

Seroprevalence of Rubella Virus among Pregnant Women and with a History of Abortion in the Red Sea Region of Sudan

Comment [CN1]: What is the type abortion? Not oblige to add history of abortion in the title, it is a finding of the study! Because if you recruit only woman with history of abortion, we should not find woman without abirption in the result

ABSTRACT

Aims: We aimed to investigate the occurrence of the rubella virus among pregnant women, including those who had a history of abortions in the Red Sea region of Sudan.

Study design: Cross-sectional study.

Place and Duration of Study: The study was conducted at the Port Sudan referral tertiary hospital.

Methodology: Blood samples were collected at random from 100 participants who were pregnant or had a history of abortion and who were referred to the hospital. Side flow chromatography immunoassay was used for detection, and the Elisa technique was used for confirmation.

Results: From a total of 100 participants, rubella virus Immunoglobulin G (IgG) was detected in 20 (20%), and 80 were seronegative (80%). The seronegative Immunoglobulin M (IgM) was 0 (00.0%), with no participant had a recent infection. The prevalence of 11 rubella virus IgG was significantly high ($P = .003$) among the females who had abortions 61 (61%). However, no statistically significant relationship was found with the frequency of IgG of rubella virus and age, gestational age, level of education, or occupation.

Conclusion: The study concluded that the prevalence of rubella infections was higher among pregnant females and who had a history of abortions in the Red Sea region. The detection of human rubella virus IgG previous infection was higher than IgG recent infection.

Keywords: IgM and IgG antibodies, rubella virus, abortion, Red Sea region, Sudan.

1. INTRODUCTION

The *rubella virus* is well known as three-day measles or Grumman measles [1]. The name "rubella" came from Latin, it means "little red." Rubeola is often used in English-speaking countries for rubella, but in the Spanish language, rubeola and rubella are used interchangeably. Rubella spreads when an infected person coughs or sneezes. Also, if a woman is infected with rubella while she is pregnant, she can pass it to her developing baby and cause serious harm. Rubella spreads when an infected person coughs or sneezes. Also, if a woman is infected with rubella while she is pregnant, she can pass it to her developing baby and cause serious harm [1].

Comment [CN2]: German measles

Comment [CN3]: Relevance of this part? Better add the type of virus

Formatted: Font: (Default) Arial, 10 pt, Pattern: Clear

This virus causes a mild disease similar to the flu that often passes unnoticed. Wang *et al.* (2019) found that this infection can end up to three days and is likely to resolve more quickly in children. However, this disease has adverse effects on pregnant women if it is contracted in the first 20 weeks of pregnancy, and it can result in a child being born with congenital rubella syndrome (CRS). This involves several severe chronic diseases, and it causes miscarriage in about 15% of reported cases [2]. Another type of this virus is not congenital. It is acquired through different ways, including the exposure of airborne droplets from the upper respiratory tract.

Formatted: Font: (Default) Arial, 10 pt, Pattern: Clear

Comment [CN4]: ??? please, Give all rubella transmission modes

¹ <https://www.cdc.gov/rubella/about/transmission.html>

There are different ways to detect the virus, such as analyzing urine, stools, and skin. According to Leung *et al.* (2019), this virus has an incubation period of two to three weeks, and it has no carrier state, as the reservoir exists entirely in the active human cases [3]. Intrauterine infection can import the syndrome (CRS) by the virus. This can cause a wide range of deficiencies, including auditory, cerebral, cardiac, ophthalmic, and auditory deficiencies [4]. The other types of deficiencies that can be caused by the virus are anemia, neonatal thrombocytopenia, premature birth, low birth weight, and hepatitis. Infections that occur in the first trimester pose a greater risk for causing defects or organogenesis. Rubella infections during pregnancy are the main reason for the development of a vaccine[5]. The disease in children can last for two days and can be accompanied by symptoms such as posterior cervical lymphadenopathy, low body temperature (less than 38.3 °C or 101°F), and a rash that begins on the face and spreads to the rest of the body [6-8].

Comment [CN5]: Rubella virus Intrauterine infection can cause CRS

The symptoms of the disease can vary according to age; for example, in adults and children, symptoms can be in the form of aching joints, coryza and swollen glands, bleeding problems, and brain infections. The antibodies can persist for more than a year, and precautions should be taken after positive test results [9]. The diagnosis can be confirmed by the characteristic rash and detection of antibodies during or a short time after the illness [10]. Early identification of these women by serologic testing might be used as part of a strategy to prevent perinatal transmission of *rubella viruses*.

The current situation regarding the identification of the virus is gloomy in Sudan because of the lack of comprehensive data on rubella, mumps, and the seroepidemiology of measles (MMR) and a lack of information about circulating genotypes of mumps virus [11]. Pregnant women and their unborn babies are vulnerable to congenital *rubella syndrome* [12]. To the best of our knowledge, no previous study has investigated the prevalence of *rubella* infections among pregnant females and who had a history of abortions in the Red Sea region of Sudan. Thus, the study aimed to investigate the prevalence of *rubella* infections in the Red Sea region of Sudan.

Comment [CN6]: It is possible that data on rubella are available as the country is conducting measles surveillance with WHO support. In measles surveillance, if a suspected case of measles is negative, it is tested for rubella

2. MATERIAL AND METHODS

2.1 Study Design and Participants

This cross-sectional study was conducted at a tertiary referral hospital in Port Sudan City, Sudan. The blood samples of 100 participants were collected at random from pregnant women and women with a history of abortion who had been referred to the hospital. A questionnaire to collect demographic data, personal information, and health and socio-economic data. The ethical committee of Omdurman Islamic University has approved the study. Participants were informed about the purpose of the study, and the samples were collected only from those who agreed to participate. The privacy and confidentiality of participants were ensured.

Comment [CN7]: Why 100 participants?

Comment [CN8]: Explain the sampling strategy before describing the blood samples collection and administration of the questionnaire

2.2 Specimen Collection

After washing hands and putting on gloves, the researcher collected a 3 ml blood sample from each participant through venipuncture. The patient was positioned with an arm extended. An appropriate vein was selected; then, about 3-4 inches above the collection site, a swab with 70% isopropyl alcohol was passed smoothly in a circle over the puncture site, moving in an outward spiral from the zone of penetration. A plain sterile vacutainer was used to collect the blood samples. The blood samples were centrifuged for 15 minutes at 3000 rpm to separate the sera. Then, they were stored at -20 °C until they were needed.

2.3 Immunological Analysis

TORCH IgG/IgM Rapid chromatographic immunoassay (On-site IgG/IgM Combo Rapid Test, CTK Biotech™, USA) was used to detect IgG and IgM antibodies of rubella virus and its differentiation. The ELISA method was used to obtain clinical findings from the reactions of specimens reactive with the TORCH IgG/IgM Rapid test [whole blood plasma/serum]. A control group of five known positive samples and five negative samples was created. Before using this technique, the room temperature was adjusted to a range of 15 °C –30 °C, and then the test, specimen, and buffer were brought in. The manufacturer's directions said that for best results, the assay should be performed within an hour. The test was removed from its sealed package and placed on a clean, level surface. The devices were labeled as belonging to a patient or from the control group for identification. Using a dropper from the test kit, we carefully transferred 1 drop (25 ul) of serum or plasma to the sample well (S) and then added 1 drop of buffer to the sample well (S). For the whole blood specimen using the provided dropper, carefully transfer 2 drops (50 ul) of whole blood to the sample well (S) and then add 1 drop of the buffer to the sample well (S). The sample migrated across the membrane, and the results read within 15 to 20 minutes.

Anti-rubella and anti-CMV IgM and IgG antibodies were assayed by an enzyme-linked immunosorbent assay (ELISA) method using an Abbott kit (AxSYM, Abbott, USA) according to the manufacturer's instructions. Anti-rubella IgM antibody titers greater than 0.6 and anti-rubella IgG antibody titers greater than 10.0 IU/ml were considered positive.

2.4 Statistical Analysis

Statistical analysis was performed using the chi-square test, and $P < .05$ was considered as a significant association.

Comment [CN9]: Which Software did you use?

3. RESULTS

3.1 Prevalence of rubella virus IgG among pregnant women and with the history of abortions

Of the 100 participants, the prevalence of rubella virus IgG was 20 (20%), while 80 were seronegative (80%). There was no evidence of recent infection, based on seronegative IgM 0 (00.0%) (Table 1). The study group was subdivided into four groups based on the subjects' ages: 18-24 years old, 25-30 years old, 31-35 years old, and 36-40 years old. The frequency of rubella virus IgG in the four groups was as follows: For those 18-24 years old, five subjects (5% of the original group of participants) were seropositive with IgG. For those 25-30 years old, 10 subjects (10%) were seropositive. For those 31-35 years old, 4 subjects (4%) were seropositive, and for those 36-40 years old, 1 subject (1%) was seropositive. These results were found to be statically insignificant ($P = .9$) (Table 2).

Table 1. The prevalence of rubella virus IgG among pregnant women and those who had a history of abortions.

RV	Frequency	Percentage (%)
IgM	0	0%
IgG	20	20%
Negative	80	80%
Total	100	100%

Table 2. Frequency of IgG of rubella virus by age.

Age		RV			P-value
		IgG Positive	IgG Negative	Total	
18-24	Count	5	16	21	.9
	%	5%	16%	21%	
25-30	Count	10	44	55	
	%	10%	44%	55%	
31-35	Count	4	15	19	
	%	4%	15%	19%	
36-40	Count	1	5	6	
	%	1%	5%	6%	
Total	Count	20	80	100	
	%	100.0%	100.0%	100.0%	

Formatted Table

3.2 Frequency of IgG of rubella virus to gestational age

The prevalence of *rubella virus* based on the stage of pregnancy also was found not to be statistically significant: 4 seropositive subjects (4%) were in the first trimester, 4 (4%) were in the second trimester, and 12 (12%) were in the third trimester. The prevalence of *rubella virus* based on gestational age was not statistically significant ($P = .8$) (Table 3).

Table 3. Frequency of IgG of rubella virus to gestational age.

Gestational age		RV			P-value
		IgG	Negative	Total	
1st trimester	Count	4	21	25	.8
	%	4%	21%	25%	
2nd trimester	Count	4	15	19	
	%	4%	15%	19%	
3rd trimester	Count	12	44	56	
	%	12%	44%	56%	
Total	Count	20	80	100	
	%	100.0%	100.0%	100%	

3.3 Rubella virus IgG according to abortion history

The frequency of *rubella virus* IgG among the females who had a history of abortions and non-aborted pregnant women was 11 (11%). The results were found to be significant for the group of women with a history of abortion 61 (61%) $P=0.003$ (Table 4). Moreover, there was no statistically significant relationship between the frequency of *rubella virus* IgG and education level ($P = .5$) or occupation ($P = .2$).

Table 4. Frequency of rubella virus IgG according to abortion history.

Abortion		RV			P-value
		IgG Positive	IgG Negative	Total	
Yes	Count	11	61	61,72	.003
	%	11%	61%	61%	
No	Count	9	19	28	
	%	9%	19%	28%	
Total	Count	20	80	100	
	%	100.0%	100.0%	100%	

Comment [CN10]: 72

Formatted: Strikethrough

Formatted: Strikethrough

Formatted: Font: 10 pt, Not Strikethrough

Formatted: Strikethrough

4. DISCUSSION

There is a high morbidity and mortality rate caused by *rubella*, especially to fetuses of mothers with congenital or neonatal infections. The result is a wide range of medical abnormalities that also create social and financial burdens to families and

countries. This has made the screening of pregnant women an important research activity. The present study found that 20 pregnant women and women with a history of abortion (20% of the participants) were positive for antibody IgG. These results agreed with those of Elamin and Khidir (2017), who found that rubella IgM and IgG antibodies were high among pregnant women in the City of Khartoum, Sudan. They also reported that 5 participants (around 5.4% of their subjects) were reactive to rubella IgM, in contrast to 48 participants (51.6%) who were reactive to rubella IgG antibodies [12]. Other researchers found rubella IgG antibodies in 35% of pregnant women in Khartoum [13]. Moreover, the seroprevalence of measles, rubella antibodies, and mumps affected 97% of healthcare workers, 93.5%, 90.8%, and 94.1%, respectively, among adult volunteers. In children, the percentages ranged from 99.2% to 63.6% [11]. In a meta-analysis of 28 studies in Sub-Saharan countries, the pooled seroprevalence of anti-rubella IgG among pregnant women was found to be about 89.0% (95% CI: 84.6–92.3%), and for rubella IgG and IgM, the pooled prevalence among pregnant women was 5.1% (95% CI: 2.6–9.9) [14].

Comment [CN11]: The only problem here is that you did not compare your results to a study where they found rubella associated with abortion.

In the present study, no anti rubella IgM was detected. This agrees with results obtained by Wafa et al., who reported IgM seronegative in their study in Khartoum [15]. In contrast, of 231 pregnant women in a study in the Western region of Sudan, 51 (65.3%) were positive for rubella IgG, and only 8 women (3.4%) were rubella IgM positive [16]. However, a study from India revealed that 39.2% of pregnant women in a sample were seropositive for rubella IgG antibodies, whereas around 20.86% of non-pregnant women were tested positive. That study also showed that of the 76 seropositive women, only 4 (5.26%) revealed IgM antibodies. This indicates that the results of both the rapid immunochromatographic test (39.20%) and the ELISA (40.61%) were low, and the specificities of the two methods were similar (79.13%) [17]. Furthermore, a study of 184 sera samples from Nigeria included 176 (95.7%) and 22 (12%) who were seropositive for IgM and rubella IgG. Twenty (11%) of those seropositive for IgM were also positive for IgG; only two (1%) were positive only for IgM.

IgG prevalence was highest among those 26–30 years old (98.3%) and IgM was highest for those 31–35 years old (18.8%) [18]. In this study, no statistically significant relationship was found between the frequency of rubella IgG and age group, gestational age, level of education, or occupation. However, Akele et al. (2020) concluded the prevalence rate for rubella IgG was highest among those 26–30 years old (98.3%) and 31–35 years (18.8%) for IgM [19]. These variations may be due to differences in sample size, study duration, or the techniques used to detect the virus.

4. CONCLUSION

In conclusion, this study found that the prevalence of the rubella virus was high among pregnant females who had a history of abortions in the Red Sea region of Sudan. The detection of human rubella virus IgG from previous infections was higher than IgM for recent infections. The prevalence of the rubella virus compared to age, level of education, and occupation was not statistically significant. However, the presence of rubella virus IgG in Sudan should be given careful attention. It is recommended that antibodies be detected among the listed tests for pre-married girls and those receiving prenatal care. This study increased public awareness and knowledge about the rubella virus, as it provided universal infant immunization to decrease the circulation of the virus.

Comment [CN12]: No case with IgM positive so the comparison seems to be not relevant. No case of recent infection was found

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study followed the ethical guidelines of the World Medical Association (WMA) Declaration of Helsinki, and the ethical committee of Omdurman Islamic University Sudan approved the study. The study does not include any human or animal experiments.

PATIENT CONSENT FOR PUBLICATION

Not applicable

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE.

ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

REFERENCES

1. Emmanuel Rudatsikira M. Moono Silitongo B. A post immunization evaluation of a measles-rubella campaign in the northern province of Zambia. *Int J Child Adolescent Health*, 2019; 12(3): 315-319.
2. Wang H, Yuan D, Wang S, Luo L, Zhang Y, Ye J, et al. Cytogenetic and genetic investigation of miscarriage cases in Eastern China. *J Matern Fetal Neonatal Med*, 2020; 33(20): 3385-3390.
3. Leung AKC, Hon KL, Leong KF. Rubella (German measles) revisited. *Hong Kong Med J*, 2019; 25(2): 134-141.
4. Ko H, Dehority W, Maxwell JR. The Impact of Maternal Infection on the Neonate. *Congenital Anomalies in Neonates-Clinical Perspectives*, 2020.
5. Herini ES, Triono A, Mulyadi AWE, Mardin N, Soenarto Y, Reef SE. Hospital-based surveillance of congenital rubella syndrome in Indonesia. *Europ J Pediatr*, 2017; 176(3): 387-393.
6. Gans H, Maldonado YA, Post T. Measles: Clinical manifestations, diagnosis, treatment, and prevention. *UpToDate*. Waltham, MA.(Accessed July 3, 2019), 2019. <https://www.uptodate.com/contents/measles-clinical-manifestations-diagnosis-treatment-and-prevention>
7. Best J, Cooray S, Banatvala J. Rubella in Topley and Wilson's Microbiology and Microbial Infections, Vol. 2. *Virology*, 2005: 960-92.
8. Staples JE, Dziuban EJ, Fischer M, Cragan JD, Rasmussen SA, Cannon MJ, et al. Interim Guidelines for the Evaluation and Testing of Infants with Possible Congenital Zika Virus Infection - United States, 2016. *MMWR Morb Mortal Wkly Rep*, 2016; 65(3): 63-7. **Formatted: French (France)**
9. Hübschen J, Bork S, Brown K, Mankertz A, Santibanez S, Mamou MB, et al. Challenges of measles and rubella laboratory diagnostic in the era of elimination. *Clin Microbiol Infect*, 2017; 23(8): 511-515.
10. Lundberg IE, Miller FW, Tjärnlund A, Bottai M. Diagnosis and classification of idiopathic inflammatory myopathies. *J Int Med*, 2016; 280(1): 39-51.
11. Adam O, Musa A, Kamer A, Sausy A, Tisserand E, Hübschen JMJIJoID. Seroprevalence of measles, mumps, and rubella and genetic characterization of mumps virus in Khartoum, Sudan. 2020; 91: 87-93.
12. Elamin BK, Khidir NBjJoML. Diagnosis. Seroprevalence of Rubella virus among pregnant Sudanese women with history of abortion. 2017; 8(2): 8-11. **Formatted: French (France)**
13. Adam O, Makkawi T, Kannan A, Osman ME. Seroprevalence of rubella among pregnant women in Khartoum state, Sudan. *East Mediterr Health J*, 2013; 19(9): 812-5.
14. Kassa ZY, Hussen S, Asnake SJHV. Immunotherapeutics. Sero-prevalence of rubella among pregnant women in Sub-Saharan Africa: a meta-analysis. 2020: 1-7.
15. ABDALLA WM, ABDALLA MA, SULIMAN SE, KHAFI SK. Serological Detection of Rubella Virus among Pregnant Women in Khartoum State. **Formatted: French (France)**
16. Hamdan HZ, Abdelbagi IE, Nasser NM, Adam IJVj. Seroprevalence of cytomegalovirus and rubella among pregnant women in western Sudan. 2011; 8(1): 217.
17. Shahapur PR, Kandi VJC. Seroprevalence of Rubella Virus-specific Antibodies in Women and the Diagnostic Efficacy of Enzyme-linked Immunoassay and Rapid Immunochromatographic Tests. 2020; 12(3).
18. Akele RY, Pam BN, Oluboyo BO, Enitan SS, Akinseye JF, Adewumi FAJAJol. Seroprevalence of Rubella IgG and IgM Antibodies among Pregnant Women Attending Antenatal Clinic in Federal Teaching Hospital Ido-Ekiti, Nigeria. 2020: 27-35.
19. Akele RY, Abelekum JT, Oluboyo BO, Akinseye JF, Enitan SS, Olayanju OA, et al. Prevalence of human parvovirus B19 IgG and IgM antibodies among pregnant women attending antenatal clinic at Federal Teaching Hospital Ido-Ekiti, Nigeria. *Afr J Infect Dis*, 2021; 15(2): 10-15.