among pregnant women with the studied risk factors. Values obtained were considered statistically significant at $p \leq 0.05$

Results and Discussion

As shown in Table 1, out of the 220 pregnant women screened for specific anti-Rubella virus IgG antibodies seroprevalence was highest (25%) among those aged ≤ 19 years. Viral infection was found to be absent among the illiterate women, those that were farmers and also those that were primiparous.

Using anti Rubella virus IgG as a seromarker for the determination of past viral infection and therefore the induction of natural immunity, a seroprevalence of 11.4% was recorded among the 220 pregnant women screened. The implication of this is that 88.6% of these pregnant women are susceptible to the viral infection. This is the lowest published rate in Nigeria. It is relatively very low especially when compared to similar studies carried out in other parts of Nigeria where there have been reports of 16.3% in Ilorin (Agbede *et al.*, 2011), 53% in Benin (Onakewhor and Chiwuzie, 2011), 97.9% and 93.1% in Zaria (Mohammed *et al.*, 2010, Olajide *et al.*, 2015 respectively), 73.5% and 79.3% in Kaduna and Adamawa respectively (Chukwuedo *et al.*, 2010), 91.5% in Ibadan (Adewumi *et al.*, 2013), 85.7% in Osogbo (Kolawole *et al.*, 2014) and 83.3% in Maiduguri (Oyinloye *et al.*, 2014). Researchers from other countries have reported 53% in India (Chopra and Mahajan, 2015), 88.6% in Cameroon (Fokunang *et al.*, 2010), 85% in Togo (Mounerou *et al.*, 2015), 65.3% in Western Sudan (Hamdan 2011), 95.0% from Burkina Faso (Tahita *et al.*, 2011) and 92.0% in Harare (Mamvura *et al.*, 2015).

It could be said that the viral prevalence differs from country to country and even from place to place within the same country. The country differences might be among other reasons a reflection of immunization policies. However in Nigeria where rubella

vaccine is not offered to the populace, the differences might be due to climatic, cultural practices, characteristics of the studied population or sensitivity of the test kits used. The high rate of IgG rubella seropositivity in some places as a result of natural immunity raises a question related to the value of vaccination based on cost/effectiveness in resource limited countries.

The distribution of rubella virus infection when stratified by age appeared to be higher in women aged ≤ 19 years (25.0%) followed by 30-34 years (11.9%), 25-29 years (11.1%), 20-24 years (10.7%) and lower among older age groups (35-39 and above 40 years). There was no statistically significant association between age and the viral infection ($p > 0.05$). A similar observation was reported by Onakwe and Chiwuzie (2011), Adewumi *et al.*, (2015), Olajide *et al.*, (2015) and Kolawole *et al.*, (2014). It is possible that most of the infection was acquired in childhood as posited by Onakwe and Chiwuzie (2011). Similarly earlier studies in some African countries reported that 80% of children are positive to rubella infection by the age of 10 years (Bamgboye *et al.*, 2004).

The viral infection was not associated with educational level as viral IgG was found only among the literate pregnant women (12.0%) while none of the illiterates tested positive to the viral infection. This non-association is similar to the observation by Kolawole *et al.*, (2014). There was no obvious reason for this observation especially as Education has been acknowledged to be of advantage in various facets of life and also helps in making informed decision and sourcing for useful information regarding health concerns. However, there was paucity of samples from illiterate women in the study population (11/220) which could have led to this observation.

Table.1 Prevalence of Rubella Virus Infection among Pregnant Women In Keffi With Respect to Some Probable Risk Factors

Variables value	No. Examined	No. Positive (%)	P
Age (Years)			p> 0.05
≤ 19	12	3 (25.0)	
20-24	56	6 (10.7)	
25-29	90	10 (11.1)	
30-34	42	5 (11.9)	
≥ 35	20	3(15.0)	
Educational level			p> 0.05
Illiterate	11	0 (0.00)	
Literate	209	25 (12.0)	
Occupation			p> 0.05
Employed	43	5 (11.6)	
Unemployed	139	19 (13.7)	
Students	28	1 (3.6)	
Farmers	10	0 (0.00)	
Locality			p> 0.05
Rural	138	14 (10.1)	
Urban	82	11 (13.4)	
Parity			p> 0.05
Primiparous	127	14 (11.0)	
Multiparous	93	9 (9.7)	
Gestation			p≤ 0.05
1 st Trimester	13	0 (0.00)	
2 nd Trimester	68	16 (23.5)	
3 rd Trimester	149	9(6.0)	

With reference to occupation none was found to be a predisposing factor to rubella virus infection. Unemployed pregnant women recorded the highest seroprevalence (13.7%), followed by those that are workers (11.6%), students (3.6%) and no evidence of infection was recorded among the farmers (0.00%). This non- statistical association between viral infection and occupation (p> 0.05) was also reported by Kolawale *et al.*, (2014) in their study.

Similarly, there was no association between locality and the viral infection (p> 0.05). The prevalence of rubella virus infection

was higher among urban (13.4%) than the rural participants (10.1%). This is similar to studies conducted in other countries such as Algeria (Ouyahia *et al.*, 2013) and Iraq (Hassan, 2011) which reported higher seroprevalence of rubella antibodies in urban pregnant women. However it is in contrast with the report of the study by Shilpi *et al.*, (2015) carried out in Bijapur which observed a higher prevalence of viral infection among women residing in rural areas as compared to those in urban areas.

The seroprevalence of rubella virus infection with respect to parity was higher among the

primiparous women (11.0%) than the multiparous women (9.7%) although this difference was not statistically significant. This suggests that viral infection might not be connected with transmission through contact with children only. Being that 80% of children in most African countries are infected early in life (Bamgboye *et al.*, 2004) they would have been a good source of infection for their pregnant multiparous mothers. Kolawole *et al.*, (2014) and Agbede *et al.*, (2015) had noted that gravidity was not a predisposing factor for rubella virus infection. In a similar study in India Gupta *et al.*, (2015) reported that anti rubella virus IgG seropositivity was not associated with parity.

There was an association between gestational age and rubella virus infection in this study ($p < 0.05$). Although there was no infection recorded among those in their first trimester, the prevalence of infection was higher among women in their 2nd trimester (23.5%) than those in their 3rd trimester (6.0%). This agrees with the works of Agbede *et al.*, (2011) and Olajide *et al.*, (2015) which reported the highest prevalence in pregnant women in their second trimester but in contrast with earlier reports by Bamgboye *et al.*, (2004) and Fokunang *et al.*, (2010) which showed the highest prevalence in pregnant women in their first trimester. There is no obvious reason for this difference. This notwithstanding, it is pertinent to note that IgG denotes past infection therefore it is very possible that these women were infected before or during pregnancy. For the former, their fetuses are protected from the viral infection because of mothers' immunity and not the latter. However, for those that were infected during their pregnancy and especially during the dangerous gestation age but were only detected now with IgG the seromarker for past infection, the

sequelae associated with the viral infection like a miscarriage, stillbirth or baby born with CRS (Al Rubai *et al.*, 2010; WHO 2011, 2013a; Kolawole *et al.*, 2014) cannot be ruled out of these pregnancy outcome.

In conclusion, a rubella virus IgG seroprevalence of 11.4% was reported in the present study. This finding revealed the susceptibility to rubella virus infection of a significant fraction (88.6%) of the pregnant women in the study population. These susceptible individuals constitute a large risk group for maintenance and further transmission of the virus with its attendant dangers. It is therefore pertinent that a rubella vaccination policy be considered for all women of child bearing age in this area.

Also, despite the non association of infection with the probable risk factors studied other than gestation age, public health education especially good hygiene should be encouraged for all women of childbearing age in the interim.

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