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Original Research Article

Immunological and Molecular Detection of Rubella Virus in Iraqi Pregnant Women with Unknown Cause of Abortions

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Abstract: ***Backgrounds*:** Rubella (German measles) is a common mild illness signs' by fever, mild intoxication, rash, swelling, and soreness of the lymph nodes. It affects children and teenagers worldwide and can also affect young adults. Rubella virus infects pregnant women, and can be transmitted to the fetus and causing birth defects or Congenital Rubella Syndrome (CRS). The study designed to scrutinize the role of the Rubella virus in spontaneous abortion by comparing Immunological and molecular tests used in diagnosing the virus in Iraqi aborted women. ***Methods*:** A total of sixty women were enrolled from two Baghdad maternity hospitals from December 2023 to February 2024. Thirty serum samples were gathered from spontaneously miscarriage women, and thirty from healthy as a control group. Serological tests, Chemi-Luminescence (TORCH) and Enzyme Immunoassay were performed for diagnosis of Rubella virus infection followed by molecular detection by Reverse Transcriptase-PCR. ***Results*:** Revealed that out of 30 specimens, 26 tested positive for rubella virus. Both Enzyme Immunoassay and Chemi-Luminescence assay detected anti-Rubella virus IgG, but the Immunoassay test showed superiority over the Chemi-Luminescence assay by 54%, and IgM anti-Rubella virus was slightly high by only 3%. ***Conclusions*:** Molecular detection was more reliable diagnostic method of rubella virus.

Keywords: Rubella Virus, Abortion, RT-PCR, TORCH, Immunoassay.

INTRODUCTION

Rubella Virus (RV) infection, is usually minor but can have significant effects especially for pregnant women and their unborn children [1-2]. Rubella virus is a member of Togaviridae family, Rubivirus genus [3]. It has a spherical shape, sized 40–80 nm, and single-stranded, positive-sense RNA genome encased in a lipoprotein envelope with surface projections that resemble spikes and contain hemagglutinin [4]. Postnatal transmission of rubella virus occurs by direct contact with the respiratory secretions of infected individuals, making humans the sole known reservoir of the virus. Despite the vulnerability of immunocompromised individuals, the primary victims are children and young adults [5]. Rubella virus (RV) infections frequently manifest as a transient fever, arthritis, lymphadenopathy, and a maculopapular rash. Studies have revealed that 20 to 50% of infections are asymptomatic. But, serious sequences might result from maternal infection during the first trimester of pregnancy, Considering a 50% chance of fetal harm if the infection progresses in the third month of pregnancy and a 90% chance if it happens in the first two [6-9]. Ocular abnormalities (retinopathy and glaucoma), deafness, and mental retardation linked to microcephaly or encephalitis are among the serious concerns of in utero infection [10, 11]. Congenital rubella syndrome remains a significant cause of birth defects in countries with low vaccination coverage. Public health campaigns focus on vaccinating children and ensuring rubella immunity among women of reproductive age to decrease congenital rubella incidence [12, 13]. Diagnosis of rubella is typically made by serologic tests (finding of IgM). The presence of IgM antibodies indicates recent rubella infection, while presence of IgG in healthy individuals approve immunity

to rubella [14]. Viral isolation or serologic tests are used to diagnose congenital rubella in neonates. The afflicted infant has antibodies in circulation, such as actively generated neonatal IgM antibody and transplacental acquired maternal IgG antibody. During first six months of life, maternal IgG antibody can be start in the newborn and wanes. Thus, a congenital rubella might be diagnosed by the presence of IgM antibody or persistence of IgG antibody for more than six months [15]. Recognizing the most practical and efficient strategies will raise the diagnostic precision, depressing the risk of rubella transmission, and improving the health of mothers and newborns [16-18]. Therefore, the current study shows that anti-rubella virus antibodies are estimated as immunological indications for diagnosing viral infection by Enzyme immunological with Chemi-Luminescence (TORCH) and confirmation by Reverse transcriptase PCR (RT-PCR).

MATERIALS AND METHODS

The study performed during December 2023 to February 2024. Sixty blood serum were collected from Baghdad maternity hospitals and divided into two groups, thirty blood serum were from aborted women, and thirty healthy women as a control group. The age of samples ranged between (17-40) years. Serological tests (IgM and IgG) were accomplished using Enzygnost Anti-Rubella Virus kit (Siemens Healthcare Diagnostics, Germany) according to the manufacturer's instruction. The levels of anti-Rubella IgG and IgM were expressed as IU/mL and COI, respectively, anti-Rubella IgG antibody values < 10.0 IU/mL are regarded as negative, and anti-Rubella IgM antibody values ≥ 10.0 IU/mL are observed as positive; while anti-Rubella IgM antibody values < 0 and 8 COI are regarded as negative, and values ≥ 1.0 are observed as positive. Molecular recognition of rubella virus was performed by reverse transcriptase PCR (RT-PCR) using Rubella Real-TM Qual kit (Sacace/ Italy) according to the manufacturer's instruction. The components of PCR mix for gene amplification are illustrated in Table 1.

Table 1: The PCR reaction components

Components	Volume (µl)
Taq PCR PreMix	4
Forward primer	8 picomols/ µl (1 µl)
Reverse primer	8 picomols/ µl (1 µl)
Distilled water	5
Total	**25**

Table 2: The PCR cycling conditions

Phase	Temperature (°C)	Time	No. of cycle
Initial Denaturation	94°C	5 min.	1
Denaturation	94°C	45 sec.	45
Annealing	55.3°C	45 sec.	
Final Extension	72°C	5 min.	1

Table 3: Primer sequences used for Rubella virus detection

Target Gene	Sequence (5′ → 3′)	Primer Name
E1 gene for Rubella virus	AGGACTGTGGACATGGTGGT	Rubella-F
E1 gene for Rubella virus	CTCCTGACCTTGAGGTTGGA	Rubella-R

Statistical Analysis

The statistical program SPSS was used to do an analysis of variance (ANOVA) on the data. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

In this study, sixty serum samples were examined for rubella virus by Enzyme Immunoassay (IgM & IgG) in aborted and healthy women. The result revealed that nineteen specimens were positive for anti-Rubella virus IgG (63.3%), two samples were positive for IgM (6.6%), and nine samples were Rubella virus negative (30.0%). However, all thirty control were negative (Table 1).

Table 1: Detection of Rubella virus by Enzyme Immunoassay test

Enzyme Immunoassay test	*No.*	*Percentage %*
IgG	19	63.3%
IgM	2	6.6%
Rubella virus (-Ve)	9	30.0 %
Total	30	100%
* (P<0.05), ** (P<0.01).		

Negative: -Ve

Chemi-Luminescence assay showed that five samples were positive for Rubella virus IgG (16.6%), one samples was positive for IgM (3.3%), and twenty four samples were negative for Rubella virus (80.0%). But, all control were negative (Table 2).

Table 2: Rubella virus detection by Chemi-Luminescence assay

Chemi-Luminescence (TORCH)	***No. Percentage %***	
IgG	5	16.6%
IgM	1	3.3%
Rubella virus (-Ve)	24	80 %
* (P<0.05), ** (P<0.01).		

Negative: -Ve

When comparing Enzyme Immunoassay and Chemi-Luminescence results, 63.3% of participants appeared IgG positive when tested by Enzyme Immunoassay, while only 16.6% of total samples were IgG positive by Chemi-Luminescence. In addition, detection of IgM by Enzyme Immunoassay test was 6.6% of tested samples in contrast to only 3.3% by Chemi-Luminescence assay (Table 3).

Despite the differing test types, it seems that both tests showed a remarkably high rubella virus frequency. This reveals the significance of rubella in abortion and the need for more precautions to understand its pathophysiology and method of transmission.

Table 3: Comparing detection of Rubella virus by Enzyme Immunoassay and Chemi-Luminescence assay

Category	***Chemi-Luminescence assay***		***Enzyme Immunoassay test***	
	No.	***Percentage (%)***	***No.***	***Percentage (%)***
IgG Rubella virus	5	16.6%	19	63.3%
IgM Rubella virus	1	3.3%	2	6.6%
* (P<0.05), ** (P<0.01).				

Single abortions were more common (53%), whereas four abortions had the lowest described rate 7%. The current study showed that infection rate was highest (37%) during the first 2 months of pregnancy in contrast to the third and fourth gestation age 20 and 13% respectively (Table 4).

Table 4: Distribution of aborted women according age group and abortion number

Variables	***Category***	***Positive of Rubella virus***
Age groups	<20	7 (23.3%)
	20-30	18 (60.0%)
	30-40	5 (16.6%)
Abortion number	Once	16 (53.3%)
	Twice	8 (26.6%)
	Thrice	4 (13.3%)
	Four times	2 (6.6%)
Gestation age	First two month	11 (36.6%)
	Third months	6 (20.0%)
	Forth months	4 (13.3%)
	Neg. Rubella virus	9 (30.0%)
*** (P<0.05), ** (P<0.01).**		

Total No. =30

Molecular detection results of rubella virus revealed that 80% of pregnant women tested positive for the virus. (Figure 1)

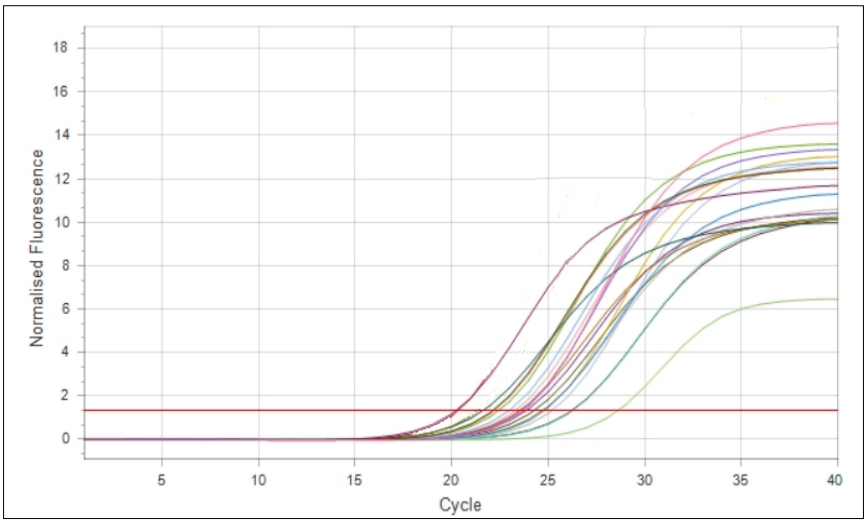

Figure 1: RT-PCR rubella virus detection curve

DISCUSSION

These results were in line with a South African study that found an upper ratio of primary Rubella virus infection through pregnancy [19]. Another study establish that pregnant women in Makkah had a significant frequency of chronic rubella infections [20]. Furthermore, a meta-analysis indicates that a high percentage of reproductive age females are prone to rubella [21]. Additionally, Olajide *et al*., revealed that both pregnant and non-pregnant women had higher rubella virus prevalence [22-24]. It was discovered that the participants' ages ranged from 15 to 40. Abortion was less common in the 31–40 age group, while the majority of instances occurred in the 20–30 age range.

These results were in line with those of Yiska *et al.,* who demonstrated a significant rate of abortions among people aged 27 to 30 [25]. Additionally, Ahmed and Kareem showed that women's abortion rates decreased as they grew older, with the age range falling between 15 and 30 [26]. However, a different group study found that women older than 33 had a considerable risk of miscarriage [27]. Also, a contrasting result conducted in Burkina Faso showed that pregnant women of 40–42years age range had a higher viral exposure rate [28]. Development of Rubella infection maybe depends upon gestational age. These results were in line with research display that the risk of vertical transmission to the baby is significantly increased when rubella occurs during the first trimester of pregnancy [29]. Furthermore, it was calculated that the fetus has a 40–60% probability of suffering several rubella-associated abnormalities if the mother contracted the infection during the first two months of pregnancy. During the third and fourth months of pregnancy, the risk decreases to 30 to 35 percent and 10 percentage, respectively. Immature host defenses during the first trimester of pregnancy may be cause of this difference in the chance and severity of fetal infection connected with gestational age [30]. Molecular detection results of rubella virus revealed that 80% of pregnant women tested positive for the virus. These results were in line with those of prior researches, which reported 63–69% of pregnant women who experience abortions had this disorder [31]. However, Zhanga *et al.,* found that 60% of pregnant women who had miscarriage had a viral genome [32].

CONCLUSION

In contrast to molecular techniques like RT-PCR, the present study shows a striking correlation between the rubella virus and abortion in Iraq. Immunological methods for diagnosing the virus are thought to be crucial, but they are not always perfect in detecting these viruses. Women who tested positive for antibodies against the rubella virus were at a higher risk of infecting their unborn children with the virus.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Conflict of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Contributions of the Authors

The study's idea and design, material preparation, data collecting and analysis, and paper drafting were all done by the authors. The completed manuscript was read and approved by all authors.

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