INCIDENCE OF RUBELLA ANTIBODIES AMONG PREGNANT WOMEN IN THIRD TRIMESTER ATTENDING ANTENATAL CLINIC AT UNIVERSITY OF MAIDUGURI TEACHING HOSPITAL (UMTH)

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ABSTRACT

Background: Rubella is caused by a positive sense RNA virus of the family Rubiviridae. This is the only non-arthropod borne virus in the family and the etiologic agent of rubella affecting people of all ages and sex. The virus has been identified as a human teratogen capable of causing a spectrum of birth defects often collectively referred to as congenital rubella syndrome (CRS) or death of a developing fetus, especially if the viral infection is acquired in the early months.

Aim: This study was aimed at determining the incidence of rubella IgG/IgM antibodies among pregnant women in their third trimester attending antenatal clinic of University of Maiduguri, Teaching Hospital (UMTH).

Methodology: Ninety (90) venous blood samples were collected from pregnant women at their third trimester, the samples were separated and the sera were screened for rubella IgG and IgM using RUB anti-RV (IgG) and (IgM) kit based on indirect Enzyme Linked Immunosorbent Assay (ELISA) method.

Results: Eighty nine (98.9%) and five (5.6%) had rubella virus specific IgG antibody (IgG sero positive) and rubella virus specific IgM antibody (IgM sero positive) respectively. Women within the age bracket of 21-25 and 26-30 had the highest incidence 2.22% of rubella specific IgM, then followed by the age groups 15-20 with 1.11%. While zero percent (0%) incidence was reported among the age groups 31-35 and 36-40. Women within the age bracket (15-20) had the least (10%) incidence of rubella specific IgG antibody, and then followed by the age group (36-40) with 12.2%, age group (31-35) with 23.3%, age group (21-25) with 24.4% and lastly the age group (26-30) with 28.9%.

Conclusion: This study revealed that there are still a percentage of women 1(1.1%) at the childbearing age that had no evidence of rubella immunity (IgG seronegative) and are at risk of being infected with the virus especially during the first trimester of pregnancy which can result to congenital defects with fatal consequences. As such, there is need for more sero-surveys on rubella in the country to support the advocacy for the inclusion of rubella vaccination in the National Programme on Immunization (NPI).

Keywords: Rubella virus, incidence, Trimester

INTRODUCTION

Rubella is caused by a positive sense RNA virus of the family Rubiviridae. This is the only non-arthropod borne virus in the family and the etiologic agent of rubella affecting people of all ages and sex (Hobman, 2007). The virus has been identified as a human teratogen capable of causing a spectrum of birth defects often collectively referred to as congenital rubella syndrome (CRS) or death of a developing foetus, especially if the viral infection is acquired in the early months, i.e. first trimester of pregnancy (Chantler, 2001).

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However, if contracted during pregnancy, it may result in miscarriage, stillbirth or an infant born with congenital rubella syndrome, characterized by deafness, heart disease, cataracts or other permanent congenital manifestations (Shah, 2010). Studies have shown that 80-90% of babies born to women infected with rubella virus during the first trimester of gestation experience birth defects (Webster, 1998).

Clinical manifestations of rubella include acute febrile illness, maculopapular rash and lymphadenopathy in adults and children. Viral-specific IgM antibodies are first detected 10 days post infection, and peaks at about 4 weeks post infection. This may persist for over 7 months after an acute infection. By three weeks post infection, anti-rubella virus antibodies are present in all immunoglobulin classes, including IgG, IgA, IgD, and IgE (Hobman, 2007). The infectious period of rubella virus is from 7 days before to 5–7 days after onset of rash. At this period, the virus remains detectable only in the Nasopharynx, where it can be isolated 1 week before, to 2 weeks after the onset of the rash (Dontigny *et al.,* 2008). In developing countries, more than 100,000 children are born with CRS each year (Binnicker *et al.,* 2010). The sero-positivity for rubella among pregnant women varies widely in different countries. As a matter of fact, in many developing countries, rubella sero-positivity among pregnant women has been reported to range from 54.1% to 95.2% (Shah *et al.,* 2010). About 10-25% of non-immunized women of childbearing age are susceptible to rubella virus infection (Dwyer *et al.,* 2001). Humans are the only known reservoir for rubella virus; hence, its maintenance requires continuous access to a susceptible population. Equally, elimination of rubella and Congenital Rubella Syndrome (CRS) with an effective vaccination program in some countries is an evidence of achievable intervention plan for rubella virus and the disease (Castillo-solarzano, 2004). Serological screening of rubella, based on the detection of IgG and IgM antibodies, remains the mainstay for diagnosis, Despite the development and administration of effective vaccines for prevention and control of rubella virus infection since the late 1960s, and prevention as well as feasibility of or elimination of the causative agent in many developed countries, the infection is still endemic in Nigeria. In fact, it has been shown that a significant number of non-immunized women of childbearing age remain susceptible to rubella virus infection in the country. Also, subclinical or clinical infections as well as continuous circulation of rubella virus have previously been reported in Nigeria (Adewumi, 2013).This study was aimed at determining the incidence of rubella IgG/IgM antibodies among pregnant women in their third trimester attending antenatal clinic of University of Maiduguri, Teaching Hospital (UMTH).

MATERIALS AND METHODS

Study Area

This study was conducted in University of Maiduguri Teaching Hospital (UMTH), Borno State, Niger ia.UMTH is tertiary health institution that serves Maiduguri metropolis as well as the whole Northeast Nigeria and partly Republics of Niger, Chad and Cameroon as referral Centre. However, the subjects enrolled in to the study were drawn from Maiduguri metropolis. Borno State has an area of 61,435sq.kg- the largest State in Nigeria in terms of land Mass. It is bound by Adamawa State to the south, Yobe State to the west and Gombe State to the Southwest. The State also shares international borders with Republics of Niger to the North, Chad to the Northeast and Cameroon to the east. Based on the National Bureau of Statistics projected report of 2016, Borno State has a population density of 5,860,200 (NBS, 2016).

Study Population

The study population comprised of ninety (90) consented pregnant women at third trimester of various gestational periods attending antenatal clinic of University of Maiduguri Teaching Hospital (UMTH).

Ethical Clearance

Ethical clearance was obtained from the medical ethics committee of UMTH before commencement of the study. Signed or thumb printed written informed consent was obtained from each consenting participant before taking the specimen. This was interpreted into local languages of Hausa, Kanuri, Fulani, Ibo e.t.c. for easy communication.

Study Design

For this study, a cross-sectional, hospital-based design was employed. The objectives and procedures of the study were discussed with the pregnant women visiting the antenatal clinic of the hospitals. Ninety pregnant women at their third trimester that consented to participate in the study were consecutively recruited. Each pregnant woman provided relevant demographic data that were obtained through questionnaires administered by an interviewer. These data included age, report of MMR vaccination, educational status, marital status, number of pregnancy, present/past experience of skin rash and knowledge of rubella.

Sample Collection

Five milliliters (5mls) of venous blood was collected from each of the women in to a sterile plain container and labeled with name, age, date of collection and serial number of the subjects. The sera were obtained centrifuging at 3000rpm for 5 minutes (Agbede *et al.*, 2011), and stored in a freezer at -20^0c until ready for use. Enzyme Linked Immunosorbent Assay (ELISA) was used to screen for rubella IgG and IgM. The samples were analyzed according to the manufacturer's instructions.

Sample Analysis

The sera were tested for anti-RV IgG and IgM using commercial ELISA kits – RUB IgG ELISA for the quantitative/qualitative determination of IgG antibodies and RUB IgM "Capture" for the determination of IgM antibodies to rubella virus in human serum (DIA.PRO, Diagnostic Bioprobes Srl, Sesto San Giovanni, Milano, Italy) as employed by Obijimi *et al.,* (2013). The serologic tests and interpretation of results were done in accordance with the manufacturer's instructions while optical signals generated were read at 450nm with ELISA plate reader (Optic Ivymen RSystem, Model 2100C). Due to the controls and calibrators included in the tests, the IgG test was performed on 89 sera while all 90 samples were screened for IgM. Interpretation of ELISA results. According to the IgG ELISA kit protocol, serum samples with anti-RV IgG concentrations < 10 WHOIU/ml were considered negative for anti-RV IgG antibody while those with concentrations ≥ 10 WHOIU/ml were considered positive. The latter titer is considered the lowest concentration of IgG that provides an effective immunological protection against a second infection of RV. Therefore, for the purpose of determining the seropositivity and136corresponding concentration of anti-RV IgG in each serum sample, the lower limit of the serum control(i.e. 18 IU/ml of anti-rubella virus IgG equivalent toOD of 0.75) was used to estimate the IgG concentration. For instance, serum sample 1 recorded OD of 1.304 which is equivalent to 31.296 IU/ml of anti-RV IgG. The pregnant woman having this sample was hence considered seropositive with protective level of anti-RV IgG. This estimation was done for each of the 89 serum samples.

For the IgM ELISA, serum samples with Sample to Cut-off (S/Co) ratio > 1.2 were considered positive for anti-RV IgM antibodies while those with S/Co ratio <1.0 were considered negative. Samples with S/Co ratio between 1.0 and 1.2 were considered equivocal as recommended by the kit manufacturer.

Data Analysis

Data obtained in this study were entered on Microsoft Excel and subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) for version 20.0. Data were summarized as percentage and frequency distribution to determine the level of prevalence. Chi-square was used to determine the relationship. P-value less than 0.05 ($P \leq 0.05$) was considered statistically significant in all statistical comparison.

RESULTS

The table below shows the age group, number of samples examined, number and percentage positive for both rubella specific IgG and IgM antibodies. Out of the 90 subjects examined in this study, rubella virus-specific IgG antibody was detected in the sera of 89(98.9%) subjects. While only one (1.11) subject was tested negative for the rubella IgG antibody. Also rubella virus-specific IgM antibody was detected in the sera of 5(5.6%) subjects, while the remaining 85(94.4%) subjects were tested seronegative for the rubella IgM antibody.

Out of the 90 samples analysed, 10 belongs to subjects in the first age group (15-20) among which 9(10%) and 1(1.1%) were tested seropositive for rubella virus-specific IgG and IgM antibodies respectively. From the second age group (21-25), 22 samples were analysed and all (24.4) were tested seropositive for rubella virus specific IgG antibody and only 2(2.22) are seropositive for the rubella virus specific IgM antibody. From the third age group (26-30), 26 samples were analysed and all (28.9) were tested seropositive for rubella virus specific IgG antibody and only 2(2.22) are seropositive for the rubella virus specific IgM antibody. From the fourth age group (31-35), 21 samples were analysed and all (23.3) were tested seropositive for rubella virus specific IgG antibody, while none is seropositive for the rubella virus specific IgM antibody. From the last age group (36-40), 11 samples were analysed and all (12.2)were tested seropositive for rubella virus specific IgG antibody, while none is seropositive for the rubella virus specific IgM antibody. According to the result of this study, subjects within the age bracket of 21-25 and 26-30 had the highest incidence 2.22% of rubella specific IgM, then followed by the age groups 15-20 with 1.11%. While zero percent (0%) incidence was reported among the age groups 31-35 and 36-40. Women within the age bracket (15-20) had the least (10%) incidence of rubella specific IgG antibody, and then followed by the age group (36-40) with 12.2%, age group(31-35) with 23.3%, age group (21-25) with 24.4% and lastly the age group (26-30) with 28.9%.

Table 1: Incidence of Rubella IgG and IgM antibodies in relation to age of subjects

Age Group	Number Tested	No. of IgG positive	% of IgG positive	No. of IgM Positive	% of IgM Positive
15-20	10	9	10	1	1.11
21-25	22	22	24.4	2	2.22
26-30	26	26	28.9	2	2.22
31-35	21	21	23.3	0	0
36-40	11	11	12.2	0	0
Total	**90**	**89**	**98.9**	**5**	**5.6**

Table 2 below shows the frequency distribution and percentage incidence of rubella IgG antibodies among the study subjects. Out of the 90 subjects examined, 89(98.9%) were seropositive for the rubella virus specific IgG antibody, while only 1(1.1%) subject is seronegative for the rubella IgG antibody.

Table: 2 Percentage frequency and distributions of rubella virus specific IgG among the study subjects.

	Frequency	Valid Percentage	Cumulative Percent
Negative	1	1.1	1.1
Positive	89	98.9	100.0
Total	**90**	**100.0**	

Table 3 below shows the frequency distribution and percentage incidence of rubella IgM antibody among the study subjects. Out of the 90 subjects examined, only 5 (5.6%) were tested positive for rubella IgM antibody, while the remaining 85 (94.4%) were tested negative. The rubella virus specific IgM antibody was detected in all the different age groups tested.

Table 3: Percentage frequency and distributions of rubella virus specific IgM among the study subjects.

	Frequency	Valid Percent	Cumulative Percent
Negative	85	94.4	94.4
Positive	5	5.6	100.0
Total	**90**	**100.0**	

DISCUSSION

The results obtained in this study showed that out of the 90 subjects examined, 89(98.9%) had rubella virus specific IgG antibody which might be due to previous natural exposure to the virus. The results obtained agreed with an earlier report of Olajide *et al.* (2015)with a 93.1%sero-positive IgG antibodies. Most studies in Africa showed that more than 80% of pregnant women were immune to Rubella (Gomwalk and Ahmad, 1989). It has been noted that there is a significant association between the incidence of rubella IgG antibody with age. The results obtained revealed that all the age groups considered had antibodies to rubella virus. The antibody incidence increased and then decreased between different age groups not following a single directional trend, this showed that individuals exposed to rubella have the tendency to develop immunity irrespective of their age groups. A closely varied finding with 77% sero-positivity for rubella IgG was reported in a previous study by Onyenekwe, (2000). Lack of protective rubella IgG antibody in 1(1.1%) of the study population and more suggest existence of susceptible population for rubella virus maintenance in the community. This finding confirms earlier reports of 3.9% sero-positivity of rubella IgM antibodies by Pennap *et al.,*(2009), which indicates that despite the development and administration of effective vaccines for prevention and control of rubella virus infection since 1969 and prevention or elimination of the causative agent in many developed countries, cases of rubella virus infection and congenital rubella syndrome are still being reported among diverse groups in Nigeria. However, since humans are the only known reservoir for rubella virus, maintenance of rubella requires continuous access to a susceptible population. Therefore, an enhanced immunization programme aimed at ensuring high level of herd immunity would facilitate the control of rubella epidemics (Vyse *et al.,* 2002). Furthermore, findings from this and previous studies in the country indicate that Nigeria has in its hands, an opportunity to eliminate the virus since the burden is low and the definite susceptible population is defined.

CONCLUSION

This study revealed that most of the pregnant women screened 89(98.9%) had evidence of Rubella immunity (IgG seropositive) which might have been acquired through natural exposure to the virus. The remaining 1(1.1%) needs to be protected from being infected with the virus especially during the first trimester of pregnancy which can result to congenital defects with fatal consequences. As such, there is need for more sero-surveys on rubella in the country to support the advocacy for the inclusion of rubella vaccination in the National Programme on Immunization (NPI).

RECOMMENDATION

Prevention or elimination of rubella virus infection has been achieved in many developed countries with the introduction of preventive vaccine such as the MMR vaccine. Therefore, to facilitate prompt and effective virus elimination in the country, immediate introduction of preventive rubella vaccination to susceptible population is essential.

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