Seroprevalence of rubella virus infection in women with recurrent miscarriage: a case control study in Jos, Nigeria

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Abstract

Background: Recurrent pregnancy loss is an emotionally and physically tasking situation for couples, especially in developing countries where lack of children is a cultural taboo. Rubella infection in early conception has a 90% probability of developing congenital rubella syndrome (CRS) or miscarriage. The aim of this study was to compare the seroprevalence of rubella virus infection in women with and without recurrent miscarriage.

Methods: It was a hospital based case control study. Sampling was done by purposive. Subjects were recruited consecutively until the required number was reached. Control subjects were selected by simple random sampling. Healthy postnatal women who consented for the study were asked to pick a piece of paper from a covered container, those who picked yes were then recruited. A semi structured researcher administered questionnaire was used to collect data. Enzyme-Linked Immunosorbent Assay (ELISA) was used to check for rubella specific IgG and IgM. We compared the prevalence of rubella virus infection in cases and controls by Chi-square analysis. A p-value < 0.05 was considered significant.

Results: The overall mean age of the participants was 30.62±3.60 years. None of the participants had received rubella virus vaccination. The seroprevalence rates of rubella IgG and IgM among the cases were 85% and 16.7%, while in the controls were 80% and 13.3%. The prevalence of Primary rubella infection (IgG^+IgM^+) was 10 (16.7%) and 8 (13.3%) among the cases and the controls respectively. Rubella virus seropositivity (IgG^+IgM^-) was 68.3% among cases versus 66.7% among controls, and rubella virus seronegativity (IgG^-IgM^-) was 15.0% and 20.0% among cases and control respectively.

Conclusion: The high seroprevalence of rubella virus infection observed in this study suggest that majority of women in our setting are exposed to rubella virus infection before pregnancy. There was no significant difference in the seroprevalence of rubella virus infection between women with and those without a history of recurrent miscarriage.

Key Words: seroprevalence, recurrent miscarriage, Rubella, viral antibodies, Jos

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Introduction

Recurrent pregnancy loss is an emotionally and physically traumatic situation for couples, especially in developing countries where lack of children is a cultural taboo. Several risk factors have been identified, and more than one contributory factor may underlie the recurrent pregnancy losses.[1] These aetiologic conditions include chromosomal, genetic, anatomical, immunological, genetic, endocrine, infectious, thrombophilic, and environmental factors.[2-4,,]

The role of infectious agents in recurrent miscarriage is unclear.[1,5] A study on the correlates of placental viral infection with rubella virus, cytomegalo - virus, parvovirus B_{19} and human herpes virus showed human rubella virus as the major pathogen in all cases of placental infection associated with fetal death.[6]

Globally, it is estimated that each year about 1 in 150 babies are born with congenital rubella infection (CRI) and about 110,000 children develop lasting disabilities caused by congenital rubella syndrome (CRS).[7] CRS is multi-organs defects, and when fetal infection occurs without birth defects it is termed Congenital Rubella Infection (CRI).[8] The actual burden in developing countries is unknown. However, a study reported rubella virus as the leading cause of vaccine-preventable congenital birth defects in developing countries.[9] Also, rubella infection occurring just before conception or during early pregnancy is a public health concern as there is up to 90% probability of developing CRS, or miscarriages.[10,11] ,This is so because there is 80% to 100% chance of viral transmission throughout the three trimesters of pregnancy.[12,13]

Rubella virus, also called German measles is passed from human to human through direct or droplet contact with infected body fluid. The incubation period range between 2 to 23 days with an average of 14 days. The virus replicates in the nasopharynx, then spread to the local lymph nodes and finally haematogenously to the placenta.[14,15] From the placenta, the virus is transmitted through the decidua glands to the vascular system of the developing fetus, causing cytopathic damage to blood vessels and developing organs.[16] Various theories including chorioamnionitis/villitis, alteration in

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immune response, direct effect of endotoxin, exotoxin or cytokines on the uterus and feto-placental unit have been proposed as possible mechanisms for pregnancy loss following rubella virus infection. However, the most accepted theory supported by several in-vitro studies is the production of anti-phospholipid antibodies, a well-known risk factor for venous and arterial thrombo-embolism.[17]

Despite the high seroprevalence of rubella virus infection in our environment,[8,19] there is paucity of data on the association of rubella infection with recurrent miscarriage in Sub-Saharan Africa. Similarly, none of the several studies on rubella virus infection from different parts of Nigeria including Jos determined the association of rubella virus infection and recurrent miscarriage.[18,20] The objective of this study is to compare the seroprevalence of rubella virus infection in women with or without a history of recurrent miscarriage.

Materials and Methods

This was a hospital based case control study conducted in the department of Obstetrics and Gynaecology over a period of nine months, from April 2017 to December 2017. The cases were 60 women recruited from among women who presented to the antenatal clinic, gynaecological emergency, and gynaecological ward with recurrent miscarriage during the study period. The controls were 60 women selected at the postnatal clinic of the hospital by simple random sampling from healthy women with successful full term delivery and without history of recurrent miscarriage history. All subjects gave written consent for obtaining their blood samples according to research purposes.

The sample bottles were given serial numbers assigned to each patient in both groups by the using a single blinded approach to protect their identity and eliminate researcher bias during laboratory analysis. A structured questionnaire was administered and privacy was ensured while interviews were being conducted. The information collected included; Age, parity, educational status, rubella virus immunization status, gestational age at time of miscarriage, number of pregnancy losses, gynecologic and medical history of other risk factors for recurrent miscarriage like retroviral disease, sickle cell disease, hypertension, diabetes and socioeconomic status. Women with risk factors for recurrent miscarriage and those who declined to give consent were excluded. A miscarriage was defined as the spontaneous loss of pregnancy less than 28 weeks of gestation. Recurrent miscarriage was defined as three or more consecutive spontaneous pregnancy losses.

For the laboratory analysis, the isolated sera of both cases and controls were stored at -70°C, and analyzed for rubella virus IgG and IgM antibodies by indirect Enzyme-Linked Immunosorbent Assays (ELISA) using the "Serion classic" rubella IgG and IgM DRG (Serion Immundiagnostica GmbH, Wurzburg, Germany) kits. As indicated in the kit prospectus, the diagnosis of acute or recent rubella infection was made when IgM is positive and IgG is negative; primary rubella virus infection when both rubella virus-IgM and IgG are positive; rubella virus seropositive when rubella virus-IgM is negative and IgG is positive; Rubella virus seronegative when both rubella virus-IgM and IgG are negative.

Determination of sample size

The sample size was calculated using the formula;

$$n \leq \frac{\{P_1(1-P_1) + P_2(1-P_2)\} \times (Z_{\alpha} + Z_{\beta})}{(P_1-P_2)^2}$$

Where:

n: number of sample size in each of the group

$P_1 \leq$ proportion of positive anti-RuV antibody among cases (0.906 in a similar study) [8]

$P_2 \leq$ proportion of positive anti-RuV antibody among controls (0.698 in the same study)[8]

$Z\text{-}_{\alpha/2} \leq$ value of standard normal distribution corresponding to a significance level of alpha (1.96 for two-sided test at the 0.05)

$Z\text{-}_{\beta/2} \leq$ value of standard normal distribution corresponding to the desired level of power (0.84 for a power of 80%)

$$n \leq \frac{\{0.906(1-0.906) + 0.698(1-0.698)\} \times (1.96 + 0.84)^2}{(0.906-0.698)^2}$$

$$n \leq \frac{\{0.0852 + 0.2108\} \times (2.8)^2}{(0.2080)^2} \leq 53.64$$

The sample size was adjusted to compensate for a non-response rate of 10% giving approximately 58 subjects. Therefore, 60 cases and 60 controls were recruited for the study.

Sampling technique

Sampling for the case group was done by purposive sampling. Subjects were recruited consecutively until the required number was reached. Control subjects were selected by simple random sampling. Healthy postnatal women without history of recurrent miscarriage who consented for the study were asked to pick a piece of paper from a covered container, those who picked yes were then recruited.

Laboratory test

Three millilitres of blood was collected from the antecubital fossa of each subject. The samples were allowed to clot and then centrifuged at 4000 revolutions per minute for 3 minutes. Serum was extracted and stored at -20^0C until adequate sample size was reached. The samples were analyzed for Rubella virus IgG and

IgM antibodies using "Serion classic" Rubella IgG and IgM DRG Enzyme-Linked Immunosorbent Assays (ELISA) kits (SERION IMMUNDIAGNOSTICA GmbH). The laboratory analysis was carried out by a consultant Chemical Pathologist in JUTH according to the kit manufacturer's specifications. The consultant chemical pathologist was blinded to the number coding used to identify the patients so as to eliminate bias.

The diagnosis of acute infection was made when ELISA rubella IgM antibody tests are positive. When IgM antibodies are negative or undetectable, a convalescent specimen 4 to 5 weeks later for rubella IgG antibody

Ethical Statement

The study protocol received ethical approval from the Human Research and Ethics Committee of Jos University Teaching Hospital.

Data analysis

All statistical analysis were performed using the Statistical Package for Social Sciences (SPSS) software version 20 (IBM Armonk, NY, USA). Frequencies and percentages were computed for demographic characteristics of cases and controls and presented in tables. Means and standard deviation were used to summarize numerical variables such as age of respondents. Chi square and Fisher's exact test as appropriate were used to determine the significant differences in proportions across cases and controls.. A p-value < 0.05 was considered statistically significant.

Results

The study comprised of a total of 120 participants. Table 1 shows the socio-demographic characteristics of the participants. The overall mean age of 30.62±3.60 years. The mean age of women that comprised the cases was 31.55±3.44 years and the control group was 29.68±3.55 years. More of the cases (60.0%) had tertiary education than the controls (48.3%). Among the cases, 35.0% were housewives, as compared to 36.7% in the control group. About 16.7% were teachers in the case group as compared to 11.7% in the control group. A total of 16 (26.7%) and 9 (15.0%) of the cases and controls respectively were aware of Rubella virus infection. None of the participants (0.0%) had received rubella virus vaccination. Table 2 shows the prevalence of rubella virus infection. The differences in the prevalence of rubella virus infection using IgM and IgG among cases and controls was not statistically significant (p>0.05). Figure 1 shows the anti-rubella serostatus among the cases and controls. Primary rubella infection (IgG+IgM+) was 10 (16.7%) and 8 (13.3%) among the cases and the controls respectively; rubella seropositivity ($IgG^{+}IgM^{-}$) was 68.3% for cases and 66.7% for controls; seronegativity ($IgG^{-}IgM^{-}$) was 15.0% for cases and 20.0% for controls.

Table 1: Demographic characteristics of cases and controls

Variables	Cases N=60 n (%)	Controls N=60 n (%)	Total N=120 n (%)	P value
Age category				
15 - 24years	1 (1.7)	5 (8.3)	6 (5.0)	0.075*
25 - 34years	47 (78.3)	50 (83.4)	97 (80.8)	
35 - 44years	12 (20.0)	5 (8.3)	17 (14.2)	
Educational level				
None	9 (15.0)	9 (15.0)	18 (15.0)	0.555
Primary	5 (8.3)	7 (11.7)	12 (10.0)	
Secondary	10 (16.7)	15 (25.0)	25 (20.8)	
Tertiary	36 (60.0)	29 (48.3)	65 (54.2)	
Occupation				
House wife	21 (35.0)	22 (36.7)	43 (35.8)	0.829*
Teacher	10 (16.7)	7 (11.7)	17 (14.2)	
Trader	10 (16.7)	13 (21.7)	23 (19.2)	
Civil servant	7 (11.7)	5 (8.3)	12 (10.0)	
Self-employed	8 (13.3)	8 (13.3)	16 (13.3)	
Student	4 (6.7)	3 (5.0)	7 (5.8)	
Applicant	0 (0.0)	2 (3.3)	2 (1.7)	
Ethnicity				
Hausa	20 (33.3)	18 (30.0)	38 (31.7)	0.824
Igbo	14 (23.3)	14 (23.3)	28 (23.3)	
Yoruba	10 (16.7)	14 (23.3)	24 (20.0)	
Others**	16 (26.7)	14 (23.3)	30 (25.0)	

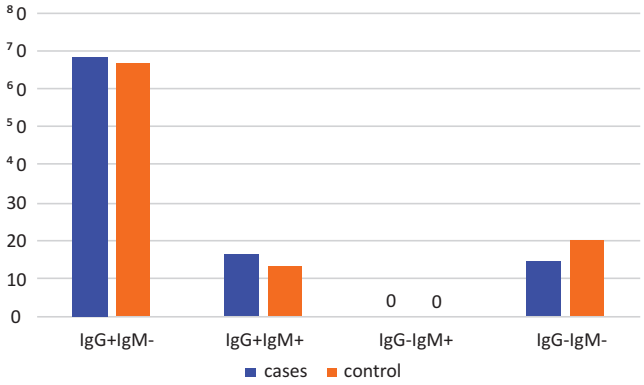
*Fishers exact test, **Berom, Ngas, Mwagavul, Jarawa, Tarok

Table 2: Prevalence of Anti-Rubella IgG and IgM

Anti-Rubella antibodies	Cases n (%)	Controls n (%)	Total n (%)	P value
IgG				
Positive	51 (85.0)	48 (80.0)	99 (82.5)	0.471
Negative	9 (15.0)	12 (20.0)	21 (17.5)	
Total	60 (100.0)	60 (100.0)	120 (100.0)	
IgM				
Positive	10 (16.7)	8 (13.3)	18 (15.0)	0.609
Negative	50 (83.3)	52 (86.7)	102 (85.0)	
Total	60 (100.0)	60 (100.0)	120 (100.0)	

Discussion

The findings from our study showed that there is no significant association between seroprevalence of rubella virus infection and recurrent miscarriage. This is in agreement with the findings by Sebastian *et al.*[19]



Chi square = 0.663; p-value = 0.718

Figure 1: Various combinations of the anti-rubella sero-status

However, the findings are at variance with other studies that found significant association between rubella virus and recurrent miscarriage.[20,21]

The high seroprevalence of rubella virus infection in both the cases and controls in our study indicates that majority of women in our environment are exposed to rubella virus infection in their early age, considering that no participant had received rubella vaccine in the past. This is similar to results from other studies carried out in Nigeria and other parts of sub-Saharan Africa. In Nigeria, Olajide et al[22] and Mohammed et al[23] reported a prevalence of 93.1% and 97.9% respectively. Abdolreza et al reported a seroprevalence of 91.2% among patients with recurrent abortion in south Iran,[21] while Elamin and Khidir in South Sudan reported a prevalence of 81.6%.[24] The findings are however at variance with that reported by Ishraq in Babylon where the seroprevalence rate for IgG in patients with abortion was 34.7%[25] and the 53% to 68.5% reported in parts of Nigeria.[26] Possible reasons for the conflicting reports can be attributed to different study populations, vaccination status of respondents, variation in sample size, sensitivity and specificity of various employed serological techniques and methods used to make diagnosis and inter- researchers differences in interpreting serological techniques.

Importantly, the 16.7% and 13.3% prevalence of primary rubella virus infection in the cases and controls respectively are similar to the 14.3% reported by Khalf et al[29] in patients with miscarriage. This is also in agreement with previous report showing that primary rubella virus infection is still active among women of reproductive age.[24] The findings are however lower than the 28.2% reported by Sebastian et al,[31] but higher than the 10.80%, 7.79% and 6.1% reported by Abdolreza et al,[24] Salman et al[30] and Al Mishaddani et al[25] respectively. On the contrary, Spano et al[7] found no correlation between abortion and active rubella virus infection after using PCR and nested PCR on tissues from abortion.[8] The possible reasons for the differences may be due to that fact that rubella IgM may persists in some individuals for one or more years following primary infection, and therefore diagnosis of primary infection may be overestimated. Furthermore, false-positive serum rubella IgM tests may occur due to the presence of rheumatoid factors or infection with other viruses cross-reacting.[31]

A major strengths of our study is that we did both IgG and IgM in one specimen unlike other studies that focus on only one antibody. However, our study could not confirm primary rubella virus infection since we did not do the 4 to 5 weeks IgG antibody avidity testing on convalescent specimen required to confirm primary rubella infection. This avidity testing is most useful in early pregnancy to help rule out a rubella infection in the first trimester, when the risk of congenital defects due to rubella is highest. Differences in low avidity and high avidity antibodies testing can be detected using protein denaturants such as diethylamine (DEA) in the washing step of an enzyme-linked immunoassay (EIA) for rubella IgG. The presence of high avidity antibodies, which develop by about three months after infection, provides evidence of remote infection. Another limitation of our study is that the exclusion criteria using historical findings is inadequate to exclude other causes of first trimester miscarriage. Specific investigations like karyo typing, viral culture, histopathological analysis of the products of conception and PCR are necessary to exclude conditions like chromosomal anomalies, anti-phospholipid syndrome and other viral infections. Also, the fact that this was a hospital-based study, and the use of a non-probability sampling technique means the findings might not be generalizable to the entire population of women, necessitating a larger population-based study in the future. Finally, this study was not powered to detect minor differences between the two groups. This call for randomized control studies.

In conclusion, majority of women in our study sample were exposed to rubella virus infection before pregnancy. Our results suggests that rubella virus infection was not associated with history of recurrent pregnancy loss. Avidity testing on 4-5 weeks convalescent specimen is required to confirm primary rubella virus infection observed in this study. Our findings also highlight the need to establish a system for community awareness on rubella-susceptibility and implementation strategy for rubella vaccination of pregnant women and women of child-bearing age.

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Erratum

The name of Alice V Ramyil, one of the authors of "Intraorbital meningioma in a young African female: A case report" published in Highland Medical Research Journal, on page 62-65, July - December 2020 [Volume 20; Issue 2; Page: 62-65] was wrongly written as **Alice A Ramyil** instead of **Alice V Ramyil**.

Corrected:

Intraorbital meningioma in a young African female: A case report: Philip O Akpa, Barka V Kwaghe, Panshak E Tenmang, **Alice V Ramyil** [Highland Medical Research Journal, July - December 2020 [Volume 20; Issue 2; Page: 62-65]

The error is regretted

Editor, Highland Medical Research Journal