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Research Article

SEROFREQUENCY OF RUBELLA AMONG PREGNANT LADIES ATTENDING OMDURMAN MILITARY HOSPITAL ANTENATAL CARE

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ABSTRACT

A total of 90 Pregnant Ladies who attending medical checkup in Omdurman Military Hospital during the period from April- May 2014, were enrolled in this study. Their age ranges from years with mean (30). The aim of this study was to detect frequency of Rubella IgM, IgG antibodies, and to determine the relationship between the presence of antibodies and certain factors such as gravidity, trimesters, past history abortion, and age). 90 serum specimens were collected from pregnant ladies, and analysed by ELISA technique. The results showed that 10(11.1%), 48 (53.3%) were positive for IgM and IgG antibody respectively, while 6 (6.7%) were positive for both. high frequency of positive IgM antibodies was observed among 25-34years age groups, and among whom had no history of abortion, in third trimester, and were multigravidae. Statistical analysis showed that there was insignificant correlation between age, Gravidity, Trimesters history Abortion and presence of rubella.

Large-scale studies in different settings and studies in Sudan are required.

Keywords: Sero-frequency, Rubella - IgM, IgG- pregnancy -ELISA-Omdorman-Sudan.

INTRODUCTION

Rubella, also known as German measles or three-day measles¹. is a disease caused by the rubella virus. The name "rubella" was derived from Latin, meaning little red. This disease is often mild and attacks often pass unnoticed. The disease can last one to three days. 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. Miscarriage occurs in up to 20% of cases².

Acquired (i.e. not congenital) rubella is transmitted via airborne droplet emission from the upper respiratory tract 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. The disease has an incubation period of 2 to 3 weeks³.

The name rubella is sometimes confused with rubeola, an alternative name for measles in English-speaking countries; the diseases are unrelated^{4, 5}. In some other European languages, like Spanish, rubella and rubeola are synonyms,

and rubeola is not an alternative name for measles⁶. Thus, in Spanish, "rubeola" refers to rubella and "sarampión" refers to measles.

Rubella has symptoms that are similar to those of flu. However, the primary symptom of rubella virus infection is the appearance of a rash (exanthem) on the face which spreads to the trunk and limbs and usually fades after three days (that is why it is often referred to as three-day measles). Other symptoms include low grade fever, swollen glands (sub occipital & posterior cervical lymphadenopathy), joint pains, headache and conjunctivitis⁷.

Rubella can cause congenital rubella syndrome in the newly born. The syndrome (CRS) follows intrauterine infection by the rubella virus and comprises cardiac, cerebral, ophthalmic and auditory defects⁸. It may also cause prematurity, low birth weight, and 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 developed⁹.

The disease is caused by Rubella virus, a togavirus that is enveloped and has a single-stranded RNA genome¹⁰. Increased susceptibility to infection might be inherited as there is some indication that HLA-A1 or factors surrounding A1 on

extended haplotypes are involved in virus infection or non-resolution of the disease^{11,12}.

In children Rubella normally causes symptoms which last two days and include the following¹³. Rash beginning on the face which spreads to the rest of the body, Low fever of less than 38.3°C (101°F) and Posterior cervical lymphadenopathy.

In older children and adults additional; symptoms including the following may be present: Swollen glands, Coryza (cold like symptoms), Aching joints (especially in young women) and Serious problems can occur including the brain infections and bleeding problems.

Rubella virus specific IgM antibodies are present in people recently infected by Rubella virus but these antibodies can persist for over a year and a positive test result needs to be interpreted with caution¹⁴. The presence of these antibodies along with, or a short time after, the characteristic rash confirms the diagnosis¹⁵.

Prevention

Rubella infections are prevented by active immunisation programs using live, disabled virus vaccines. Two live attenuated virus vaccines, RA 27/3 and Cendehill strains, were effective in the prevention of adult disease. However their use in prepubertile females did not produce a significant fall in the overall incidence rate of CRS in the UK. Reductions were only achieved by immunisation of all children¹⁶.

The immunisation program has been quite successful. Cuba declared the disease eliminated in the 1990s, and in 2004 the Centers for Disease Control and Prevention announced that both the congenital and acquired forms of rubella had been eliminated from the United States^{17,18}.

Screening for rubella susceptibility by history of vaccination or by serology is recommended in the United States for all women of childbearing age at their first preconception counseling visit to reduce incidence of congenital rubella syndrome (CRS)¹⁹.

MATERIALS AND METHODS

This was descriptive- cross sectional study which had been conducted in Khartoum state during period from April to May 2014, ninety pregnant ladies were enrolled, Data was collected by using direct interviewing questionnaire; ethical clearance was obtained from research ethical committee of faculty of graduate studies and ministry of health Khartoum state, written consent also was obtained from Pregnant ladies.

Experimental work

Samples collection:

blood samples were collected from 90 pregnant ladies, under direct medical supervision by medial vein puncture using 5 ml syringe into plain tube to obtain serum by centrifugation at 5000 rpm for 10 min. serums was kept in -20°C till serological study was performed.

Specimens were processed by Enzyme linked immune sorbent assay (ELISA) (3rd generation ELISA) (Weka- China) for detection IgM and IgG

Enzyme linked immune sorbent assay for detection anti rubella IgM and IgG (the same method for both)

All reagents and samples were allowed to reach room temperature for 15minutes before use.

Washing buffer was prepared 1:40 from buffer concentrate with distilled water.

100µl of sample diluents was added into appropriate wells except the blank well and negative well.

10µl from each sample was added to the appropriate wells and mixed by pipette repeatedly until liquids turn blue. 50µl from negative and positive control was dispense and added to the negative and positive wells separately without dispensing liquid into the blank control well.

Microtiter wells was flicked for 30 seconds and mixed well, then plate was covered and incubated for 20 minutes at 37⁰ C

Plate was taken out and wash buffer was added to each well (Washing 1) and aspirated off after 20 seconds. This step was repeated for 5 times until each well become dry.

50µl of HRP-Conjugate Reagent was added in to each well except the blank, the plate was mixed well and covered with the plate cover and incubated for 20 min at 37°C.

The plate cover was removed and discarded. The liquid was aspirated and each well was rinsed in wash buffer (Washing 2). This step was repeated for 5 times until each well become dry.

50µl of substrate A and 30µl substrate B solution was added in to each well including the Blank and mixed by tapping the plate gently. The plate was incubated at 37°C for 10 min.

50 µl Stop solution was added into each well and mixed gently.

Measuring the absorbance: The plate reader was calibrated with blank well and the absorbance was read at 450nm. The results were calculated by relating each sample optical density (OD) value to the Cut off value of plate. Calculation of Cut off (C.O) value.

$$C.O = *Nc*2.1$$

*Nc= the mean absorbance value for the three negative controls.

The absorbance was read with micro well reader at 450nm.

Interpretation of Results

Negative results: samples giving absorbance less than Cut-off value are negative for this assay. Positive result: sample giving absorbance equal to or greater than Cut-off considered initially reactive. Borderline: sample with absorbance to Cut-off value are considered borderline and retesting of these samples in duplicate is recommended.

Data analysis: Data was analyzed by SPSS (Statistical Package of Social Science) software program version 16

RESULTS

A total of 90 Pregnant Ladies who attending medical checkup in Omdurman Military Hospital during the period from April-May 2014, were enrolled in this study. Their age ranges from 15-45 years with mean 30 (fig 1) , most of them were multigravidae (72.5%) , in third trimester of pregnancy (53.8%), and had no history of past history of abortion (64.6%) (fig 2,3, and 4), The aim of this study was to detect frequency of Rubella IgM and IgG antibodies, and to determine the relationship between the presence of antibodies and certain factors such as (Gravidity, Trimesters history Abortion and age). 90 serum specimens were collected from pregnant ladies, and analyzed by ELISA technique. The

results showed that 10(11.1%), 48 (53.3%) were positive for IgM antibody and IgG respectively (fig 5,6) , while 6 (6.7%) were positive for both. high frequency of positive IgM results was observed among 25-34 age groups (table 1), and whom had no history of abortion,in third trimester, and were

multigravidae (as demonstrated in tables 2,3, and 4). Statistical analysis showed that there was insignificant correlation (P value more than 0.05) between age, Gravidity, Trimesters history Abortion and presence of rubella.

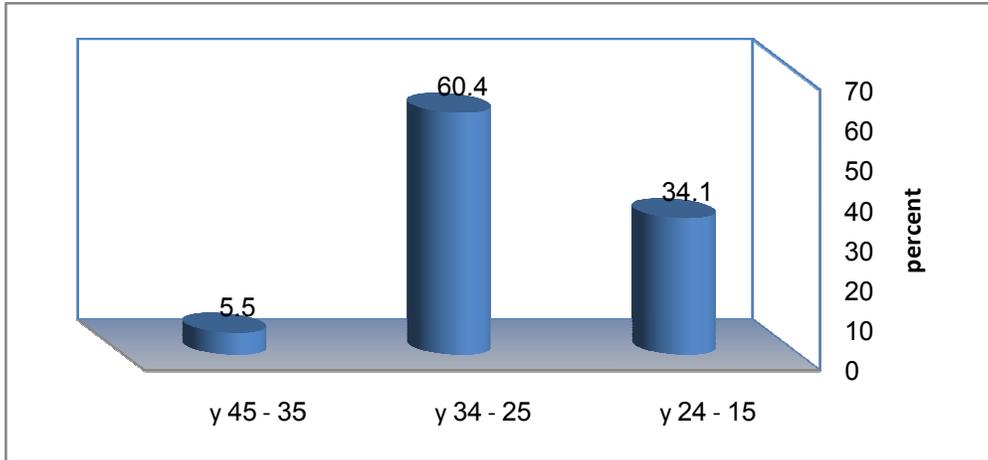


Figure 1: distribution of study population according to their age (n=90)

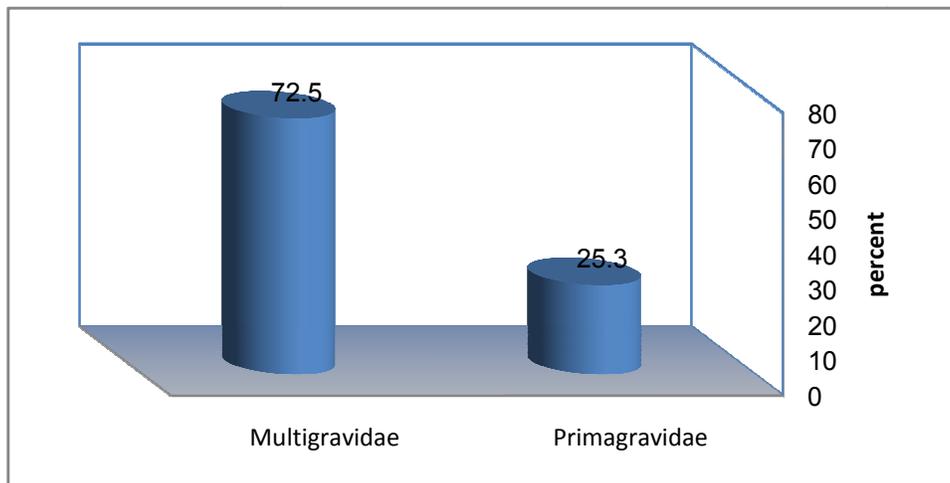


Figure 2: distribution of study population according to their gravidity (n=90)

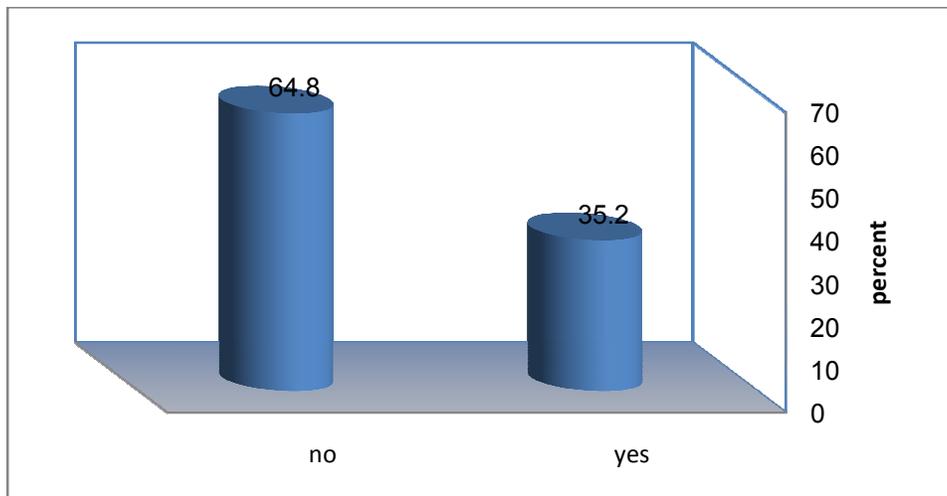


Figure 3: distribution of study population according to the past history of abortion (n=90)

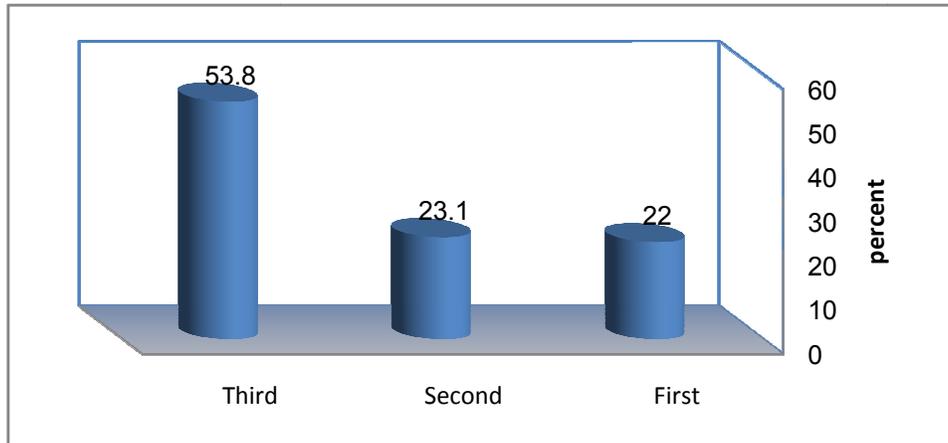


Figure 4: distribution of study population according to the trimester of pregnancy (n=90)

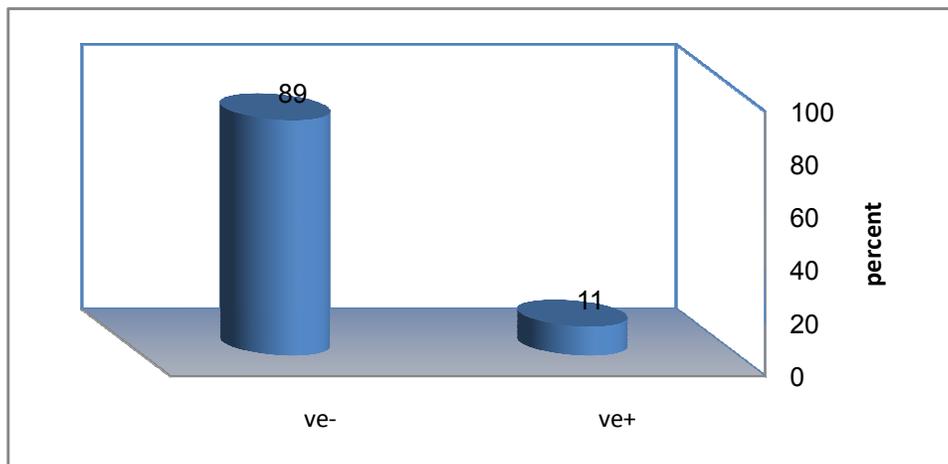


Figure 5: frequency of anti rubella IgM among study population (n= 90)

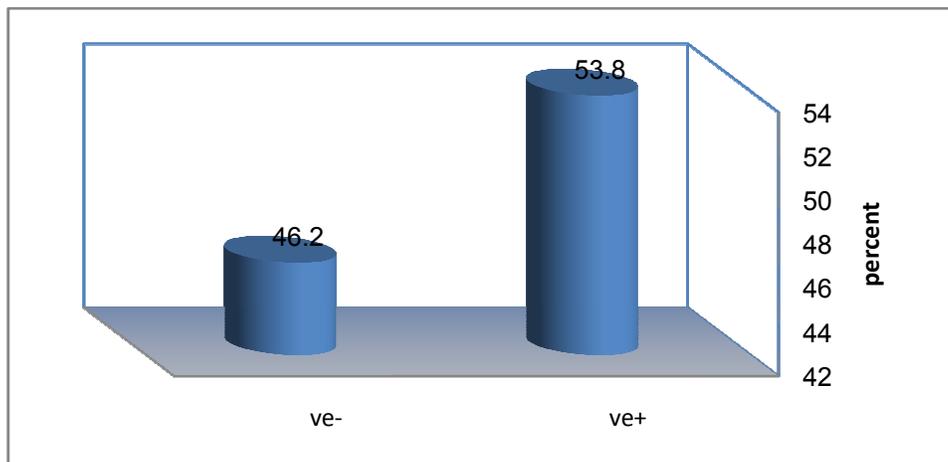


Figure 6: frequency of anti rubella IgG among study population (n= 90)

Table 1: Serofrequency of rubella according to age group

Age groups	Results			
	IgM seropositive	IgG seropositive	IgM-IgG seropositive	Negative
15-24	3 (3.3%)	17 (18.9%)	3 (9.7%)	14 (45.2%)
25-34	7 (7.8%)	30 (33.3%)	3 (5.6%)	20 (37.0%)
35-45	0 (0.0%)	1 (1.1%)	0 (0.0%)	4 (80.0%)
Total	10 (11.1%)	48 (53.3%)	6 (6.7%)	38 (42.2%)

Table 2: Serofrequency of rubella according to past history of abortion

Abortion	Results			
	IgM seropositive	IgG seropositive	IgM-IgG seropositive	Negative
yes	5 (5.6%)	15 (16.7%)	1 (3.1%)	13 (40.6%)
no	5 (5.6%)	33 (36.7%)	5 (5.6%)	25 (43.1%)
Total	10 (11.1%)	48 (53.3%)	6 (6.7%)	38 (42.2%)

Table 3: Serofrequency of rubella according to trimesters of pregnancy

Trimesters	Results			
	IgM seropositive	IgG seropositive	IgM-IgG seropositive	Negative
First	2 (2.2%)	11 (12.2%)	2 (10.5%)	8 (42.1%)
Second	0 (0.0%)	10 (11.1%)	0 (0.0%)	12 (54.5%)
Third	8 (8.9%)	27 (30.0%)	4 (8.2%)	18 (36.7%)
Total	10 (11.1%)	48 (53.3%)	6 (6.7%)	38 (42.2%)

Table 4: Serofrequency of rubella according to gravidity

Gravidity	Results			
	IgM seropositive	IgG seropositive	IgM-IgG seropositive	Negative
Primagravidae	2 (2.2%)	11 (12.2%)	1 (4.0%)	13 (52.0%)
Multigravidae	8 (8.9%)	37 (41.1%)	5 (7.7%)	25 (38.5%)
Total	10 (11.1%)	48 (53.3%)	6 (6.7%)	38 (42.2%)

DISCUSSION

This study presented the most recent data on the serofrequency of Rubella in the pregnant ladies in terms of age groups and gravidity and trimester and history of abortion, thereby for the prevention of rubella infection.

The present study results revealed that 10(11.1%), 48 (53.3%) were positive for IgM antibody and IgG respectively, when compared with others finding, regarding it is lower than which obtained by Adam et al., Khartoum Sudan- 2013, who reported Rubella IgG antibodies were detected in 95.1% among pregnant ladies, and agreed with their result in insignificant association with age¹⁹, but it is similar to results of study in Benin city Nigeria (2011) in which they found IgG seroprevalence was 53%, while 10.0% of all IgG seropositive women were IgM seropositive. Most infections were acquired before the age of 35. None of the women ever had previous rubella vaccination Rubella vaccine is scarce in Nigeria²⁰. In Sudan there is no rubella vaccine.

Similar study targeting IgG was conducted in Turkey-2007- by Aksakal *etal*, reported seropositivity was 95.5% for the total group and 96.2% among pregnant women²¹.

We presume this high serofrequency indicates a high circulation of wild rubella virus in Khartoum. Similar studies in other Sudanese states would be important for informing a decision to introduce rubella vaccine to Sudan.

CONCLUSION

Prevalence of rubella seromarkers for previous and current infection is high. Facilities for routine diagnosis and vaccination are lacking. Initiation of organized screening and vaccination programs is limited by lack of vaccine. We recommend immunization of children and women of child-bearing age as a cost-effective public health intervention strategy for managing the sequelae of the congenital rubella syndrome.

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