Sero-Frequency of Rubella Virus among Pregnant Women Attending Omdurman Maternity Hospital

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Abstract:

   Background: *Infection of mothers with Rubella virus during pregnancy can be serious; if the mother is infected within the first 20 weeks of pregnancy she is likely to have miscarriage, stillbirth, or baby with congenital rubella syndrome. This study was carried out to detect sero-frequency of Rubella virus among pregnant ladies attending Omdurman maternity hospital, Sudan.*

   Methods: *This is a cross-sectional study, serum specimens were collected and analyzed by ELISA for rubella IgG and IgM antibodies among pregnant women, and the result were correlated with Age, history of abortion, trimester of pregnancy and history of chronic illnesses. Generated data were analyzed by using SPSS*

   Results: *Out the total of 90 pregnant ladies tested 43 (47.8%), 8 (8.9%) were positive for IgG & IgM respectively and 45 (50.0%) were negative for both. The result showed higher sero-frequency (46.5% for IgG and 62.5% for IgM) among group age 20-30 year. Rubella sero-frequencies was significantly associated with history of abortion but*

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insignificantly associated with age, gestational age, history of chronic illnesses.

  Conclusions: As the immunity gap in the studied population was high, rubella vaccination should be provided for all women of child-bearing age and children.

Key words: Rubella, IgG, IgM, ELISA, Pregnant Women, Omdurman Maternity Hospital, Sudan.

Introduction:

Rubella virus, a member of the Togaviridae family is the sole member of the genus Rubivirus. It is enveloped and has a single stranded ribonucleic acid genome[1.] *Rubella virus* causes a disease called Rubella commonly known as German measles. The virus is transmitted through the respiratory route, it replicates in the nasopharynx, followed by multiplication in the cervical lymph nodes. Virus then enters the bloodstream and is disseminated[2] .The disease has an incubation period of 2-3 weeks[3.] The Virus causes a mild rash-like disease that is associated with low-grade fever, lymphadenopathy and a short-lived morbilliform rash[4] .The rash starts on the face, extends to the trunk and extremities and rarely last more than 3 days[5]. Other symptoms include swollen glands (post cervical lymphadenopathy), joint pains, headache and conjunctivitis[6]. Rubella Is predominantly a childhood disease which is endemic throughout the world[7,8] .In contrast to the mild infections caused in early childhood or adult life, rubella in pregnant women causes Congenital Rubella Syndrome (CRS), Which is the infection of a developing foetus following transplacental transmission of rubella virus from the mother to the foetus[4] .

  When a pregnant mother is infected within the first 20 weeks of pregnancy, she might have miscarriage, stillbirth, or baby born with Congenital Rubella Syndrome (CRS). The syndrome (CRS) follows intrauterine infection by *Rubella virus*

and comprises cardiac, cerebral, ophthalmic and auditory defects[9.] It may also cause prematurity, low birth weight, and neonatal thrombocytopenia, anemia and hepatitis[10] .Two specific antibodies are associated with rubella. The first to appear is immunoglobulin (Ig) M antibody, which rises and peaks 7–10 days after infection and then reduces after several weeks. The IgG antibody develops more slowly, but remains positive for life, hence conferring immunity against repeat infection. Therefore, the presence of IgM antibody indicates a recent infection, while IgG antibody indicates an old infection and immunity[11.]

Rubella has a worldwide 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 [12].It is estimated that worldwide more than 100 000 children with CRS are born each year[13]. Rubella vaccines are not included in the Sudanese national immunization programme, and data on the prevalence of rubella among women of childbearing age are inadequate.[14] Furthermore, there is no routine surveillance for CRS, and data on its incidence are extremely scarce[15]. Sudanese surveillance for measles and rubella since 2006 has reported that rubella infection is a frequent cause of none-measles rash[16]. This study aimed at detecting the presence of both anti-rubella IgM and IgG antibodies in pregnant women attending Omdurman Maternity Hospital. This study will also help to possible risk factors associated with the spread of the virus and determine the level of awareness of infection among pregnant women. More importantly, this study, combined with the findings of other studies on rubella in Sudan, will provide information necessary for health care administrators and health care providers in Sudan to address rubella.

Material and Methods:

This was descriptive- cross sectional study which had been conducted in Omdurman Maternity Hospital during period from March to April 2015, 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 was also obtained from Pregnant ladies.

Experimental work

Samples collection:
Blood samples were collected from 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) (Foresight- Germany) 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 1:25 from buffer concentrate with distilled water. 100µl of sample diluents was added into appropriate wells except the blank well and negative well. 5 µl from each sample was added to the appropriate wells and mixed by pipette repeatedly until liquids turn from green to blue.

100µl 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 30 minutes at 37°C .Plate was taken out and 350µl of 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 100µl of Peroxidase-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 30 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 became dry. 50µl of substrate A and 50µl substrate B solution was added in to each well including the Blank and mixed by tapping the plate gently. Then 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 450 nm. 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
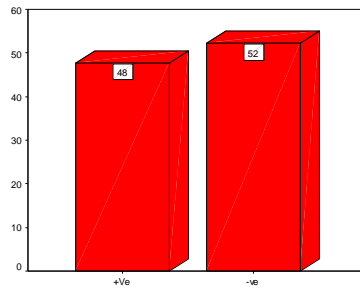
Result:

A total of 90 pregnant women were enrolled in the study. Their ages ranged from 15 to 46 years, with 28 years mean. Most of them were in third trimester of pregnancy (54.4%), had no chronic illnesses (78.9%) and had no history of past history of abortion (80.0%). Among the total studied pregnant women 8 (8.9%) and 43 (47.8%) were sero-positively for rubella IgM and IgG antibodies, respectively (fig1,2). However 6(6.7 %) were sero-positive for both IgG & IgM.

Highest sero-frequency of IgM and IgG results was observed among 21-30 age groups (table 1), and among whom had no history of abortion, in third trimester, and who had no history of chronic illnesses (as demonstrated in tables 2,3).

Statistical analysis showed that there was significant association (P value less than 0.05) between history of abortion and presence of rubella antibodies, But there is insignificant correlation (P value more than 0.05) between age, Trimesters and history of chronic illnesses.
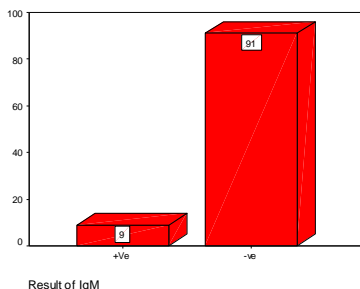
Figure 1: frequency of anti rubella IgM among study population (n= 90)

Figure 2: frequency of anti rubella IgG among study population (n= 90)

Table 1: Serofrequency of rubella among pregnant women according to age group(n=90)

Age groups in year	Result			
	IgM seropositivity	IgG seropositivity	IgM – IgG seropositivity	Negative
10-20	1 (1.1%)	8(8.9%)	1(1.1%)	8(8.9%)
20-30	5(5.6%)	20 (22.2%)	3(3.3%)	25(27.8%)
30-40	1(1.1%)	13(14.4%)	1(1.1%)	12(13.3%)
40-50	1(1.1%)	2(2.2%)	1(1.1%)	0(0.0%)
Total	8(8.9%)	43(47.8%)	6(6.7%)	45(50.0%)
p-value	.156**	.406**	.052*	

**Not significant at the 0.05 level.

Table 2: Sero-frequency of rubella among pregnant women according to past history of abortion(n=90)

History of abortion	Result			
	IgM seropositivity	IgG seropositivity	IgM – IgG seropositivity	Negative
Yes	4(4.4%)	13(14.4%)	4(4.4%)	5(5.6%)
No	4(4.4%)	30(33.3%)	2(2.2%)	40(44.4%)
Total	8(8.9%)	43(47.8%)	6(6.7%)	45(50.0%)
	026*	020*	.001*	

 *Significant at the 0.05 level.

Table 3: Sero-frequency of rubella among pregnant women according to trimesters of pregnancy(n=90)

Trimester of pregnancy	Result			
	IgM seropositivity	IgG seropositivity	IgM – IgG seropositivity	Negative
First trimester	5(5.6%)	16(17.8)	4(4.4%)	16(17.8%)

second trimester	0(0%)	4 (4.4%)	0(0.0%)	4 (4.4%)
Third trimester	3(3.3%)	23(25.6%)	2(2.2%)	25(27.8%)
Total	8(8.9%)	43(47.8%)	6(6.7%)	45(50.0%)
p-value	.242**	.982**	.311**	

**Not significant at the 0.05 level.

Table 4: Sero-frequency of rubella according to history of Chronic illnesses

History of chronic illnesses	Result			
	IgM seropositivity	IgG seropositivity	IgM – IgG seropositivity	Negative
Yes	2(2.2%)	6(6.7%)	2(2.2%)	13(14.4%)
No	6(6.7%)	37(41.1%)	4(4.4%)	32(35.6%)
Total	8(8.9%)	43(47.8%)	6(6.7%)	45(50.0%)
p-value	.778**	.822**		

**Not significant at the 0.05 level.

Discussion:

Infection with *Rubella virus* can be disastrous during pregnancy. The virus may affect all organs and can cause a variety of congenital defects in the fetus if a susceptible pregnant woman is exposed to it, especially in the early gestational weeks. This condition is called CRS and has a very high estimated lifetime cost for both parents and governments[16].

The result of this study revealed a *Rubella virus* overall sero-frequency rate of 50.0% among pregnant as 43 (47.8%) for IgG and 8 (8.9%) for IgM. We presume this indicates a circulation of wild rubella virus in Sudan, since rubella vaccination is not yet practiced in the country. If this compared with related findings, this closely similar to the prevalence of 53.3%, 11.1% for IgG and IgM respectively reported in Omdurman, Sudan[10], Also as the IgG prevalence of 54.1% in Maiduguri, Nigeria [17] and IgG prevalence of 53% in Benin City, Nigeria [18]. But lower than the prevalence of 95.1% reported in a similar study in march 2009 in Khartoum state, Sudan.[19]

Higher IgG prevalence of 93.5% was reported in Malatya, Turkey [20] and IgG prevalence of 76% in Lagos, Nigeria [21].

Possibly this difference between our report and report of *Adam, et al* in Khartoum state 2009 is due to long period between these two studies, since rubella occurs in a seasonal pattern with epidemics every 5- 9 years [19]. Sero-frequency based on age groups, trimester showed an insignificant association with *Rubella* infection and that agreed with findings of *Adam, et al*[19] and *Olatunji, et al*[16]. But the result showed a significant association between *Rubella* infection and pregnant women whom have had history of previous abortion and that not agreed with report of *Abdelrhman, et al*[10.]

Conclusion:

This high frequency of rubella IgG & IgM antibodies among pregnant ladies suggesting a sustained past and current infections in the population and indicating endemicity Also Outbreaks and possibly reinfections may occur. While sero-negativity of IgG antibody indicate susceptibility of high proportion of the population to the infection and this necessitate the introduction of rubella vaccine.

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