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Immunological detection of Rubella virus among aborted women in Diyala province, Iraq

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ABSTRACT

German measles is a public illness that can cause congenital rubella syndrome (CRS) and abortion, among other devastating consequences for expectant mothers. The sickness is brought on by the rubella virus. The goal of this study was to determine the prevalence of rubella virus antibodies among aborted women in Diyala province/ Iraq. In the current study, 120 samples were taken from three groups; pregnant women with previous abortion, non-pregnant women with previous abortion, and healthy pregnant women without abortion as a control group. Each group consisted of 40 women. (ELISA) technique was used to measure rubella virus IgG and IgM antibodies in serum samples obtained from the Al-Batool Hospital laboratory. A questionnaire was used to collect data on clinical and demographic characteristics. The results indicated that the seropositivity of RV-IgM among pregnant women with abortion and healthy pregnant women was 0%, but the seropositivity of RV-IgM among non-pregnant women with prior abortion was 2.5%. The RV-IgG levels were 80% among pregnant women who have had prior abortions, 75% in non-pregnant women with prior abortion, and 82.5% in healthy pregnant women without abortion. The study found that women with a history of miscarriage had high levels of rubella IgG antibodies, indicating that they had previously been exposed to the rubella virus or vaccination state. The low incidence of IgM antibodies suggests a limited number of recent infections. These findings highlight the necessity of rubella immunization and surveillance in women of reproductive age to avoid rubella-related problems during pregnancy.

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1. INTRODUCTION

Rubella is an extremely infectious disease often regarded as a mild rash ailment. However, it remains a significant worldwide public health concern because it can lead to miscarriage or fetal abnormalities in the early stages of pregnancy [1]. The disease was described by two German physicians, De Bergen in 1752 and Orlow in 1758, leading to the moniker "German measles" [2]. Scottish researcher Henry Veale first used the name "rubella" in 1866 [2]. The term "rubella" comes from the Latin word "rubellus," which means "reddish" or "little red". The rubella virus is an enveloped, positive-sense; single-stranded RNA virus with a single serotype and it belongs to the Matonaviridae family and is categorized under the genus Rubivirus genus [3]. Rubella virus, which is spread through the respiratory system, is only known to exist in humans [4]. Once the virus infects the respiratory tract, it spreads through the bloodstream to other locations, such as the skin, where it causes a rash [5]. Although rubella usually heals in most cases, infection during pregnancy can result in stillbirth, fetal death, or congenital rubella syndrome (CRS), which is marked by severe birth abnormalities [6]. During the maternal viral phase, the fetus becomes transplacentally infected, leading to a range of abnormalities depending on the stage of pregnancy, such as the triad of cataracts, congenital heart problems, and sensorineural deafness [4]. Antibodies are often detected 14-18 days after rubella virus infection, about the time the maculopapular rash develops and immunoglobulin M (IgM) and G (IgG) titres rise immediately; IgG remains elevated, however IgM antibody titres decline and are generally undetectable by 8 weeks following infection [7].

A rubella-specific T-cell response develops one week following the humoral response, and cell-mediated immunity appears to last throughout life [7]. However, sporadic re-infections have been documented. Rubella IgG antibodies of ≥10 IU/mL are thought to be protective, although no formal correlation has been established. Assay sensitivity and cut-off values vary [8].

Maternal immunity, whether acquired spontaneously or through immunization, protects against intrauterine rubella infection [9]. After infecting the placenta in a susceptible gravid patient, the virus may cross it and proceed into the developing fetus's vascular system [10, 11]. Necrosis in the chorionic villi epithelium, direct viral damage of infected cells through apoptosis, viral inhibition of mitosis and restricted precursor cell development, and cytopathic damage to blood vessel endothelial cells with consequent ischemia in developing organs is some of the possible causes of fetal damage [10].

The maternal rubella infection during pregnancy does not always result in vertical transmission of the virus to the fetus [12]. The timing of the mother's illness affects the risk of infection in the fetus; the rate of fetal infection is around 80% during the first trimester, and 25% during the late second trimester [13]. It's crucial to remember that even when a baby is infected, fetal malformations don't always result [13]. The estimated probability of fetal deformity is around 90%, 33%, 11%, 24%, and 0% when the maternal infection is before 11 weeks, between 11 and 12 weeks, between 13 and 14 weeks, between 15 and 16 weeks, and after 20 weeks of gestation, respectively [9]. This can be explained by the gradual development of fetal humoral and cell-mediated immune responses as well as the passive transfer of maternal antibodies [10].

Rubella has been eradicated in the US, but it is still widespread in many other nations, accounting for 100,000 cases of CRS births every year worldwide [7, 14]. By incorporating this live-attenuated rubella vaccine into national immunization programs, the virus has been eradicated from the US as well as many other nations around the world [5]. Despite this success, a significant number of individuals remain unvaccinated globally, resulting in between 30,000 and 50,000 CRS cases predicted for 2020 [15]. CRS and rubella eradication are the goals of the World Health Organization [6]. Rubella-containing vaccine (RCV), which is widely used, is both safe and effective, with a single dose being approximately 97% effective in preventing rubella [16].

2. METHODS

2.1. Study Configuration and Design

This cross-sectional study included 120 women, divided into three groups: 40 pregnant women with a history of previous abortion, 40 non-pregnant women with a history of miscarriage, and 40 healthy pregnant women with no such history. These subjects attended Al-Batool Teaching Hospital in Diyala from November 2023 to March 2024.

2.2. Sample collection

Each women provided her consent to a document outlining the details of the study, either in writing or orally. A well-prepared questionnaire was used to collect data such as age, residency, education, past abortions, and immunization status through direct interviews. All patients, and healthy women in this study, had (3 mL) of venous blood drawn by venipuncture. This blood was collected in a gel tube (without anticoagulant) and kept at room temperature until it clotted. After centrifuging at 2500 rpm for 10 minutes, the serum was separated and kept at -20°C in a deep freezer for later use of the samples.

2.3. Serological diagnosis of Rubella virus

A commercial qualitative enzyme-linked immunosorbent kit (NovaLisa®, Germany) was used to assess serum levels of rubella IgG and IgM. The manufacturer's instructions were followed exactly. The positive threshold value for IgG was ≥15 IU/mL, while for IgM, it was >11 NTU. ELISA reader was used to detect optical density at 450 nm, and the results were read using a microwell reader and compared in parallel with controls.

2.4. Statistical Analysis:

The frequency of antibodies against Rubella infection was statistically evaluated in both the patient and control groups. The findings were analyzed using SPSS version 29. Standard deviations and means were used to report quantitative data, whereas percentages and numbers were used to indicate qualitative values. P-values were deemed statistically significant if they were less than 0.05.

3. RESULTS AND DISCUSSION

3.1. Rubella IgG

Table 1 and Figure (1), showed the results of the Enzyme-linked immunoassays applied for all study groups. Regarding the anti-Rubella IgG, the results found that 32 (80%) of the pregnant women with abortion were positive versus 8(20%) were negative. In the non-pregnant group, 30 (75%) were positive versus 10 (25%) were negative. In contrast, 33 (82.5%) of the healthy pregnant women were positive and 7 (17.5%) were negative. As a result, (P= 0.702) indicates that there was no statistically significant difference between the study groups.

Table 1. Distribution of serological markers among study groups

Serological Detection		Pregnant with abortion	Non-pregnant with abortion	Healthy pregnant women	P value
		No. (%)	No. (%)	No. (%)	
Rubella IgG	Positive	32 (80.0)	30 (75.0)	33 (82.5)	0.702
	Negative	8 (20.0)	10 (25.0)	7 (17.5)	

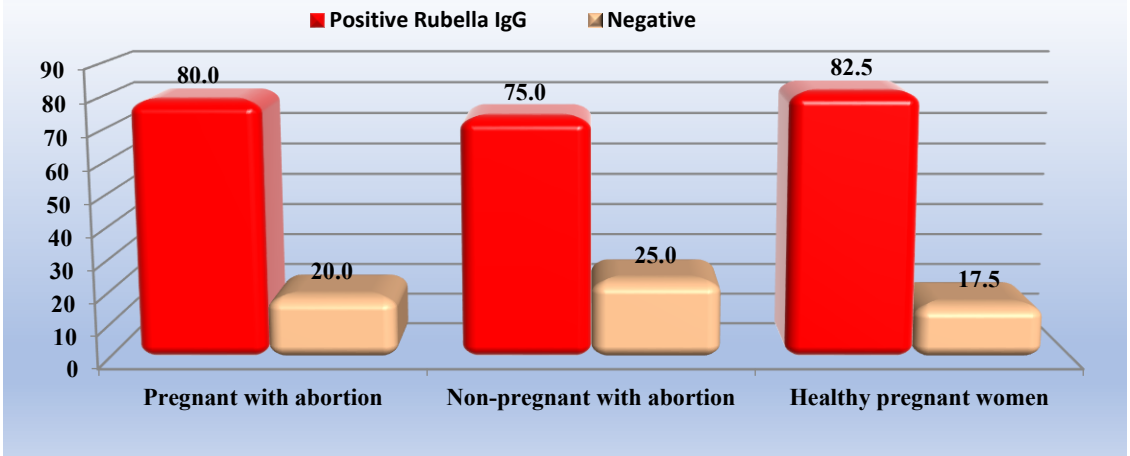

Figure 1. Diagram of Rubella IgG positivity among study groups.

3.2. Rubella virus association with age categories:

Since the Rubella vaccine was introduced in the Iraqi expanded program immunization (EPI), thus all women should be positive for anti-Rubella IgG. The results in Table 2 showed that the 8 anti-Rubella IgG negative pregnant women were distributed in all age categories with the greatest percentage (42.9%) falling in the ≥ 35 years. There was no statistically significant difference (P= 0.518). On the other hand, the 10 anti-rubella IgG-negative non-pregnant women were also distributed over the age categories with the highest rate being in the 30-34 years with a statistically insignificant difference (P= 0.258). Furthermore, in the healthy pregnant women, there were 7 women negative for anti-Rubella IgG aged 25 years and upward. The statistical significance threshold was not met by the difference (P=0.069).

Table 2. Association of anti-Rubella IgG with age categories in study groups.

Age categories (ys)	Anti-Rubella IgG					
	Pregnant with abortion		Non-pregnant with abortion		Healthy pregnant women	
	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)
< 20 years	2 (100)	0 (00)	2 (100)	0 (00)	3 (100)	0 (00)
20---24	8 (80.0)	2 (20.0)	3 (75.0)	1 (25.0)	7 (100)	0 (00)
25---29	11 (84.6)	2 (15.4)	11 (78.6)	3 (21.4)	11 (91.7)	1 (8.3)
30---34	7 (87.5)	1 (12.5)	5 (50.0)	5 (50.0)	8 (80.0)	2 (20.0)
≥ 35years	4 (57.1)	3 (42.9)	9 (90.0)	1 (10.0)	4 (50.0)	4 (50.0)
P value	0.518		0.258		0.069	

3.3. Rubella virus association with education and residence:

The results found that in pregnant women, the highest anti-Rubella positivity rate (100%) was among those with a primary level of education, while the highest negativity rate (31.8%) was among those possessing a secondary education. There was no statistically significant difference (P= 0.090). In the non-pregnant women, the highest positivity rate (90.9%) was among those with university levels of education, while the highest negativity rate (37.5%) was among those with primary levels of education, the variation was not statistically significant (P= 0.318). Additionally, in healthy pregnant women, the highest positivity rate (96%) was among those with a secondary level of education versus the high negativity rate among those with a primary educational level. A statistically significant difference was detected (P= 0.011).

Concerning the residence, anti-Rubella IgG positive rates in rural versus urban residency in all three study groups were close to each other; for instance, in pregnant women, it was 86.7% versus 76 % and in non-pregnant women, it was 70.6% versus 78.3% and in healthy pregnant women it was 78.9% versus 85.7% and of course, all were statistically non-significant (P= 0.414), (P= 0.580) and (P= 0.574) respectively. All data are displayed in Table 3.

Table 3. Rubella virus association with education and residence of study groups

Variables	Anti-Rubella IgG					
	Pregnant with abortion		Non-pregnant with abortion		Healthy pregnant women	
	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)
			Education			
Primary	11 (100)	0 (00)	5 (62.5)	3 (37.5)	3 (50)	3 (50)
Secondary	15 (68.2)	7 (31.8)	15 (71.4)	6 (28.6)	24 (96.0)	1 (4.0)
College	6 (85.7)	1 (14.3)	10 (90.9)	1 (9.1)	6 (66.7)	3 (33.3)
P value	0.090		0.318		0.011*	
			Residence			
Rural	13 (86.7)	2 (13.3)	12 (70.6)	5 (29.4)	15 (78.9)	4 (21.1)
Urban	19 (76.0)	6 (24.0)	18 (78.3)	5 (21.7)	18 (85.7)	3 (14.3)
P value	0.414		0.580		0.574	

3.4. Anti-Rubella IgG association with Rubella vaccine:

Results in Table 4 found that in pregnant women who were positive anti-Rubella IgG levels, (78.4%) were vaccinated with the Rubella vaccine versus 100%) were non-vaccinated. The difference was statistically insignificant (P=0.368). In the non-pregnant group, (83.9%) anti-Rubella IgG women were vaccinated which was significantly higher than (44.4%) of women who were non-vaccinated (P=0.016). In the healthy pregnant group, 88.6% of positive women were vaccinated versus 40% who were non-vaccinated, with a significantly higher difference (P=0.008).

Table 4. Anti-Rubella IgG association with Rubella vaccine.

Rubella vaccine	Anti-Rubella IgG					
	Pregnant with abortion		Non-pregnant with abortion		Healthy pregnant women	
	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)
Yes	29 (78.4)	8 (21.6)	26 (83.9)	5 (16.1)	31 (88.6)	4 (11.4)
No	3 (100)	0 (00)	4 (44.4)	5 (55.6)	2 (40.0)	3 (60.0)
P value	0.368		0.016*		0.008*	

3.5. Rubella IgM

Regarding the anti-Rubella IgM, all 40 (100%) pregnant women were negative, while in the non-pregnant women, only one (2.5%) was positive, was in the 20-24 age group versus 39(97.5%) were negative. Likewise, all healthy pregnant women 40 (100%) were negative for anti-Rubella IgM. The research groups' differences were not statistically significant (P= 0.365). Table 5 and Figure 2, showed the results.

Table 5. Distribution of serological markers among study groups.

Serological Detection		Pregnant with abortion	Non-pregnant with abortion	Healthy pregnant women	P value
		No. (%)	No. (%)	No. (%)	
Rubella IgM	Positive	0 (00)	1 (2.5)	0 (00)	0.365
	Negative	40 (100)	39 (97.5)	40 (100)	

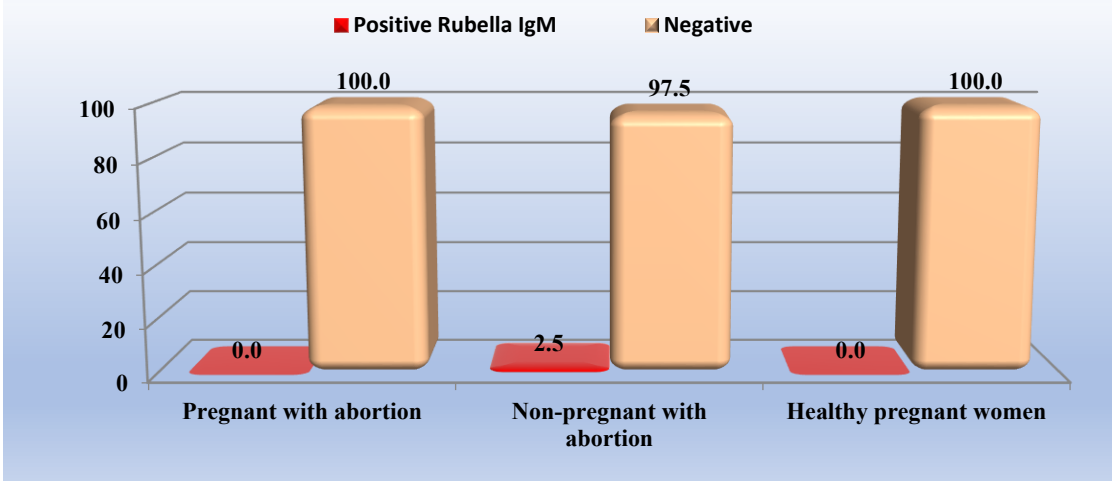

Figure 2. Diagram of Rubella IgM positivity among study groups.

The low detection rate was due to the low infection rate by Rubella virus during pregnancy of Iraqi women [17]. In Iraq, routinely, two doses of MMR vaccines are recommended for the prevention of these diseases; usually between the ages of one and six years old. Because this vaccine contains live attenuated virus, it was recommended to avoid giving it to pregnant women [18]. It is worthy to know that the efficacy of rubella vaccine among Iraqi children to be 94% [19]. Therefore our results are consistent with those reported by other Iraqi researchers [20, 21].

Rubella eradication and congenital rubella syndrome (CRS) prevention are becoming more important public health issues across the world. Both require rubella screening among pregnant women and rubella immunization for the entire population [22]. Therefore, many countries have conducted serosurveys among pregnant women to assess the prevalence of Rubella virus (RV) infections. Characterizing infection in a particular location requires an understanding of the immunoglobulin profiles of IgM and IgG [23]. The presence of IgG seropositivity suggests previous exposure to the rubella virus, indicating acquired protective immunity while Seropositivity to IgM indicates recent or acute with the rubella virus [24].

The current study examined pregnant women's levels of immunity against rubella virus infection as well as acute/recent infections by analyzing both rubella-specific IgM and IgG antibodies. However, the overall seroprevalence was 0.8% for IgM antibodies and 79.2% for IgG antibodies against rubella. These results are close to other previous studies, for example; several studies were conducted in Diyala province. This prevalence of Anti-Rubella antibodies (IgM and IgG) between individuals undergoing premarriage testing. The result showed that all males and females were negative for anti-rubella IgM, but 97.7% of males and 91.4% of females were positive for anti-rubella IgG recommended anti-rubella IgG screening of girls and vaccination of those with negative results to minimize the rate of Rubella infection during pregnancy and child-bearing period [25]. Another local study examined the seroprevalence among pregnant women who had not previous abortions, as well as the ones who had. The seroprevalence rate was found to be 96.1% among women without previous abortion and 76% among women with previous abortion. The general proportion of susceptible to infection of rubella was 10.7%. The study found that age, level of education, residence, as well as presence of previous abortion, had no significant impact on the levels of Anti-Rubella IgG [26].

Furthermore, the current results are primarily concordant with studies conducted in other provinces; for instance, in Thi-Qar province 81% of aborted women were positive for anti-Rubella IgG, meaning that 19% were susceptible to Rubella infection [20]. These findings were also consistent with earlier research conducted in Zakho Region [27].

However, these results are higher than results recorded in other regions for example, a study carried out in Hilla City, revealed that 58% of pregnant women during the first month of pregnancy lack immunity to infection of Rubella (antirubella IgG negative), which makes them susceptible to Rubella infection and consequently CRS, the majority of them were reside in rural areas [21].

Similarly, a study in Iran reported that 75.3% of miscarriage-affected women had positive rubella IgG antibodies, with 1.2% testing positive for IgM [28]. Another study in Sudan found that 72% of women were seropositive for rubella IgG antibodies, while 3.4% tested positive for IgM [29]. Nevertheless, the rate of seropositivity for IgG antibodies to rubella virus among pregnant women in this study was lower compared to several previous studies conducted in other countries, including Turkey [30, 31], Italy [32], and Australia [33]. Variations in rubella IgG antibody seropositivity among different countries may stem from differences in rubella virus endemicity and the incorporation of the rubella vaccine into national immunization initiatives [27]. Variations in the populations being investigated may also have an impact on these differences, differences in the reference ranges of diagnostic tests, and the laboratory techniques employed [27]. According to World Health Organization (WHO) guidelines, it is crucial to implement supplementary immunization activities and regularly monitor progress at the national level [7].

To prevent rubella transmission and lower the risk of CRS, selective vaccination of adolescent girls and women of reproductive age who are vulnerable to rubella, as well as vaccination in the postpartum period, should be implemented [34]. The preferred approach for introducing RCV into national immunization programs is to launch a vaccination campaign that targets the vast majority of people who may not have been exposed to rubella naturally, typically children and teenagers under the age of 14 [7]. This approach has been used to eradicate CRS and rubella in the region of the Americas (AMR) [35]. According to WHO guidelines, nations implementing RCV should aim for and sustain a minimum coverage of 80% with at least one dose administered through regular services or campaigns [7]. The foundations for eradicating rubella and congenital rubella syndrome (CRS) are high immunization rates and surveillance systems [36].

4. CONCLUSION

Finally, our findings shed light on the incidence of Rubella virus infections in women with a history of miscarriage in the Diyala region, Iraq. The recruited patients tested positive for high anti-Rubella IgG and low IgM. Screening women who have had a miscarriage or stillbirth may help to determine the cause. Further research with bigger sample numbers is needed to evaluate the link between these infections and pregnancy outcomes.

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