

Serological Detection of Rubella Virus among Pregnant Women in Khartoum State

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Abstract:

This study aimed to detect sero-prevalence of rubella virus among pregnant women in different hospitals in Khartoum State.

A total of 293 blood samples were collected from major Khartoum hospitals to detect rubella antibodies serologically by ELISA. All the participants were non vaccinated and most common age group were 20-30 years and 31-40 years. Two (0.7%) were IgM+ve and 262(89.4%) were IgG+ve and there is association between rubella infection and miscarriage and recurrence of miscarriage. Also the gravidity and age were associated with rubella infection and no significant differences were noticed in antibody prevalence regarding the family size and trimester with rubella infection. Moreover, there was association with history of skin rash which likely due to rubella

infection. The importance of rubella derives from the potentially harmful effects on the fetus that can occur when a woman who is pregnant is infected with rubella. Accurate diagnosis of acute primary rubella infection in pregnancy is necessary and requires serologic testing, since an important number of cases are subclinical. Serology by ELISA to measure rubella-specific IgG and IgM is convenient, sensitive, and accurate. The sero-prevalence of rubella in Khartoum State is tremendous and pregnant women were highly exposed to rubella virus. Unfortunately there are no protective prophylaxis tools.

Key words: Rubella virus, serology, ELISA, pregnant women, Khartoum State

INTRODUCTION:

Rubella virus (RV) is the sole member of the Rubivirus genus of the Togaviridae family which has a single-stranded RNA and positive-polarity. It causes a mild childhood disease; on the other hand it is a potent teratogenic agent when contracted by a pregnant woman ⁽¹⁾.

The main route of postnatal virus transmission is by direct contact with nasopharyngeal secretions ⁽²⁾. Postnatal RV infection is a generally mild and self-limited illness ^(2, 3, 4, 5), but primary RV infections during the first trimester of pregnancy have high teratogenic potential leading to severe consequences, known as congenital rubella syndrome (CRS) which may occur in 80–85% of cases ^(4, 5).

The national elimination plan recommends strengthening surveillance of rubella and congenital rubella cases ⁽⁶⁾.

It is therefore strongly recommended by the World Health Organization that serological surveys on rubella virus infection in women of childbearing age be done ⁽⁷⁾.

But it is a vaccine preventable disease and in developed countries outbreaks are mostly confined to unvaccinated communities ⁽⁸⁾.

The seroprevalence of rubella in Khartoum state was 95.1%, while the susceptibility to rubella among the examined women was 4.9% ⁽⁹⁾.

Two important techniques used for diagnosis rubella which are; the sero-detection of rubella IgM and the detection of RV RNA by conventional and real time reverse-transcription polymerase chain reaction assays ⁽¹⁰⁾.

This study aimed to detect rubella viral IgG and IgM antibodies in the blood of the pregnant women and find out the risk factors predisposing to the infection with rubella among pregnant women in Khartoum State (socioeconomic situation, family size, gravidity, and trimester)

MATERIALS AND METHODS:

Study design:

Observational, cross-sectional study

Study area:

This study was conducted as a hospital-based study in different hospitals of different geographical locations in Khartoum State (Omdurman Maternity Hospital, China Friendship Hospital, Alsaudi Hospital, Ibrahim Malik Teaching Hospital, Turkish Hospital, Saad Aboalula Hospital, Haj Elsafi, Aldrooshab Specilized Hospital and East Nile Model Hospital).

Study population:

The study was scoped all pregnant women attending the study areas were considered eligible to participate irrespective of race, age, residence and parity.

Method of data collection:

Data was collected through direct interview with pregnant women. The interview instrument (Questionnaire) consists of 14 questions. It was consisted of three parts including general information on women.

Sample size:

A total of 293 pregnant women were randomly selected. The blood samples were collected from vein (vein puncture) of pregnant women by sterile syringes and sera were collected in sterile containers and stored at -70° C until tested.

Laboratory tests:

Enzyme-Linked Immunosorbent Assay (ELISA):

ELISA for detection of RV IgM and IgG antibodies:

Commercial ELISA Kits (EUROIMMUN, Medizinische labordiagnostika AG, Germany) were used as described by the manufactures. In brief, the sample diluents X100 concentrate was diluted 1:101 in sample buffer for the assay run. According to the plan of working, 100 µl of the negative control, 100 µl of calibrator, 100 µl of positive control and 100 µl of diluted samples (1:101) were incubated in microplate well coated with rubella virus glycoprotein antigen at room temperature for 30 minutes. The wells were washed three times manually by washing buffer (which diluted firstly by distilled water 1:9) to remove residual plasma. Then 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgM and IgG) were added to each well and incubated at room temperature for 30 minutes. After another washing step to eliminate unbound material, an enzyme substrate solution (TMB Substrate) was added (100 µl /well) and the plate was incubated for 15 minutes. The blue color changed to yellow after adding of the stop solution (100 µl). The optical density (O.D) in a microplate reader was read within 10 minutes at 450 – 630 nm.

Calculation and interpretation of the results:

The following formula was used to calculate the ratio:

Extinction of the control or patient sample/ extinction of the calibrator = ratio

Interpretation of results was done as follows:

Ratio < 0.8: negative

Ratio ≥ 0.8 to < 1.1: borderline

Ratio ≥ 1.1: positive

RESULTS

Table (1) showed that the most age group of the pregnant women was 20-30 years (64.8%) followed by 31-40 years group (25.6%), less than 20 years (7.8%) and above 40 years were 5 (1.7%).

Table (1): Age group of pregnant women in different hospitals in Khartoum State

Age group/ year	Frequency	Percent
Less than 20 years	23	7.8%
20-30 years	190	64.8%
31- 40 years	75	25.6%
More than 40 years	5	1.7%
Total	293	100.0%

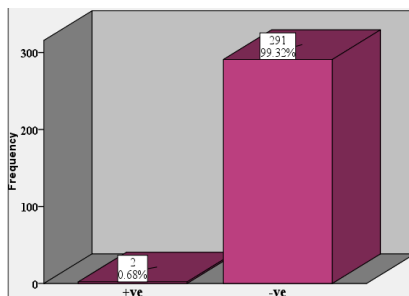


Figure (1): Detection of anti-rubella IgM positive cases among the pregnant women in different hospitals in Khartoum State

Figure (1) exhibited that 2 of pregnant women were IgM + ve (0.68%) and 291 were IgM -ve (99.32%).

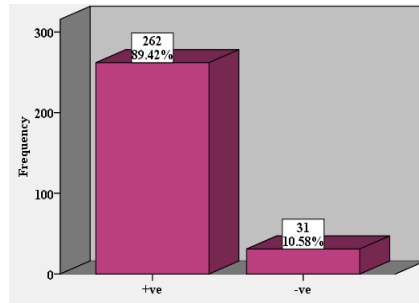


Figure (2): Detection of anti-rubella IgG positive cases among the pregnant women in different hospitals in Khartoum State

In figure (2) 262 pregnant women (262/293) were anti-rubella IgG positive (89.42%) and 31(31/293) women were anti-CMV IgG negative (10.58%)

Table (2): Sero-detection of anti-rubella IgM among pregnant women with history of miscarriage in different hospitals in Khartoum State

Miscarriage	ELISA IgM Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
Yes	2	2.6%	76	97.4%	78	100.0%
No	0	0.0%	215	100.0%	215	100.0%
Total	2	.7%	291	99.3%	293	100.0%

P-value: .018

In table (2) there was statistically significant differences ($p > 0.05$) between pregnant women who had miscarriage and anti-rubella IgM+ve (2.6%), and who had no miscarriage and anti-rubella IgM+ve (0.0%).

Table (3): Sero-detection of anti-rubella IgG among pregnant women with history of miscarriage in different hospitals in Khartoum State

Miscarriage	ELISA IgG Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
Yes	72	92.3%	6	7.7%	78	100.0%
No	190	88.4%	25	11.6%	215	100.0%
Total	262	89.4%	31	10.6%	293	100.0%

P-value: 0.333

In table (3) there was a significant difference ($p > 0.05$) between pregnant women who had miscarriage and anti-rubella IgG+ve (92.3%) and who had no miscarriage and anti-rubella IgG+ve (88.4%).

Table (4): The relation between number of miscarriage of pregnant women and IgM Percentage in different hospitals in Khartoum State

Number of miscarriage	ELISA IgM Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
Once	1	2.0%	50	98.0%	51	100.0%
Twice	0	0.0%	16	100.0%	16	100.0%
More than twice	1	9.1%	10	90.9%	11	100.0%
-	0	0.0%	215	100.0%	215	100.0%
Total	2	.7%	291	99.3%	293	100.0%

P-value: .003

Table (4) showed that there was a significant difference ($p < 0.05$) between pregnant women who had once miscarriage (2.0%), twice miscarriage (0.0%) and more than twice miscarriage (0.7%) and IgM+ve.

Table (5): The relation between number of miscarriage of pregnant women and IgG Percentage in different hospitals in Khartoum State

Number of miscarriage	ELISA IgG Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%

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Once	46	90.2%	5	9.8%	51	100.0%
Twice	16	100.0%	0	100.0%	16	100.0%
More than twice	10	90.9%	1	9.1%	11	100.0%
-	190	88.4%	25	11.6%	215	100.0%
Total	262	89.4%	31	10.6%	293	100.0%

P-value: .532

In table (5) there was a significant difference ($p < 0.05$) between pregnant women who had once miscarriage (90.2%), twice miscarriage (100.0%) and more than twice miscarriage (90.9%) and IgG+ve.

Table (6): The association between trimester and IgM Percentage of pregnant women in different hospitals in Khartoum State

Trimester	ELISA IgM Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
First trimester	1	1.4%	69	98.6%	70	100.0%
Second trimester	1	.8%	127	99.2%	128	100.0%
Third trimester	0	.0%	95	100.0%	95	100.0%
Total	2	.7%	291	99.3%	293	100.0%

P-value: .536

Table (6) showed that there was no meaning differences ($p > 0.05$) between the trimester and anti-rubella IgM +ve.

Table (7): The association between trimester and IgG percentage of pregnant women in different hospitals in Khartoum State

Trimester	ELISA IgG Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
First trimester	64	91.4%	6	8.6%	70	100.0%
Second trimester	112	87.5%	16	12.5%	128	100.0%
Third trimester	86	90.5%	9	90.5%	95	100.0%
Total	262	89.4%	31	10.6%	293	100.0%

P-value: .631

In table (7) there was no meaning difference ($p > 0.05$) between the trimester and anti-rubella IgG +ve.

Table (8): The association between family size and IgM percentage in different hospitals in Khartoum State

Family size	ELISA IgM Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
1-5 members	1	.5%	183	99.5%	184	100.0%
6-10 members	1	1.1%	89	98.9%	90	100.0%
> 10mmbers	0	.0%	19	100.0%	19	100.0%
Total	2	.7%	291	99.3%	293	100.0%

P-value: .808

Table (8) the size of family had no effect ($p > 0.05$) on acquiring new rubella infection (IgM).

Table (9): The association between family size and IgG percentage in different hospitals in Khartoum State

Family size	ELISA IgG Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
1-5 members	162	88.0%	22	12.0%	184	100.0%
6-10 members	82	91.1%	8	8.9%	90	100.0%
> 10mmbers	18	94.7%	1	5.3%	19	100.0%
Total	262	89.4%	31	10.6%	293	100.0%

P-value: .547

Table (9) exhibited that the size of family had no effect ($p > 0.05$) on rubella infection.

Table (10): The relation between history of skin rash and IgM percentage in different hospitals in Khartoum State

History of skin rash	ELISA IgM Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
Yes	0	.0%	4	100.0%	4	100.0%

No	2	.7%	287	99.3%	289	100.0%
Total	2	.7%	291	99.3%	293	100.0%

P-value: .867

Table (10) revealed that there was no significant difference ($p > 0.05$) between women who skin rash previously (0.0%) and who had never skin rash in the past (0.7%) concerning anti-rubella IgM +ve.

Table (11): The relation between history of skin rash and IgG percentage in different hospitals in Khartoum State

History of skin rash	ELISA IgG Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
Yes	3	75.0%	1	25.0%	4	25.0%
No	259	89.6%	30	10.4%	289	100.0%
Total	262	89.4%	31	10.6%	293	100.0%

P-value: .345

Table (11) showed that there was significant difference ($p > 0.05$) between pregnant women who had history of skin rash (75.0%) and who had no skin rash previously (89.6%) among IgG+ve results.

Table (12): Sero-detection of anti-rubella IgM among pregnant women according to age incidence in different hospitals in Khartoum State

Age/ year	ELISA IgM Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
Less than 20 years	0	0.0%	23	100.0%	23	100.0%
20-30 years	1	0.5%	189	99.5%	190	100.0%
31-40 years	1	1.3%	74	98.7%	75	100.0%
More than 40 years	0	0.0%	5	10.0%	5	100.0%
Total	2	0.7%	291	99.3%	293	100.0%

P-value: .866

There was no significant difference ($p > 0.05$) between various age groups (Table 12) on new rubella infection (IgM).

Table (13): Sero-detection of anti-rubella IgG among pregnant women according to age incidence in different hospitals in Khartoum State

Age/ year	ELISA IgG Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
less than 20 years	22	95.7%	1	4.3%	23	100.0%
20-30 years	166	87.4%	24	12.6%	190	100.0%
31-40 years	69	92.0%	6	8.0%	75	100.0%
more than 40 years	5	100.0%	0	.0%	5	100.0%
Total	262	89.4%	31	10.6%	293	100.0%

P-value: .406

There was a significant difference ($p < 0.05$) between various age groups on past rubella infection (IgG) (table 13); in which the highest age group was more than 40 years (100.0%) and the lowest group was 20-30 years (87.4%).

Table (14): Sero-detection of anti-rubella IgM among pregnant women according to gravidity in different hospitals in Khartoum State

Gravidity	ELISA IgM Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
Monogravida	0	.0%	88	100.0%	88	100.0%
Multigravida	2	1.0%	203	90.0%	205	100.0%
Total	2	.7%	291	99.3%	293	100.0%

P-value: .352

In table (14) there was a significant difference ($p < 0.05$) between monogavida pregnant women(0.0%) and multigravida women (1.0%) on new rubella infection (IgM).

Table (15): Sero-detection of anti-rubella IgG among pregnant women according to gravidity in different hospitals in Khartoum State

Gravidity	ELISA IgG Percentage				Total	
	+ve		-ve			
	No.	%	No.	%	No.	%
Monogravida	76	86.4%	76	13.6%	88	100.0%
Multigravida	186	90.7%	19	9.3%	205	100.0%
Total	262	89.4%	31	10.6%	293	100.0%

P-value: .265

Table (15) show that there was a significant difference ($p < 0.05$) between monogavida pregnant women (0.0%) and multigravida women (1.0%) on rubella past infection (IgG).

DISCUSSION

Rubella virus is teratogenic and it is transmitted to a fetus producing a serious complication called congenital rubella syndrome (CRS). The mother is able to transmit the virus even if she is asymptomatic. Fetal injury varies according to the time of infection. It is generally accepted the infection in the first trimester is most likely to induce miscarriage or multiple permanent defects in the newborn such as cardiac abnormalities, ocular lesions, deafness, and mental and physical retardation. Less drastic sequelae that usually resolve in time are anemia, hepatitis, pneumonia, carditis, and bone infection ⁽¹¹⁾.

Congenital rubella syndrome (CRS) is more frequently manifest in developing countries due to vaccination against rubella is not common practice ⁽¹²⁾.

In the present study, 0.7% of pregnant women were IgM +ve (recent infection) and 89.4% were IgG+ve (past infection) for rubella antibodies using ELISA technique (figure -1).

Our results were in agreement with Barah and Ghehda (2010) ⁽¹³⁾ in Syria, they found that 85.6% of females of child bearing age were IgG+ve.

These findings are less than the results obtained by Adam *et al.* (2014) ⁽⁹⁾ which found that 95.1% of women were IgG+ve in Khartoum State , 94.1 % among pregnant women and young females in KSA by Fadwaa *et al.* (2010) ⁽¹⁴⁾ and 92% among pregnant women in Harare, Zimbabwe achieved by Mamvura *et al.* (2013) ⁽¹⁵⁾. Also in other studies carried in Republic of Iran, 91.1% anti-rubella IgG among pregnant women. In addition, 95.3% has been reported in some African countries, such as South Africa and Mozambique ⁽⁹⁾, besides, a slightly higher prevalence of 97.9% among Nigerian pregnant women ⁽⁹⁾. That may be due to rubella have a seasonal pattern with epidemics every 5–9 years ⁽¹⁶⁾.

This study was high compared with the results obtained by Hamdan *et al.* (2011) ⁽¹⁷⁾ and in western Sudan yield the following results: IgG (65.3%) and IgM (3.4%).

In our study, there is significant association between miscarriage (table 2 and 3) and rubella infection in the past and in recent time (p-value= 0.33 and .018 in sequence). Also there is significant differences in the result of IgM and the number of miscarriage (P-value: .003) and no meaning association (table 4) between number of miscarriage and IgG results (P-value: .532) that may be due to CMV or other agents (table 5).

In view of trimester, there is no sense relation between trimester of pregnancy and rubella infection (p-value: 0.536 and 0.631) (table 6 and 7). Also there is no significant association between family size and rubella infection (p-value: 0.808 and 0.547) as other factors related (table 8 and 9) to crowdedness uncontrollable like public transport or resident areas.

There is significant relation between skin rash formerly and rubella infection (p-value: 0.345) which may be appear (table 11) in symptomatic ladies and no significant relation between skin and recent rubella infection (p-value: 0.867) as Rubella is asymptomatic in 25 to 50 % of cases ⁽¹⁸⁾.

Our study showed that there is a significant difference between age groups: less than 20 years, 20-30 years, 31-40

years and more than 40 years, which with prevalence; 95.7%, 87.4%, 92.0% and 100.0% respectively (table 13). This findings were in harmony with the results attained by Adam *et al.* ⁽⁹⁾ with age group ≤ 25 years and > 25 years, Fadwaa *et al.* (2010) ⁽¹⁴⁾ which done in small ages (<5 years, 5- <10 years, 10-15 years, 15-20 years, and 20-25) and Mamvura *et al.* (2013) ⁽¹⁵⁾ with the groups: 16-20, 21-25, 26-30, 31-35, 36-40, 41-45, and 46-50 years. But no significant differences (table 12) with recent infection (anti-rubella IgM +ve).

This study showed (table 14 and 15) a significant association between rubella infection and gravidity (P-value: .265 and 0.352) which dissent with the results yielded by Adam *et al.* ⁽⁹⁾.

CONCLUSIONS:

Pregnant women in Khartoum State were highly exposed to rubella virus and possibly related to miscarriage of them. Rubella infection is associated with age and gravidity.

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