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Full Length Research Paper

Survey of the serro-prevalence of IgM antibodies in pregnant women infected with *Rubella* virus

***Eleazu Chinedum Ogbonnaya, Eleazu Kate Chinedum, Amajor John and Amajor Esther**

Department of Biochemistry, National Root Crops Research Institute, Umudike, Abia State, Nigeria.

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One hundred and ninety pregnant women attending Plateau State Specialist Hospital, Jos, were screened to detect the prevalence of IgM antibodies in their serum using the Biotech Diagnostic Enzyme Linked Immunosorbent Assay Antigen Kit Method. A well-structured questionnaire was administered to the subjects to obtain socio-demographic data. Results show that out of the 190 patients that were screened, the IgM antibody was detected in 6.8% of the patients indicating that they are the population in which the rubella virus is likely to occur whereas 93.2% of the study population were not likely to be susceptible to the virus as the IgM antibody was not detected in them. Age was found to be a serious factor for the prevalence of the virus as was the case for the pregnant women who fell within the ages of 25-34years, who were observed to have the highest prevalent rates to the virus unlike other pregnant women who weren't within that age bracket. Furthermore, women who were in their first trimesters of pregnancy recorded the highest prevalence rate to the antibody compared with the women who were in their 2nd or 3rd trimesters. In addition, pregnant women who were either single or divorced were observed not to be vulnerable to the virus as the IgM antibody was not detected in their serum unlike the pregnant women who were married. The pregnant women who had secondary education recorded the highest prevalence to the virus compared with the illiterates or those who had primary and tertiary education. Finally, the pregnant women who were farmers recorded the highest prevalence compared with the housewives, traders and civil servants.

Key Words: Sero-prevalence, *Rubella* virus, Pregnant women, ELISA, Antigen, Antibody

INTRODUCTION

Rubella, commonly known as 'German measles' is a disease that is caused by *Rubella* virus. The name is derived from the Latin word and it means little red. It is so called because the disease was first discovered by German physicians in the mid eighteen century (Lee and Bowden, 2000). This disease is often mild and attacks often pass unnoticed. The disease can last one to three days hence the term '3-days measles'. Children recover more quickly than adults. Infection of the mother by *Rubella* virus during pregnancy can be serious. If the mother is infected within the first 20 weeks of pregnancy, the child may be born with *Congenital Rubella Syndrome* (CRS), which entails a range of serious incurable illnesses. Spontaneous abortion occurs in up to 20% of cases (Siegel *et al*, 1971).

Rubella is a common childhood infection usually with minimal systemic upset although transient arthropathy may occur in adults. Acquired (i.e. not congenital) *Rubella* is transmitted via air borne droplet emission from the upper respiratory track of active cases. The virus may also be present in the urine, feces and on the skin. There is no carrier state. The reservoir exists entirely in active human cases (Richardson *et al*, 2001) since human is the only known natural host for the virus (Francis and David, 1976). The viral infection occurs in both adults and children and has an incubation period of 2-3 weeks before onset of symptoms. However, it may persist for some months post partum in infants surviving the CRS. These children are significant sources of infection to other infants and most importantly to pregnant female contacts (CDC, 2004). After an incubation period of 14-21 days, the primary symptoms of *Rubella* virus infection is

*Corresponding author. Email: eleazon@yahoo.com.

the appearance of rashes (exanthema) on the face which spreads to the trunk and limbs usually fades after three days. Other symptoms include low grade fever, swollen glands (post cervical lymphadenopathy), joint pains, headache and conjunctivitis (Edilich *et al*, 2005).

Complication of *Rubella* infection occurring after birth is uncommon but tends to occur more often in adults than in children. Arthritis and joint pains may occur in up to 60% or more adult women who contact *Rubella*. Swelling of the brain occurs rarely and is more frequently found in adults especially in females than in children. *Rubella* can cause Congenital *Rubella Syndrome* in the newly born. The (CRS) follows intrauterine infection by *Rubella* virus and comprises of cardiac, cerebral, ophthalmic and auditory defects (Atreya *et al*, 2004). It may also cause prematurity, low birth weight, neonatal thrombocytopenia, anemia and hepatitis. The risk of major defects or organogenesis is highest for infection in the first trimester. CRS is the main reason a vaccine for *Rubella* was developed. *Rubella* has a world wide distribution with varying incidences of out breaks. The virus tends to peak during the spring in countries with temperate climates. Before the vaccine to *Rubella* was introduced in 1969, widespread out breaks usually occurred every 6-9 years in the United States and 3-5years in Europe, mostly affecting children in the 5-9 years age group (Reef *et al*, 2002).

Since the introduction of vaccines, occurrences have become rare in those countries with high prevalent rates. Outbreaks however, still arise, usually in developing countries where the vaccine is not accessible (Reef, 2006). Some prevalence of *Rubella* (German measles) have significantly been recorded in Russia, Western Europe and Netherlands (Odland *et al*, 2001). Other areas with evidences of *Rubella* include, Asia and Africa (Cutts *et al*, 2000) especially Nigeria.

Studies have shown that *Rubella* infection in early stages can be disastrous, where the virus infects the fetus and causes severe abnormalities, premature birth or fetal death. These malformations are related to the chronic stage of the infection and the inhibition of fetal cell multiplication.

This work is targeted at detecting the presence of IgM antibodies in pregnant women attending Plateau State Specialist Hospital, Jos as an indication that they were recently infected with *Rubella* virus.

MATERIALS AND METHODS

The ELISA IgM Kit used for this test was prepared and manufactured by Biotech Laboratories UK. The study was conducted in 2009 in the Virology and Biochemistry Laboratories of the Federal College of Veterinary and Medical Laboratory Technology of National Veterinary Research Institute, Vom, Jos, Plateau State. Pregnant women attending antenatal screening at the Plateau State Specialist Hospital, Jos, Nigeria were chosen as the study population. Ethical approval was obtained from the Ethical Committee of the Plateau State Specialist Hospital, Jos, Plateau State.

A total of 190 blood samples were collected from 190 subjects who fell within the ages of 15-45 years. 5mls of the blood samples were collected via the antecubital vein of the subjects using sterile needles and vacutainer into plain tubes. The sera obtained were harvested into clean sterile bottles which were covered and labeled accordingly. Questionnaires were administered and filled by the subjects. The assay was carried out using the Enzyme Linked Immunosorbent Assay (ELISA) method. ELISA is a sensitive and reliable procedure that can be used for the detection of IgM.

Principle of the test

The principle is based on the interaction or binding of antibodies to antigens (Foreign substances which may be harmful that evoke an immune response). The *Rubella* antigens are fixed to the interior surface of micro wells. When the patient's serum is added, the antibodies (Fight antigens) present in it bind to the *Rubella* antigens. The micro wells are washed to remove unbound serum proteins. Antigens conjugated with *Horseradish* peroxidase enzyme and directed against human IgM (Immunoglobin M or the antibody that is produced by B cells and the largest antibody in human circulatory system) are added and they in turn interact with any human IgM present. The micro wells are washed to remove unbound conjugates and the chromogen/ substrate is added. In the presence of peroxidase enzyme, the colorless substrate is hydrolyzed to a colored end product. The color intensity is proportional to the amount of antibodies present in the patient's serum.

Assay of IgM antibodies

The micro wells were placed in a micro well holder, one end of each strip was marked for orientation. The sample was diluted 1:100 with serum diluents (one hundred micro-liter serum to one thousand micro-liters serum diluents). The diluted sample was incubated for 30 minutes at room temperature. One hundred micro-liters of negative control, low positive standard, high positive standard and specimens were pipetted into subsequent wells. They were incubated at room temperature for 30 minutes. After the incubation, the micro wells were washed with buffer and blotted with absorbent paper. One hundred micro-liters of the enzyme conjugate was pipetted into each well. It was incubated for 30 minutes at room temperature. The micro wells were washed again while one hundred micro-liters of Tetramethylbenzidine (TMB) substrate was pipetted into each well. It was incubated at room temperature for 10 minutes. Then, one hundred micro-liter of stop solution was pipetted into each well. The color intensity in each well was measured with a micro well reader at a wavelength of 450 nm (Before the reading, the exterior of the wells were carefully wiped and checked to ensure that there was no residue or scratches that may give erroneous reading). A standard curve was prepared and the results were calculated from the curve.

RESULTS

Age distribution of pregnant women with IgM antibodies

Of the 190 pregnant women that were screened, 13

Table 1. Age distribution of pregnant women with IgM antibody

Age	Total No. of patients screened	No. of positive (%)	No. of negative (%)
15-24	57	2(1.05%)	55(28.95%)
25-34	111	10(5.26%)	10(5.26%)
35-44	22	1(0.53%)	21(11.05%)
Total	190	13(6.84%)	177(93.16%)

π^2 = 1.993 df = 2 P value = 0.369

Table 2. Sero-prevalence of IgM antibody among pregnant women according to stages of pregnancy.

Stages	Total no of patients screened	No of positive (%)	No of negative (%)
1st trimester	21	2(1.05%)	19(10%)
2nd trimester	77	6(3.16%)	71(37.37%)
3rd trimester	92	5(2.63%)	87(45.79%)
Total	190	13(6.84%)	177(93.16%)

π^2 = 0.632 Df = 2 P value = 0.729

Table 3. Sero-prevalence of IgM antibody among pregnant women according to their marital status.

Marital status	Total no of patients screened (%)	No of positive (%)	No of negative (%)
Married	183	13(6.84%)	170(89.47%)
Single	3	0	3(1.58%)
Divorced	1	0	1(0.53%)
Widow	1	0	1(0.53%)
Separated	2	0	2(1.05%)
Total	190	13(6.84%)	177(93.16%)

π^2 = 0.534 Df = 4 P value = 0.970

(6.8%) were positive while 177 (93.2%) were negative for the IgM antibodies. The highest prevalence of the virus was recorded among the women that fell within the ages of 25-34 years (Table 1).

Sero-prevalence of IgM antibodies amongst pregnant women according to their stages of pregnancy

IgM antibodies were identified in all three trimesters of pregnancy (Table 2). Of the 21 women screened in the first trimester, 2 (1.05%) were positive for the IgM antibodies while 19 (10%) were negative. This however decreased during the second and third trimesters with respect to the number of persons screened where 6 (3.16%) out of the 77 screened were observed to be positive for the antibodies and 5 (2.63%) were observed to be positive among the 92 patients that were screened.

Sero-pevalence of IgM antibodies among pregnant women in relation to their marital status.

IgM antibodies were only detected in the pregnant women that were married. Of the 183 women that were screened, 13 (6.8%) were positive and none was found to be positive among the singles, divorced, widows and separated pregnant women as indicated in Table 3.

Sero-prevalence of IgM antibodies among pregnant women in relation to their educational level.

IgM antibodies were detected in all the women at their

Table 4. Sero-prevalence of IgM antibody among pregnant women according to their educational level.

Educational level	Total No. of patients screened	No. of positive (%)	No. of negative (%)
None	74	6(3.16%)	68(35.79%)
Primary	39	1(0.53%)	38(20%)
Secondary	42	4(2.11%)	38(20%)
Tertiary	35	2(1.05%)	33(17.37%)
Total	190	13(6.84%)	177(93.16%)

$\pi^2 = 1.850$ Df = 3 P value = 0.604

Table 5. Sero-prevalence of IgM antibody among pregnant women according to their occupation.

Occupation	Total no of patients screened	No. of positive (%)	No. of negative (%)
Housewife	98	7(3.68%)	91(47.89%)
Trading	48	1(0.53%)	47(24.74%)
Civil servant	40	4(2.11%)	36(18.95%)
Farming	4	1(0.53%)	3(1.58%)
Total	190	13(6.84%)	177(93.16%)

$\pi^2 = 4.414$ Df = 3 P value = 0.220

different educational levels (Table 4). However, the women with secondary education recorded the highest prevalence to the virus.

Sero-prevalence of IgM antibodies among pregnant women according to their occupation.

IgM antibodies were detected in all the categories of women studied. However, the highest prevalence was recorded among the farmers compared to other groups studied (Table 5).

DISCUSSION

Rubella is a *toga* virus that has been implicated in human diseases. *Rubella* poses an important public health problem because of its frequency of congenital infections which may lead to severe congenital abnormalities. Humans are the only known hosts of the virus and transmission requires close contact (Frank and David, 1976). Primary maternal infections during pregnancy are responsible for most cases of *Rubella* congenital syndrome.

Results obtained from this study indicate that out of the 190 pregnant women that were screened from Plateau State Specialist Hospital, Jos, IgM antibodies were detected in 6.8% of them while 93.2% were negative to the antibodies indicating that 6.8% of the study population could be recently infected with the *Rubella* virus as the IgM antibody is the body's first line of defense against an antigen and it is found in the blood and lymph fluids. This confirms earlier reports by Cutts *et al.* (2000) who reported that the proportion of women who are sero-positive for *Rubella* virus are less than 10%. However, the results are reported as activity index (AI) values. The AI compares the binding activity (positivity) of the test sample to the cut-off level of activity that is defined as positive (Activity Index Value = 1.0) (Engvall and Periman, 1972). Thus the percentage of the women (6.8%) that were recently infected with the virus and whose activity index were less than 1.0, it cannot make this disease to be a serious threat to lives in Nigeria.

Sero-prevalence of IgM antibodies increased gradually with age and was higher among the women who fell within the ages of 25-34 years. It can be seen that more than half of these 6.8% women fell within the ages of 25-34 years at which the infection is likely to occur (Table 1). The women who were in their first trimesters of pregnancy were observed to record the highest prevalence to the virus (Table 2). Our findings contrasts that of previous researchers like Barbara *et al.*(1987) who reported that the average transmission of *Rubella* virus infection from mother to fetus is greater during the period of maternal viremia (ie in the first 3-4 months of pregnancy). Best (2007) reported that IgM antibodies can persist in the body for over a year. Thus the 6.8% of these pregnant women in whom the IgM antibodies were detected could be said to be either lacking IgG antibodies (fight bacteria and viruses) or the quantities available may not be able to protect their fetus since with the persistence of the IgM antibody, the body's natural production of IgG in response to an antigen ought to

overwhelm the *Rubella* virus leading to the disappearance of the IgM antibody.

The categories of women observed in Tables 3 to 5 give an idea of which category stands the risk of having their unborn babies being infected with the *Rubella* congenital defects. Results obtained suggest that there is a higher prevalence of the infection among married women compared with divorced or singles, among women with secondary education compared with illiterates or women with primary and tertiary education and among farmers compared with housewives, traders and civil servants. The detection of the antibody in all categories of women investigated (except singles, divorced, widows and separated) could be attributed to the way in which the virus is acquired (through respiratory mode).

Conclusion

The serological evidence of *Rubella* virus found in the pregnant women in this study is an indication that *Rubella* is endemic in Nigeria though it may not really pose a serious threat. This lends credence to earlier findings of Miller (1991) who reported that *Rubella* is endemic in most developing countries, although their study showed a low proportion of 13(6.3%). There is therefore need to immunize pregnant women during the first trimesters of their pregnancy to create resistance to this virus. In addition, routine prenatal screening and post partum vaccination is highly encouraged in order to reduce the incidence of *Congenital Rubella Syndrome.*

Finally, more research work needs to be geared towards this area especially since we only investigated the pregnant women who were coming to the hospital for antenatal screening. Its also possible that there may be non pregnant women with this virus who could not be covered during the study.

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