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Molecular Detection of Rubella Virus among Asymptomatic Pregnant Women In Khartoum State

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Abstract
Background: *Rubella or 'German measles' is transmitted by droplets, direct contact or vertically from pregnant woman to the fetus. The virus is worldwide distributed and of public health importance due to its teratogenic effects. Most infections are asymptomatic and there is no routine screening for rubella among pregnant women. Unfortunately there is no vaccine program in vaccination schedule in Sudan.*
Aim*: This study conducted aimed to detect rubella virus infection by Reverse Transcriptase-PCR among asymptomatic pregnant women.*
Materials and Methods:
One hundred and eighteen (118) blood samples were collected from pregnant women in Khartoum State, plasma were separated and stored till use. RNA was extracted using column based kit and then was converted into cDNA using RT kit. PCR was used to amplify cDNA and gel electrophoresis was done to detect PCR product comparing to DNA ladder. Data were analyzed using SPSS 16.0 by Chi-squire test.
Results: *Out of 118 plasma samples, 4 (3.4%) specimens were positive by RT-PCR.*
Keywords: Rubella virus, PCR, pregnant women, Khartoum State.

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I. Introduction

Rubella virus remains an important public health problem due to the teratogenic effects and risk of miscarriage and stillbirth that may result from congenital infection, particularly when the mother becomes infected during the first trimester of pregnancy(1). Infections during pregnancy, especially before week 12 of gestation, can cause severe birth defects known as congenital rubella syndrome (CRS). Clinical signs of CRS include cataract, glaucoma, heart disease, loss of hearing, brain dysfunction, and pigmentary retinopathy (2). The encumbrance of CRS in developing countries is undervalued and few reports documenting the incidence of CRS are available. In 2009, only 165 CRS cases were reported worldwide with the majority being from the World Health Organization (WHO) African and Eastern Mediterranean regions (3).

Rubella infection may present as an acute, mild or asymptomatic illness; therefore the outbreaks may occur without clinical recognition or may be misdiagnosed as measles cases (4, 5).

A prenatal diagnosis of fetal infection could be proposed. Although progress has been made, the prenatal diagnosis of rubella is not always easy. The incidence of rubella has significantly decreased in many countries because of vaccination campaigns; however, rubella has not disappeared in developed countries and is a significant source of disability (6).

The only reliable evidence of acute rubella infection is a positive viral culture for rubella or detection of rubella virus by polymerase chain reaction (PCR), the presence of rubella-specific IgM antibody, or demonstration of a significant rise in IgG antibody from paired acute- and convalescent-phase sera (7).

Despite the availability of an effective vaccine for rubella since the 1960s, the virus is still a global health concern with over 100,000 babies born with congenital rubella syndrome every year (8).

Rubella vaccination is not yet included in the immunization schedule for Sudan (9). So we conducted this study to explore the burden of rubella on Sudan.

II. Materials And Methods

Study design

It was descriptive cross sectional study.

Study area

This study was conducted as a hospital-based study in different hospitals of different geographical locations in Khartoum State.

Study population

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

Sample size

The sample size was 118.

Sampling technique

This study was based on non-probability convenience sampling technique. Samples were taken from attended agreed women.

III. Methods

Method of data collection

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

Ethical consideration

Permission to conduct the study was taken from research committee of College of Medical Laboratory Science; Sudan University of Science and Technology and then from research committee of Ministry of health. A written informed consent was obtained from each participant.

Specimen collection

The blood specimens were collected from vein (vein puncture) by sterile syringes. Aliquots of 3 ml blood were collected into EDTA containers. Then the samples were centrifuged at 3000 rpm for 5 minutes and plasma were collected in sterile cryogenic vial containers and were stored at -70º C till time of analysis.

RNA Extraction

Extraction of RNAwas done by RNA extraction kit (analytic Jena) following the manufacturer's instructions**.**

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Extracted RNA was converted into cDNA by reverse transcriptase enzyme by RT kit (Intron Biotechnology, Korea). In which 10µl of RNA was added to 10µl of D.D.W and cDNA synthesis reactions was performed by incubation of the reaction mixture for 1 hour at 45°C, followed by 5 min at 95°C using PCR machine (Applied Biosystems).

After cDNA was synthesized; it was amplified by PCR machine (Applied Biosystems, Roche Diagnostic Systems) by using ready premix (Intron Biotechnology, Korea). The primers were used to amplify the entire E1 gene region of 1,446 nucleotides (nt) (nt 8258 to 9703), which includes the 739 nt (8731 to 9469) corresponding to the minimum acceptable window defined by WHO for routine molecular epidemiology (10). The primers design as follow: E1.1n (forward primer) **5**´CTAGCTACGTCCAGCACCCT**3**` (8691–8710 position) and E1.2Ra (reverse primer)**3**´ACTGGTAGCACCCGGTCACA**5**` (9292–9311position)(11).

The reaction conditions were: 95°C for 3 min; 35 cycles of 95°C for 30seconds, 57°C for 30seconds, and 72°C for 45seconds; and finally 72°C for 5 min, followed by 4°C for 10 min.

Gel electrophoresis

Amplicons of RT-PCR was detected by gel electrophoresis. Agarose gel powder (1.5 g) was weighted by sensitive balanced and was dissolved in 100 ml1x TBE buffer, then was dissolved by microwave for 2 minutes ; after cooling ethidium bromide was added and then poured in a gel tank contains comb which was removed after polymerization of gel. In first lane marker (100 bp) was added, negative control and the samples. Then the gel was submersed by 1x TBE buffer and run for 45 minutes at voltage 75. The gel was visualized by trans-illuminator. The length of band was 621 bp.

Statistical analysis

Data were computed and analyzed by SPSS software program version 16.0. Significance of differences was determined using Chi-square test and statistical significance was set at p-value < 0.05. Data were presented in form of tables.

IV. Results

A total of 118 pregnant women were enrolled in the study, most of them were in age groups 20 to 30 years and all of them are non-vaccinated.

Out of 118, 4 (3.4%) were positive by RT-PCR and 114 (96.6) were negative (table-1) and there was association between family members and detection of rubella virus by RT-PCR (table-2). there was no association between trimester, gravidity and presence of rubella virus (table-3 and 4).

Table -1: Frequency of rubella virus by RT-PCR among pregnant women

PCR Results	Frequency	%
Positive	4	3.4
Negative	114	96.6
Total	118	100

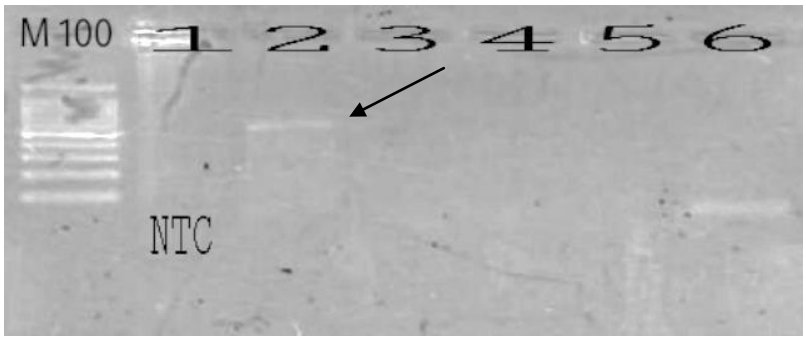

Figure (1): PCR product for E1 gene of rubella virus on 1.5% agarose gel.

M= DNA marker 100bp NTC= negative test control (lane 1)
Lanes 2,3,4,5 and 6 are tested samples Lane 2= positive with band length 621 bp

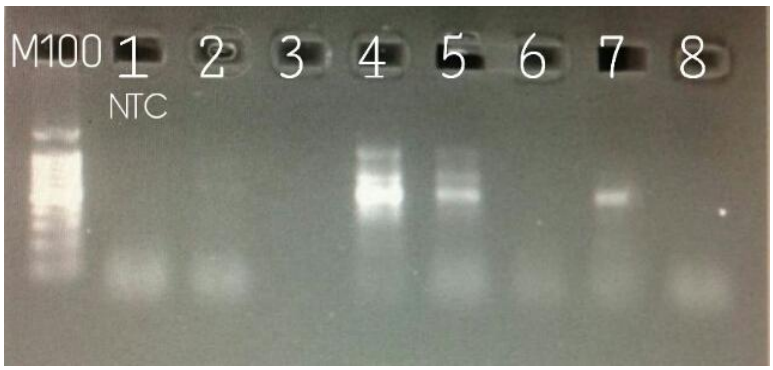

Figure (2): PCR product for E1 gene of rubella virus on 1.5% agarose gel.

M= DNA marker 100bp lane 1=NTC (negative test control)
Lanes 2,3,6, and 8 are negative samples Lanes4,5 and 7 are positive samples with band length 621 bp

Table -2: Association between family members and RT-PCR results

PCR results		Family members		Total	*P-value*
		2-5 members	>5 members		
	Positive	0	4	4	
	Negative	66	48	114	0.022
Total		66	52	118	

Table –3: Association between age of gestation and RT-PCR results

PCR results		Trimester			Total	*P-value*
		First trimester	Second trimester	Third trimester		
	Positive	0	2	2	4	
	Negative	19	48	46	113	0.669
Total		19	50	48	17	

Table – 4: Association between number of gestation and RT-PCR results

PCR results		Gravidity		Total	*P-value*
		Primagravida	Multigravida		
	Positive	2	2	4	
	Negative	28	86	114	0.251
Total		30	88	118	

V. Discussion

The currant study showed that 4(3.4%) of 118 apparently healthy pregnant women were positive by RT-PCR, that means the prevalence of rubella is low and there is silent or in-apparent disease which is not routinely detected. So the disease can be transmitted via infected droplets to others without any clinically obvious symptoms that gives chance to spread the infection easily and may lead to miscarriage, still birth or CRS. Our finding is inconsistent with result obtained by Zanga *et al.*, (2017) in Democratic Republic of Congo (12), in which viral genome was detected in 60% of pregnant women. We anticipate these variations in results to; the type of specimens '' throat swab is better than plasma'' and apparently healthy pregnant, while Zanga *et al.*, (2017) follow up pregnant women concerning clinical signs or complications. This study found there is an association between family members and presence of viral genome by RT-PCR (*p-value* = 0.022) in which larger family is more crowded than small size family, as infection acquired through droplets (13).Our study also showed that neither, gravidity, nor trimester was significantly associated with presence of rubella genome(*p-value* =0.251 and 0.669 respectively). This is similar to results achieved by Zanga *et al.*, (2017) (12).

VI. Conclusion

Rubella virus genome is detected in few apparently health pregnant women and there is association between infection and family members but no association with trimester and gravidity.

Competing Interests

The authors declare that they have no competing interest**.**

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