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SEROPREVALENCE SURVEY OF RUBELLA ANTIBODIES AMONG PREGNANT WOMEN IN MAIDUGURI, BORNO STATE, NIGERIA

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ABSTRACT

Rubella is a vaccine- preventable viral infection. Its etiologic agent, rubella virus was identified as a human teratogen capable of causing spectrum of birth defects described as congenital rubella syndrome (CRS) if the pregnant mother is infected within the first trimester of pregnancy. A total of 90 pregnant women attending a secondary health care facility in Maiduguri were screened for IgM and IgG antibodies using enzyme linked immunosorbent assay (ELISA) kit (Cortez Diagnostics Inc. USA). Of these, 37.8% (34/90) and 83.3% (75/90) were seropositive for anti-rubella IgM (x^2 Cal 5.1; p=0.05) and IgG respectively. Chi-square analysis (x^2 Cal 38.38, p=0.05// x^2 tab 31.41, p=0.05) revealed an association between miscarriage and IgG antibody level in twenty-one subjects. Pregnant women within 20-24years had the highest prevalence of 40% (36/90)(x^2 Cal 4.22, p=0.05) : 44.4% (16/36) of them were seropositive for IgM (x^2 Cal 4.31, p=0.05). A marked surge in IgG antibody level, which tantamount acute infection, was observed in 15.6% (14/90)(x^2 Cal 19.85, p=0.05) of the pregnant women. Pregnant women in the first trimester seropositive for anti-rubella IgM were 36.4% (4/11), inferring that the fetuses of these women are susceptible to sequelae of rubella. This result highlights the consequence of rubella infection and confirms continuous circulation of rubella virus in the study area. There is need for vaccination of vulnerable population in order to ensure the control /elimination of rubella virus in Nigeria.

Key words: Rubella virus, teratogen, antibodies, Maiduguri

SURVEILLANCE DE SEROPREVALENCE DES ANTICORPS ANTI-RUBEOLE CHEZ LES FEMMES ENCEINTES A MAIDUGURI, ETAT DU BORNOU, NIGERIA

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RESUME

La rubéole est une infection virale évitable par la vaccination. Son agent étiologique, virus de la rubéole a été identifié comme un tératogène humain capable de provoquer le spectre de malformation congénitale décrite comme le syndrome de rubéole congénitale (SRC) si la femme enceinte est infectée au cours du premier trimestre de la grossesse. Au total, 90 femmes enceintes fréquentant un établissement de soins de santé secondaires à Maiduguri ont été dépistées pour le dosage des anticorps IgM et IgG à l'aide de kit immunoenzymatique (ELISA) (Cortez Diagnostics Inc. USA). Parmi elles, 37,8% (34/90) et 83,3% (75/90) étaient séropositives respectivement pour les anticorps anti-IgM (X^2 Cal. 5,1, *p=0,05*) et IgG de la rubéole. L'analyse Chi-carré (X^2 Cal. 38,38, *p=0,05* /X^2Tab. 31,41, *p=0,05*) a révélé une relation entre la fausse couche et le niveau d'anticorps IgG dans vingt-et-un sujets. Les femmes enceintes de 20 à 24 ans ont eu la plus forte prévalence de 40% (36/90) (X^2 Cal. 4,22, *p=0,05*): 44,4% (16/36) d'entre elles étaient séropositives pour les IgM (X^2 Cal. 4,31, *p=0,05*). Une augmentation remarquable de taux d'anticorps IgG,

équivalent à l'infection aiguë, a été observée chez 15,6% (14/90) (X^2 Cal. 19,85, *p=0,05*) de femmes enceintes.Les femmes enceintes au premier trimestre, séropositives aux IgM anti-rubéole, ont été de 36,4% (4/11), déduisant que les fœtus de ces femmes sont sensibles aux séquelles de la rubéole. Ce résultat souligne la conséquence de la rubéole et confirme la circulation continue du virus de la rubéole dans la zone d'étude. Il est nécessaire de vacciner la population vulnérable afin d'assurer le contrôle et/ou l'élimination du virus de la rubéole au Nigeria.

Mots clés: virus de la rubéole, tératogènes, anticorps, Maiduguri

INTRODUCTION

Infection with rubella virus during pregnancy, especially during the first trimester, can result in congenital rubella syndrome (CRS) (1). The burden of rubella infection in most developing countries is however not well documented because of lim-ited epidemiological data (2).

The symptoms of rubella infection include a rash, low-grade fever, arthralgia, and lymphadenopathy. In most cases, the disease is self-limiting and rarely causes complications (3). Complications of CRS may include miscarriage and severe abnormalities of the fetus, such as cataracts, retinopathy, heart defects, neurological deficits, and deafness (4).

No antiviral drugs are available for treating rubella or preventing transmission to the fetus. Vaccination programs are regarded as an effective tool to eliminate rubella and congenital rubella (5).

WHO estimates that worldwide more than 100,000 children are born with CRS each year, most of them in developing countries (6).

In some African Countries, 80% of children have been found to be positive for rubella antibodies by the age of 10 years (7). Post-epidemic rubella antibody prevalence in Ghana has been found to be 92% among pregnant women, with susceptibility associated with a younger age. In Eritrea, the prevalence of antibodies to rubella has been reported to be as high as 99% in some female population (3).

In Nigeria, past studies have revealed 14.3%, 3.9%, 10%, 16.3%, prevalence of anti-rubella IgM in Abuja, Benue, Benin and Ilorin respectively (2, 8, 9, 10) while 53%, 62.7%, 68.5%, 54.1% prevalence of anti-rubella IgG in Benin, Zaria, Ibadan, and Maiduguri respectively (9, 11, 12, 13) have been reported.

Till date there is no national program to vaccinate children and women against rubella (14). This indicates that rubella immunization is not included in Nigeria immunization schedule and thus infections during pregnancy may still occur.

The possibility of occurrence of rubella in Nigeria is corroborated by report of "a Nigerian woman who arrived in the United States in early March 2012 in approximately week 32 of pregnancy. In the United States, her pregnancy was complicated with oligohydramnios and severe growth retardation. She did not recall having had a rash illness during her pregnancy. Maternal serum collected 3 days after she had given birth tested negative at CDC for rubella IgM and positive for rubella IgG with a high avidity index. In March 2012, she was delivered of an infant in Alabama by cesarean delivery at 33 weeks' gestational age. At birth, the infant had generalized hemorrhagic purpura (a blueberry muffin rash) over the entire body, patent ductus arteriosus, cardiomegaly, thrombocytopenia, pneumonitis, anemia, and liver dysfunction. Approximately 1 month later, the infant was transferred to a pediatric hospital, where the infant died in April 2012. Cause of death was recorded as CRS (1).

No serological evaluation of combined IgG and IgM antibodies for previous and current rubella infections has been reported in a single study in the study area.

Therefore this study was carried out to determine the sero-prevalence of maternal anti-rubella IgG and IgM in pregnant women attending a secondary health facility in Maiduguri, Nigeria and to generate baseline data which can serve to prompt relevant health authority to appreciate the need to formulate feasible, implementable policy to mitigate the sequelae of rubella virus infection, not only in the study area but the country at large.

MATERIALS AND METHODS

Study area

This research was carried out in Fatima Ali Sheriff health center, Maiduguri, Borno State, Nigeria. The research spanned January – November 2013. Maiduguri is the capital of Borno State located in the north eastern region of Nigeria. The indigenes are predominantly Kanuri by tribe.

Ethical Clearance

Ethical approval for the study was obtained from the Ethical Board of the hospital. Informed oral consent was obtained from all subjects recruited into the study.

Study design

To allow for fair representation of sample, a cross-sectional, hospital –based design was utilized. Out of the one hundred and twenty antenatal attendees intended for the study, only ninety (75%) whose consent was sought and obtained participated in the research. A structured questionnaire was administered on each pregnant woman to obtain necessary demographic data which included age, trimester of pregnancy, history of measles, mumps, rubella (MMR) vaccination and experience of skin rash.

Exclusion criteria

Women who are not pregnant were excluded from the research.

Inclusion criteria

Only pregnant women were included in the research.

Specimen collection

Three milliliter (3ml) of blood was aseptically collected by venipuncture and serum was obtained by centrifuging at 3000rpm for 5 minutes (Agbede *et al.*, 2011). Serum was kept frozen at -20^{0}C in the Department of Immunology, University of Maiduguri Teaching Hospital until analysis was done.

Specimen Analysis

The Enzyme linked immunosorbent assay technique was employed for both IgM and IgG antibodies. Abiding strictly by manufacturer's instruction (Diagnostic Automation, Inc. U.S.A), one in forty (1:40) dilutions of specimen, negative control, positive control and calibrator were prepared by adding 5µl of the aforementioned to 200µl of sample diluent and mixed well. One hundred microliter (100µl) of diluted sera, calibrator and controls were dispensed into the appropriate wells. One hundred microliter (100µl) of absorbent solution was dispensed in 1A well position for the reagent blank. The holder was tapped to remove air bubbles from the liquid and was mixed well and incubated for 30 minutes at room temperature. Liquids from all wells were removed and washed three times repeatedly with washing buffer. One hundred microliter (100µl) of enzyme conjugate was dispensed into each well and incubated for 30 minutes at room temperature. The enzyme conjugate was then removed from all wells and washed repeatedly three times with washing buffer. One hundred microliter (100µl) of TMB Chromogenic Substrate was dispensed to each well and incubated for 15 minutes at room temperature. Then 100µl of 2 N HCl was added to stop reaction. While ensuring there were no air bubbles in each well, O.D. at 450 nm was read with a microwell reader.

Interpretation of Result

Based on manufacturer's instruction (Diagnostic Automation, Inc. U.S.A), the mean value of Rubella G Index for each specimen was calculated by dividing the mean absorbance value of each sample by the cut off calibrator mean value. A sample was then considered positive for anti-Rubella IgG and IgM antibody whenever a Rubella G and M Index value is equal to or greater than 1.0, and considered negative whenever a Rubella G and M Index value is equal to or less than 0.90.

Criteria for the validity of the IgM assay

The assay was considered valid:

1. When the OD450nm of the A1 blank well was <0.150
2. When the OD450 nm of the calibrator was >0.250

Criteria for the validity of the IgG assay

The assay was considered valid:

1. When the OD450nm of the A1 blank well was <0.250

2. When the OD450 nm of the calibrator was >0.250

RESULTS

An overall prevalence of 37.8% and 83.3% for anti-rubella IgM and IgG antibodies respectively were obtained in this study (Table 1). Pregnant women within the age bracket 15-19years and 20-24years combined, constitute 58.88% of the respondents (Table 1) while majority, 62.22% to be precise, of the pregnant women sampled were in the third trimester of pregnancy. None of the respondent (0%) was 40years old (Table 1). Prevalence of pregnant women in the first trimester seropositive for rubella IgM was 36.4% (Table 1). There was evidence of marked surge in IgG antibody level among fourteen (15.6%) pregnant women (Table 2). Chi-square analysis (x^2 Cal 38.38, p=0.05// x^2 tab 31.41, p=0.05) revealed an association between miscarriage and IgG antibody level in twenty-one pregnant women (Table 1).

TABLE 1: DISTRIBUTION OF ANTI-RUBELLA ANTIBODIES AMONG PREGNANT WOMEN

Variable	n	IgM(+ve)	IgG(+ve)
	Age		
15-19	17	06	14
20-24*	36	16	30
25-29	15	04	14
30-34	16	07	14
35-39	06	01	03
≥40	00	00	00
	Trimester		
First	11	04	10
Second	23	10	20
Third	56	25	45
	History of Miscarriage		
Yes	27	13	21**
No	63	27	56
	History of MMR Vaccination		
Yes	0	0	0
No	90	34	75
	Experience of Rashes		
Recent	90	34	20
Past	90	56	75

*** x^2 Cal = 4.22, P=0.05; ** x^2 Cal 38.38, P =0.05**

TABLE 2: MARKED SURGE IN ANTI-RUBELLA IGG ANTIBODY AMONG PREGNANT WOMEN

Sample No	Rubella G Index of Convalescent(RGIC)	Rubella G Index of Pre-vaccination(RGIP)	Ratio of RGIC/RGIP
17	3.150	0.9	3.5
23	3.527	0.9	3.9
24	4.435	0.9	4.9
31	5.532	0.9	6.1
54	3.561	0.9	3.9
79	3.536	0.9	3.9
80	3.381	0.9	3.7
81	3.092	0.9	3.4
86	3.054	0.9	3.3
87	3.728	0.9	4.1
89	3.158	0.9	3.5
96	3.117	0.9	3.4
99	3.364	0.9	3.7
144	3.096	0.9	3.4

Manufacturer: RGIC/RGIP should be >1.5 to be suggestive of significant rise in antibody level.

DISCUSSION

This is the first serological evaluation of combined IgM and IgG antibodies among pregnant women in Maiduguri, Nigeria. The result of this study reveals 37.8% and 83.3% IgM and IgG seroprevalence respectively. The 37.8% IgM prevalence in this study is high compared to the 14.3%, 3.9%, 10%, and 16.3% prevalence in Abuja, Benue, Benin and Ilorin respectively (2, 8, 9) while the 83.3% IgG prevalence obtained in this study is also comparatively higher than the 53%, 62.7%, 68.5% prevalence in Benin, Zaria and Ibadan (9, 11, 12, 13,). Bukbuk *et al*., (2002) had earlier reported a 54.1% IgG seroprevalence in Maiduguri, but eleven years on, the prevalence of 83.3% in this study implies that more women have been and are perhaps still being exposed to rubella virus.

The age-stratified prevalence of anti-rubella antibodies among the pregnant women within the 20-24years revealed the highest prevalence of 40% (36/90)(x^2 Cal 4.22, p=0.05). We observed also that a combination of the 15-19years and 20-24years age bracket constituted a 58.88% of the pregnant women sampled in this study. This is instructive in that it shows that majority of the women in the area of study enter into childbearing early i.e. between 15-24years of age. This is in consonance with the tradition of early marriage in the northern part of Nigeria. None of the pregnant women sampled in this study was up to forty years of age. This is not unexpected since at such age, in northern Nigeria, many a woman would have become a grandmother due to early marriage.

The predominance of first time visitors (62.22%) to antenatal clinics at the third trimester of pregnancy was observed in this study (Table 1). This implies that majority of pregnant women either do not attend at all or present late at the antenatal clinics for routine medical attention. This attitude would impact negatively on any possible future effort to conduct surveillance on the prevalence of, and mitigate occurrence of children born with congenital rubella

syndrome in the study area in particular and Nigeria in general. This highlights the utmost importance of educating women of childbearing age in the study area on the need to enroll immediately in antenatal clinics whenever they are pregnant.

Rubella virus infection usually causes a mild disease in humans, but infection during early pregnancy often leads to severe congenital abnormalities (15). Therefore, the fetuses of 36.4% (4/11) of the pregnant women in the first trimester who were seropositive for IgM are at risk of severe congenital abnormalities. It also implies that had the pregnant women in the second trimester (76.9%; 10/13) and those in the third trimester (80.6%; 25/31) seropositive for IgM contracted this virus earlier, their fetuses would have also been at great risk of malformation (Table 1).

In this study, 10% (9/90) of the pregnant women were IgM and IgG anti-rubella antibodies negative. This means that they constitute population at risk. They are susceptible to infection by rubella virus. Among these nine, three (33.3%; 3/9) had history of pregnancy loss. This is indicative of miscarriage of non-rubella aetiology. The loss could only be attributed to other teratogenic organisms. However, 23.33% (21/90) of the pregnant women seropositive for rubella IgG had history of miscarriage while Chi-square analysis (x^2 Cal 38.38, p=0.05// x^2 tab 31.41, p=0.05) revealed an association between miscarriage and IgG antibody level in these category of pregnant women. This further corroborates the assertion by previous researchers that miscarriage is a sequelae of rubella virus infection.
Six point seven percent (6.7%; 6/90) of the pregnant women were IgM seropositive and IgG negative teratogenic agent responsible for several unreported cases of its sequelae- congenital rubella syndrome.

We recommend, therefore, that relevant authority devise drastic measure to be taken to protect women of childbearing age and fetuses from this scourge by, beside other measures, including rubella vaccination in routine antenatal screening exercise in Nigeria. There is also the need to health-educate women of childbearing age on how they could protect themselves from contracting this virus.

inferring that these pegnant women are undergoing acute infection. None of them was found to be in the first trimester of pregnancy, although two (2/6) of them had history of pregnancy loss. Even though they were IgM positive, rubella virus could not have been responsible for the past pregnancy loss due to the IgG seronegative status.

A marked surge in anti-rubella IgG antibody level was observed in fourteen of ninety pregnant women sampled (15.55%; x^2 Cal 19.85, p=0.05 // x^2 tab 22.36, p=0.05). This marked rise in antibody level is the ratio between the Rubella G Index of convalescent sample and that of pre-vaccination sample, which should be greater than 1.5 (Diagnostic Automation, Inc, USA). Manufacturer's manual specified Rubella G Index of 0.9 or less to be seronegative for IgG antibody to Rubella virus. None of the pregnant women in this study had any history of Rubella vaccination, therefore we assume pre-vaccination value to be 0.9 while the Rubella G Index of convalescent sample (after natural exposure, as the case in this study) is value obtained after analysis of sera (Table 3). The high ratio obtained for this samples (Table 3) is suggestive of recent infection even in the absence of IgM antibodies especially had further analysis revealed IgG with high avidity index. Due to scarce resources, however, avidity indices for these IgG antibodies were not determined.

In conclusion, the result of this work has revealed that rubella virus is still in circulation in the study area and that more women have been and are perhaps still being exposed to this potentially hazardous

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