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Serological and Molecular Detection of *Rubella Virus* In clinical sample of pregnant women and its impact in miscarriage

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ABSTRACT

Rubella virus infection is one of the common viral conditions during pregnancy that may lead to miscarriage, 100 blood samples were collected from a woman with miscarriage and recurrent miscarriage. The patient's age ranged between 15-44 years. ELISA technology was used for serological test which is the primary analysis for rubella. qRT-PCR technique used to determine the type of infection-causing strain due to its high accuracy and short time period in determining the type of strain. The results showed that strains (1E) were more prevalent in rubella patients than strains (2B), which were less prevalent, while strains (1D) didn't recorded. The results obtained from the serological confirmed miscarriages, of which 48 were distributed over the age groups 15-44 years. As for the search by gene expression, the total number of cases was 87, and the number of miscarriages was 48, distributed between two strains: the first (1E) was 69, and the second (2B) was 18. The third (1D). The strain did not. The difference between the two methods was very slight, amounting to 0.22, where the results were (11.48) in the PCR technique, while it was in the ELISA machine (11.26). For the second table, we found that the number of infections varies with age. And that the age group most exposed to infection was between (24-29) years in the polymerase chain reaction assay, and the first strain (1E) represented (20) positive cases, and the second strain (2B) represented (9) cases. Of the total number (29) of the total number of injuries (93) for all age groups.

1. INTRODUCTION

The first clinical diagnosis of German measles was authored by a German chemist in the mid-eighteenth century, or in 1740, and scientists have later confirmed it. Rollick, which means in German, was coined by three German scholars [1- 5]. The London Smith International Congress of Medicine, JL, officially recognized it in 1881. Based on the work with monkeys, Alfred Fabian Hess has assumed that rubella was caused by a virus in 1914, and Hero and Osaka has confirmed this in 1938 by killing children with filtered a nasal wash from acute cases. The German measles epidemic has spread out in Australia in 1940 [4], [5]. The

idea (German measles parties) was promoted by some popular magazines, and it was intended to spread the disease to other children (especially girls) in order to immunize them for a life and protect them from contracting the disease later in pregnancy [9]. Between 1962 and 1965, the German measles pandemic spread across Europe, soon reach the United States [6]. The United States had an estimated 12.5 million cases of rubella in 1964 and 1965. There would have been 11,000 miscarriages or surgical abortions and 20,000 cases of congenital rubella syndrome as a result of this [10]. Two research groups isolated rubella virus in 1962 based on this report [11], [12]. The fetus was not fully developed and had major birth defects at birth. The condition is known as congenital rubella syndrome (CRS), and sepsis and diabetes are symptoms of rubella that develop later in pregnancy [13], [14]. Rubella is a major global disease that causes severe diarrhea. It is known as rubella (rubella, little red measles, or measles) but is highly contagious [15]. Humans are the only reservoir of the virus [16]. The incubation period of the virus is 2-3 weeks. In the postpartum and placental pathway during pregnancy, transmission occurs by air [17]. Up to 50% of rubella cases are early onset [18], if not in pregnant women, the infection Rubella is relatively harmless and in most scenarios the infection is mild and bodily [19]. RV has a slow replication rate, which is reflected in the long latent duration of the virus from 8 to 12 hours [20], [21].

Four distinct types of viral RNA can be detected during RV infection. There is a 40S RV single-stranded genomic RNA [22] and a 24S sub-genomic RNA (1.2103 kDa) that corresponds to a 3′-third genomic RNA. Polyadenylated tail at tip 3. The 40S RV genomic RNA serves as a messenger for non-structural proteins (ns) and as a template for the synthesis of the 40S polar negative RNA strand during viral replication. The deficient strand in turn acts as a guide for both 40S and 24S RNA transcripts [23]. Nucleocapsids are formed when the nascent 40S RNA is packaged with the RV capsid protein. In terms of viral distribution, RV is unable to infect any cell at any given minute, regardless of virus titer [22- 25]. Furthermore, the proportion of cells infected with RV at any given time depends on the cell type [23], [24], [82- 97]. Rubella is an acute infectious disease caused by the German measles virus. It is usually mild. Rubella is transmitted to children and young adults mostly through the respiratory tract. The infection may be subclinical or cause a self-limiting illness with symptoms including low-grade fever, lymphadenopathy, and rash E. Martinez Quintana, 2015. Rubella may also be transmitted to unborn children by infected pregnant mothers. Miscarriage, stillbirth, miscarriage, congenital rubella syndrome (CRS), or asymptomatic infection in a baby are all possible outcomes of a congenital rubella infection (CRI). Symptoms of CRS include heart, brain, ocular, and auditory defects [26]. Depending on the gestational age of the fetus at the time of infection, the risk of developing a birth defect ranges from 10% to 90%. Rubella infection occurs early in pregnancy, especially during the first 12 weeks, which increases the risk of more serious outcomes. This study aimed to determine the frequents of strain that more common and it is related with miscarriage and recurrent miscarriage, serological test.

2. Materials and methods

A group of pregnant women who underwent abortion and samples were examined. Total number of samples collected from 100 samples, depending on the following criteria: miscarriage, recumbent miscarriage, serological test.

2.1 Sample collection

A blood sample was collected from pregnant women who underwent miscarriage by drawing (3-5 ml) of each woman and the tubes were divided into two parts: Eppendorf tube for gene expression plain tube for serological test. for 30 min before centrifugation. The Centrifuge plain tube at 5000 rpm for 5 minutes, “Serum It was collected and kept in the freezer until use. the plain tube was centrifuge for at 5000 rpm for 5 minutes.

2.2 Serological assay

The technique detects the total IgM -RUV EIA to measure quantitatively the level of IgM and depend on a Sandwich ELISA in microplates.

2.2.1 Total RNA Extraction kit

Virus DNA/RNA Extraction kit used for DNA extraction from the serums.

According to the manufacturer's instruction. total RNA was conversely translated to cDNA utilizing AccuPower RT PreMix Kit. The system was completed in a response volume of 20 ul. Three main step were applied to conversion by thermo cycler (step 1: 42C for 60 min, step 2: 94C for 5 min, and 4Cfor 5min:one step).1E,2B,1D genes were detection by real–time polymerase chain reaction (RT-PCR) analytic jena. To ensure that the test genes is expressed, GoTaq *qPCR Master Mix(SYBR) was used. Primes design and preparation were created using the Primer 3 web version (online at website http: primer 3. ut,ee) for qRT-PCR process.

2.3 Measurement of RNA concentration and purity

using a Niño drop apparatus device (AppendorfBio photometer plus). It is based on measuring the optical density of a RNA sample (2 µl) at two wavelengths (260 and 280 nm). In most of the samples, RNA preparation gave an A260/A280 ratio between 1.7 and 2.0, which was considered suitable for further analysis in viral gene identification.

2.3.1 Gene Expression

The reaction program was on the first gene 1E: 95°C for 5 min/1 cycle, Intervention: 95°C for 40 secs, annealing: 57°C for 40 secs, Extension: 72°C for 40 sec F: 5-, done. Carry out the scale using 35 cycles and then press 4°C for 1 cycle. 1E sequence sets F: 5′-TCGTGCAATGTCACCACTGA3′, R: 5′-CTGGTAACCCCGTGACAC-3′ while 2B and 1D as the same reaction program: 95 °C for 5 min/1 cycle, Interference: 95 °C for 40 s, annealing: 58 °C for 40 seconds, extension: 72°C, for 40 seconds, F: 5-, run, do 35 revolutions, then continue at 4°C for 1 turn. The 2B primers were F: 5′GCGACCTGGTGAGTACAT3′, R: 5′- AAGATGGCAGTTCCCTCCTC-3′ and 1D gene F: 5′-CATTTGCGAGATCCCCCCCCACTG3′, R: 5′- GGTAGGGTGTGTAG′T,

2.4 Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to statistical analysis 1. The statistical analysis system was (38) Used to determine the effect of different factors in Study parameters. Test T. Pressure between means. Value P for all tests It was considered statistically important if P <0.05 2. Expressive levels Calculated according to Livak and Schmittgen [28] Equation The genetic for the breeds showed The results were derived in identifying the causative breeds (P<0.01) significant compare between percentage (0.05 and 0.01 probability) in this study.

3. Results and Discussion

Serological and genetic expression tests were conducted on samples taken from pregnant women between the ages of 15-44 years, some of whom had miscarried, and the results obtained through the research were that most of the age groups exposed to miscarriage are limited to (24-40) years, and this It applies to both methods.

3.1 Serological study

In the serological examination, the total number of tests used in the research was (186), and the total number

of abortions was 50, distributed among all age groups, which were the most in the aforementioned category as in (Table 1). As shown in the table through the relationship between injury and the number of abortions and the results in both methods.

Table (1): Finding a relationship between the level of infection with Rubella of different types with the number of miscarriage

Age (year)	**Dedication by ELISA test to Patients group No. & %**						**Dedication by RT-PC Strains of RUV**				
	Total	**miscarriage**	**Rubella IgG**	**IgM**	**Sub total**	**Total**	**miscarriage**	**1E strains**	**2B strains**	**1D strains**	**Sub total**
15 – 19	18	6	9	9	24	8	8	7	1	0	**16**
20 – 24	48	7	24	24	55	26	7	24	3	0	**34**
25 – 29	60	13	30	30	73	30	13	20	6	0	**39**
30 – 34	26	11	13	13	36	13	11	8	3	0	**22**
35 – 39	20	5	10	10	25	10	5	8	1	0	**14**
40 - 44	14	6	7	7	20	7	6	4	3	0	**13**
Total	**186**	**48**	**93**	**93**	**233**	**94**	**50**	**71**	**17**	**0**	**138**
Chi-χ^2	**17.03 ******	**7.81 ******	**11.26 ******	**11.26 ******	**18.34 ******	**10.75 ******	**7.84 ******	**11.48 ******	**4.79 ***	**NS**	**14.37 ******
*** (P≤0.05), ** (P≤0.01), NS: Non-Significant.**											

3.2 moliculor study

As for the examination by the method of gene expression by RT-PCR, the results were close to the results of the serological examination, as the total tests performed on the samples were distributed between two strains (1E, 2B) except strain (1D). figure (1,2,3).

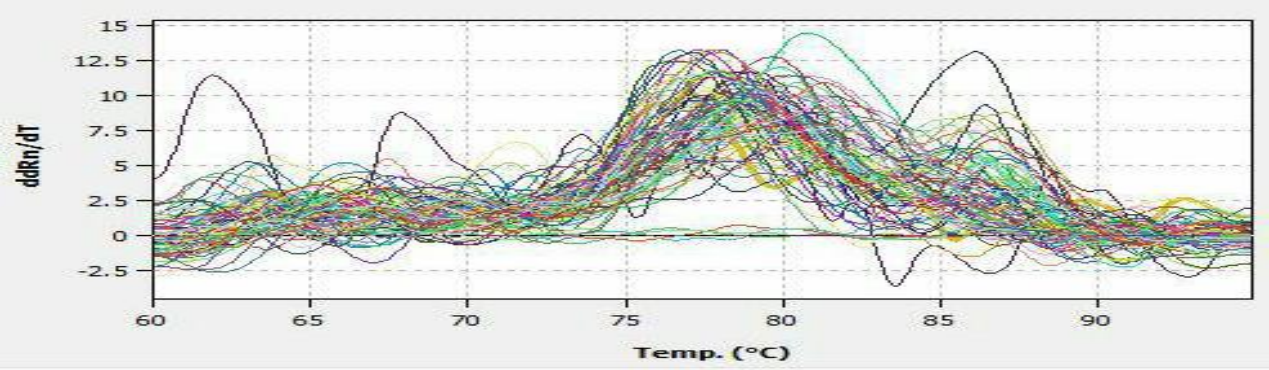

Figure (1) :1E gene amplification plots by qPCR. Ct values ranged from 16.81 to 35.15. this plot for samples (1-30) another plots in appendix (32). The photograph was taken directly from Rotor-Gene Software Version 2.1.0.9, Threshold (0.210), gain Green: 9.33

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Figure (2): 1E gene dissociation curves by qPCR Samples included all study groups. Melting temperature ranged from 83°C to 87°C, No primer dimer could be seen. The photograph was taken directly from Rotor-Gene qPCR machine Rotor-Gene Software Version 2.1.0.9

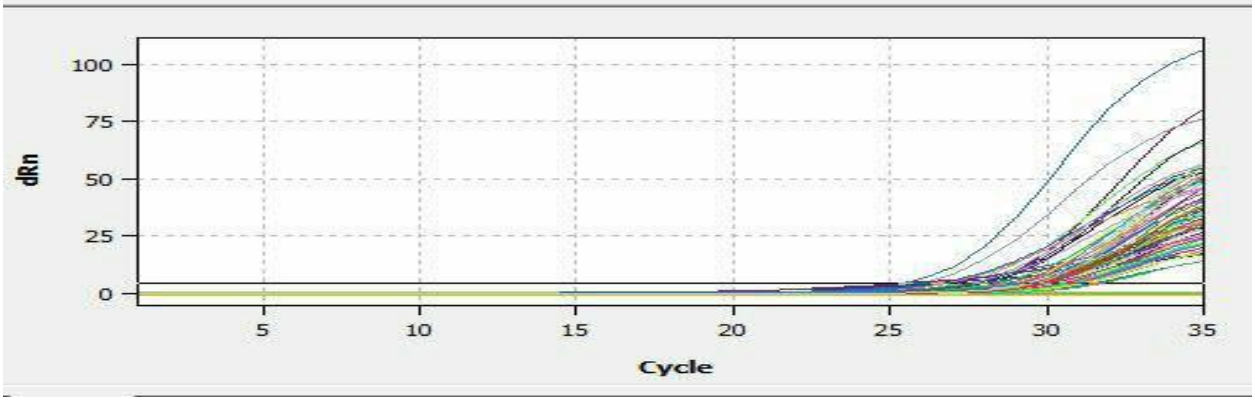

Figure (3): 2B gene dissociation curves by qPCR Samples included all study groups. Melting temperature ranged from 83°C to 87°C, No primer dimer could be seen. The photograph was taken directly from Rotor-Gene qPCR machine Rotor-Gene Software Version 2.1.0.9

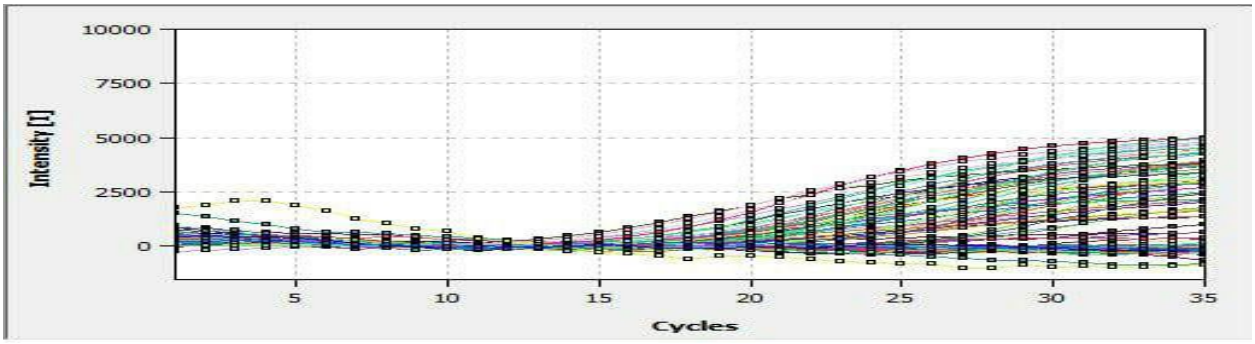

Figure (4): 1Dgene dissociation curves by qPCR Samples included all study groups. Melting temperature ranged from 83°C to 87°C, No primer dimer could be seen. The photograph was taken directly from Rotor-Gene qPCR machine Rotor-Gene Software Version 2.1.0

In the table (1). Type (1E) more than others, so that the total number of tests reached (38), the percentage of 1E strain 69, and the strain (2B) was (39), and the total number of miscarriage was 48 cases, where the first cause of abortion was from strain (1E). By 32 cases, and (2B) 16 cases. As shown in the table

Table (2) Finding a relationship between age and infection rate in general

Age (year)	Dedication by ELISA test to Patients group No. & %				Dedication by RT-PCR to Strains of RUV. & %				
	Total	Rubella IgM	IgG	Sub total	Total	strains (1E)	strains (2B)	strains (1D)	Sub total
15 – 19	18	9	9	18	9	8	1	0	9
20 – 24	48	24	24	49	23	21	2	0	23
25–29	60	30	30	60	29	20	9	0	29
30 – 34	30	15	15	30	15	12	3	0	15
35 – 39	18	9	9	18	9	8	1	0	9
40 - 44	18	9	9	18	8	6	2	0	8
Total	192	86	86	192	93	75	18	0	93
Chi-χ^2	18.39 **	13.58 **	13.08 **	18.39 **	8.91 **	8.63 **	4.59 **	NS	8.91 **
* (P≤0.05), ** (P≤0.01). NS: Non-Significant.									

In the second table in which the most affected age group or its relation to infection was determined, when the rubella serological examination was most susceptible to infection, it was confined between 24 - 29 years, where the total infection for all ages was 192. The mentioned group represents 60 of the total number distributed Between IgM and IgG, each has 30 positive cases. As for the examination by polymerase chain reaction, the total number of infections reached 93, and the aforementioned age group constituted 29 of the total number. It was distributed among two strains out of the three on which the research was conducted. The first strain represented 1E and the second 2B, while the third that did not appear to us The results of any infection with it, the distribution of cases of infection was, where the first strain constituted 20 cases and the second 9 cases out of the total, as shown in the second table. In this study, we can discuss the results in both ways in terms of accuracy and speed. Through this table, it became clear to us during the research that working with the gene expression method is more accurate than the serological method. ELISA testing in miscarriage patients is high (84.00%), but molecular technology such as PCR is sensitive and highly specific compared to other diagnostic methods such as serological assays, by its speed, characteristics and accuracy of results [40]. In Hefei, China, 16.29% of the measles IgM antibody test result was reported as positive. Yashodara and colleagues in Hyderabad reported that 11 cases (12.5%) tested positive for rubella IgM antibody. Mathur et al reported that 13.8% were positive for IgM antibodies to rubella [41]. At Azad Medical College, New Delhi, the seropositivity for rubella IgM antibody was 8.3% with a previous normal delivery [42]. Several studies in India and other countries have shown that the seroprevalence of rubella ranges from 4.66% to 28.6% of women of childbearing age. In India, pregnant women are exposed to poor economic and social conditions which lead to a variety of infections due to poor environment and hygiene. This is an important factor in poor pregnancy outcomes [43].

4. Discussion

In this study, 11.26% of women were positive for Rubella IgG and IgM antibodies with poor postpartum history and the results were similar to those performed previously. By comparing ELISA and PCR. Serological tests used to determine the type of virus take a long time (1 to 2 weeks) and this in turn affects immunocompromised patients [44]. Moreover, up to 50% of infections during pregnancy are subclinical, and many of them go unnoticed. As a consequence, the true incidence of deafness associated with rubella (along with other CRS defects) is probably lower than the estimated incidence [45], but also in the second and third trimesters of pregnancy [46]. Seroprevalence of Rubella was determined in 580 women including 80 women of medical community of district Amritsar, by ELISA test. The overall Rubella IgG seropositivity was 68.8% while in women of medical community it was 80%. Maximum number of women were seropositive (77.2%) in age group 26-35 years. Significantly higher rates were observed in women of urban areas and those belonging to lower socioeconomic class. Although the incidence of seropositivity was more in women with history of adverse pregnancy outcome than those with normal obstetric performance, the difference was

statistically not significant ($p > 0.05$). Serologically, immune status showed poor correlation with history of past Rubella virus like infection. Epidemiologists link appearances. New infectious diseases and their spreadFor a high density of people and animals [47- 50]. The proximity between people and interaction is one of the main factors of urbanization; Urbanization. Urban forgives through Density, lack of space [51]. Urbanisation is increasingly problematised as the increasing densities and interconnections are argued to facilitate the rise and proliferation of infectious diseases [52]. but, These public health crises, which mainly affected Cities, also raised awareness that poor hygiene. The conditions of the poor were also of the rich Hence a collective problem [53].

We have noticed during the research that miscarriage may not be the main cause of rubella virus infection. Abortion may be due to a functional or mechanical defect inside the uterus, such as the widening of the vagina, exposure to a specific virus, for example, herpes simplex virus or cytomegalovirus, or infection with a parasite such as Toxo-plasma gondi, which is transmitted to the uterus in some stages of pregnancy and this is what Confirmed [54], the risk of reproductive failure lies in the first trimester of pregnancy when exposed to infection [55]. During pregnancy due to a weakened immune system, the mother's immunity can reduce the transmission of the virus to the fetus and this was confirmed by [56]. In it, it is one of the cellular sites of infection in humans and many others [56]. Infection with the parasite Toxoplasma condae is the primary cause of spontaneous abortion during pregnancy [57] due to its spread within the family. Fetuses of a woman infected during the first trimester of pregnancy with the virus are 30% to 50%. During pregnancy, it is possible for the virus to be transmitted to the baby through the placenta [58]. As for rubella virus infection during the first trimester of pregnancy, the possibility of miscarriage is low compared to confirmed congenital malformations such as deafness and/or vision loss [59]. In this table, we review the results obtained by searching for the extent of the impact of injury on different age groups and who are the most vulnerable to it. Through the table below, we find that the ages most affected by miscarriage are between 25-29 years, compared to the rest of the ages. There may be reasons that are far from the main reason we are looking for. However, it becomes clear to us that age has nothing to do with infection and abortion, as all ages are exposed to infection with the virus and abortion if the appropriate conditions are available for that to happen. Although the rates of infection vary. Perhaps among those reasons is the lack of vaccination due to social ignorance, the presence of genetically transmitted diseases, or due to a disease of the era such as diabetes, for example, which is associated with pregnancy. In 1986, [60] Black and colleagues compared the prevalence of rubella antibodies in pregnant women in 15 cohorts in Brazil, Chile, Ecuador, India, Nigeria, Jordan, South Africa, Taiwan, and the United States. The percentages of women with rubella aged 15-30 years in serum increased with age. This confirms the existence of a high risk of infection during the childbearing years. In other words, the prevalence of rubella antibodies in tropical Africa among women of reproductive age varies with different countries [61]. In The Gambia, Ethiopia, Upper Volta and Uganda they had 93% or more at age 14; This is a possible explanation for the absence of CRS cases in The Gambia, [62].

But in Nigeria, Ghana and Togo, 25-50% of women of childbearing age are unaffected. In a study of three northern Indian cities (Delhi, Chandigarh and Lucknow) and one eastern city (Calcutta) found that antibody to rubella was present in 80% by age. Serological studies in children in the last ten years in Jordan [63], Nigeria [64], [65], Saudi Arabia [66] Libya) 67and Taiwan [67] all showed increased incidence with age. That rubella affects children in many developing countries of all ages and that varying proportions of women did not acquire antibodies when they reached reproductive age, women in the North compared to only 57% of women in the East Unless there are more than 70% reliable vaccination records, measles vaccination may be limited. Restricting vaccination to a specific age group increases the age of infection and thus the incidence of CRS. To avoid this, girls are vaccinated before puberty, as well as women before or after pregnancy, and the vaccination of infants should precede or accompany it; Vaccine so that the goal is to register age groups.

This common policy has now been adopted in both the USA and the UK [68], [69]. The results that appeared to us according to the age groups most exposed to infection (35-39 years) were more prevalent by (60%) of patients, and this is consistent with a study that showed results that the age group 21-30 years was the most prevalent among (67.9%) of patients, and showed Other studies found that (44.5%) of injuries in Iraq were in the age of 21-30 years, (22.7%) of injuries in Kosovo were between 20-25 years of age, and (81.7%) of injuries in China were aged 29.20 years [70- 74]. The age groups ranged from 31-40 years and from 11 to 20 years (16.9%) and (13.07%), respectively, (7.8%) of injuries in Greece were older than 35 years, and in Iraq the study showed that the percentage ranged between 17 and 45 years old [75], [76]. Younger women are at risk of contracting primary toxoplasmosis, CMV. [77] and rubella during pregnancy [78], [79]. Other researchers in the study revealed severe rubella virus infections in Turkey with an average age of (30.7) years in Iraq. [80] Rubella virus [81]. The spread of the disease for age groups between (24-29 years) lies for reasons related to weak immune system or exposure to chronic diseases that make women more.

5. Conclusions

According to the results of the current study, the following concluded: Total IgM antibodies showed a higher frequency in patients with exacerbation of miscarriage and The sensitivity and specificity of the ELISA test was lower than the PCR technique for detecting the rubella virus. During the research, we found that the infection rate of the type 1E strain of rubella is more prevalent than the rest of the strains.

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