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-square test.

Results: Out of 118 plasma samples, 4 (3.4%) specimens were positive by RT-PCR.

Keywords: Rubella virus, PCR, pregnant women, Khartoum State.

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 pigmented 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 RNA was 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'CTAGCTACGTCCAGCACCCT3' (8691–8710 position) and E1.2Ra (reverse primer) 3'ACTGGTAGCACCCGGTCACAS5' (9292–9311 position)(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.
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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

<table>
<thead>
<tr>
<th>PCR Results</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>4</td>
<td>3.4</td>
</tr>
<tr>
<td>Negative</td>
<td>114</td>
<td>96.6</td>
</tr>
<tr>
<td>Total</td>
<td>118</td>
<td>100</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>PCR results</th>
<th>Family members</th>
<th>Total</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-5 members</td>
<td>&gt;5 members</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>66</td>
<td>48</td>
<td>114</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>52</td>
<td>118</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>PCR results</th>
<th>Trimester</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First trimester</td>
<td>Second trimester</td>
<td>Third trimester</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Negative</td>
<td>19</td>
<td>48</td>
<td>46</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>50</td>
<td>48</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>PCR results</th>
<th>Gravidity</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primagravida</td>
<td>Multigravida</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>28</td>
<td>86</td>
<td>114</td>
</tr>
<tr>
<td>total</td>
<td>30</td>
<td>88</td>
<td>118</td>
</tr>
</tbody>
</table>

V. Discussion

The current study showed that 4(3.4%) of 118 apparently healthy pregnant women were positive by RT-PCR, which 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 specimen “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 healthy 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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References


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