

Research Article



## Evaluation of the MicroRNA184 Levels in Women with Miscarriage Association with Toxoplasmosis, Rubella, and CMV Infections

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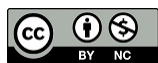
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### ABSTRACT

MiR-184 plays a significant regulatory role in immunity during pregnancy. Its dysregulation in toxoplasmosis and rubella infection is strongly associated with increased risk of abortion. Further clinical studies are needed to validate miR-184 as a diagnostic marker and therapeutic target in pregnancy complications. One hundred specimens of blood were taken from the miscarriage ladies, with age ranges between 15 and 50 years, who were complaining of TORCH infections. The findings showed that the age range group (<20-29 years) was the highest at (59.0%), followed by (30-39 years) at (36.0%), and then (40-46 years) 5 (5.0%). Distribution of infections according to residency showed the patients that living in rural areas were higher 59 (59.0%) than those who lived in urban areas (41.0%), while the number and percentage of acute infections with rubella were higher 29 (29%) than toxoplasmosis (4.0%) in aborted women with TORCH infections. However, 4 (4.0%) of Toxoplasmosis was recorded compared to 0 (0.0%) CMV infection. The evaluation of IgM antibodies showed that Rubella IgM levels were higher at 29 (29.0%) in comparison to CMV IgM levels of 0 (0.0%). The number and percentage of rubella IgM levels among the age group (<20-29) were higher (18.0%) than in other age groups, while it was (9.0%) among the age group (30-39) years and 4 (4.0%) among Toxoplasmosis. Out of 100 patients, gene expression of MicroRNA-184 was done on 33 patients, and the results revealed that 10 (27.2%) of the aborted women were carriers of Toxoplasmosis, CMV, and Rubella IgM, and they had positive MicroRNA-184.



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## 1. Introduction

Abortion is one of the most complicated economic and social issues. Child loss during pregnancy can be related to many factors, including human reproduction. The most common causes of abortion are uterine and genetic abnormalities, immunologic and endocrine dysfunctions, environmental pollution, endometriosis, and psychogenetic factors, as well as infectious factors (El Hachem *et al.* 2017). The abortion rate due to fetal infections by some infectious factors, such as TORCH (Toxoplasma, Rubella, Cytomegalovirus, Herpes Simplex virus) is thought to be 10-15% (Salman 2016).

Toxoplasma gondii is one of the most widely spread parasites throughout the world that leads to abortions. The intracellular protozoan Toxoplasma gondii can infect human beings and other warm-blooded animals (Rouatbi *et al.* 2019). The parasite is transmitted to humans via accidental drinking of water, ingestion of soils or foods contaminated with *T. gondii* oocysts or eating undercooked or raw meat containing *T. gondii* cysts (Marques *et al.* 2020). In immunocompetent adult individuals, the disease is of no clinical significance. The encystment of the parasite (latent toxoplasmosis) follows the immunological response to primary infections, providing lifelong immunity to the patient. Expected latent infection reactivation among a highly immunosuppressed population, however, will make toxoplasmosis an important opportunistic infection.

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In addition, toxoplasmosis has long been recognized as a main cause of perinatal morbidities (Pereira *et al.* 2020). Possible transmission of *T. gondii* infection from pregnant women to their fetuses may lead to blindness, mental retardation, epilepsy, or death (Manuel *et al.* 2020). Human cytomegalovirus is a main cause of congenital infection. The clinical features of CMV range from asymptomatic to severe fetal damage and, in a few cases, death because of abortions (Aljumaili *et al.* 2014). During pregnancy, cytomegalovirus infection has more complexity than other infections because of the viral ability to be frequently reactivated during the period of childbearing and be transmitted to the fetus in spite of maternal immunity (Leruez-Ville *et al.* 2020). In general, rubella infection is an asymptomatic disease of childhood, but during the first trimester of pregnancy, it may lead to serious congenital disabilities [congenital rubella syndrome] or fetal death (CRS) (Bukasa *et al.* 2018). The risk of rubella-related disorders is increased among infants whose mothers are infected with the rubella virus during the first 4 months of pregnancy (Vueba and Sousa 2020; Mohamed *et al.* 2013). Herpes genitals are transmitted sexually, as fears of the spread of herpes during pregnancy are increasing, with possible damaging effects on the fetuses (Mohamed *et al.* 2013).

Our study discusses the epidemiology and pathophysiology of Toxoplasma, Rubella, and CMV, but it does not explain the role of MicroRNA-184 in pregnancy-related miscarriage, leaving the rationale for including MicroRNA-184 in this study unclear. Herpes simplex virus was not excluded of the data; therefore, this section can be revised to focus only on the pathogens examined in the study. To strengthen the introduction, the author can add a brief description of the role of MicroRNA-184 in pregnancy, a clear knowledge gap, and specific objective statements (Mirabedini *et al.* 2025). The present study aimed to conduct a screening study about abortion in the country of Iraq and the major roles played by Toxoplasma, Rubella, and Cytomegalovirus.

## 2. Materials and Methods

In this study, blood samples were collected from (100) aborted women, with age ranges between (<15-50) years, who were complaining of Toxoplasmosis, Rubella virus and Cytomegalovirus infections and attended the medical city (Al-KarKh Hospital Hospital)/Baghdad, Iraq during the period from 15<sup>th</sup> March to 15<sup>th</sup> November

2020. Serum samples were separated by centrifugation and stored in small tubes at -20C until used. An Enzyme-Linked Immunosorbent Assay (ELISA) was used of detect Toxoplasma IgM, and results were recorded as mean optical density (OD) readings. Common kit contents: Coated microplates or test cassettes, IgM controls (positive/negative) Conjugate reagent Wash buffer, and substrate Instructions for use Quantitation of cDNA Quantus Fluorometer was used to detect the concentration of extracted DNA to determine the goodness of samples for downstream applications. For 1 µL of DNA, 199 µL of diluted Quant Flour Dye was mixed. After 5 minutes of incubation at room temperature, DNA concentration was measured using the MiR-RT for Micro-RNA 185 Quantus Fluorometer to assess the quality of the extracted RNA for downstream applications. For 1 µL of RNA, 200 µL of diluted QuantiFlour Dye was mixed. After 5min of incubation at room temperature in the dark place, RNA concentration values were detected. GTTGGCTCTGGTGCAGGGTCCGAGGTATTC GCACC AGAGCCAACACCCTT.

## 3. Results

### 3.1. Distribution of the Study Group According to the Age Group

Table 1 showed that the age range group (<20-29) years was the highest 59 (59.0%), followed by (30-39) years 36 (36.0%), then the age group (40-46) years 5 (5.0%).

The prevalence of infections by residency showed that patients living in rural areas had a higher prevalence (59/59, 59.0%) than those living in urban areas (41/41, 41.0%), as shown in Table 2.

The prevalence of Toxoplasma and Rubella IgM was higher in acute Rubella infection (29/29, 100%) than in acute Toxoplasmosis (4/4, 100%) in abortion cases with TORCH, as shown in Table 3.

There were 4 (4.0%) cases of Toxoplasmosis recorded in aborted women in comparison to 0 (0.0%) CMV infection, as explained in Table 4.

### 3.2. Distribution of the Study Groups According to Rubella and CMV IgM

A highly significant variation was revealed ( $P < 0.01$ ) between Rubella IgM 29 (29.0%) and CMV IgM 0 (0.0%), as observed in (Table 5).

Table 1. Prevalence of infections by age group (year)

Age (year)	No.	%
(<20-29)	59	59.0
(30-39)	36	36.0
(40-46)	5	5.0
Total	100	100.0

Table 2. Prevalence of the study group by residency

Resident	No.	%
Urban	41	41.0
Rural	59	59.0
Total	100	100.0

Table 3. Prevalence of infections according to the Toxoplasma IgM and Rubella IgM

Toxo IgM		Rubella IgM		Total
		Negative	Positive	
-Ve	No.	67	29	96
	%	67%	29%	96%
+Ve	No.	4	0	4
	%	4%	0%	4%
Total	No.	71	29	100
	%	71%	29%	100%

Table 4. Prevalence of infections according to Toxoplasma and CMV IgM

Toxo IgM		CMV IgM		Aggregate
		Negative	Positive	
Negative	Number	96	0	96
	percentage	96%	0.0%	96%
Positive	Number	4	0	4
	percentage	4%	0.0%	4%
Aggregate	Number	100	0	100
	percentage	100%	0.0%	100%

Table 5. Prevalence of the infections according to Rubella and CMV IgM

Rubella IgM		CMV IgM		Total
		-Ve	+Ve	
Negative	Number	71	0	71
	percentage	71%	0%	71%
Positive	Number	29	0	29
	percentage	29%	0%	29%
Aggregate	Number	100	0	100
	percentage	100%	0%	100%

### 3.3. Distribution of the Study Groups According to Age Groups of Toxoplasma IgM, Rubella IgM & CMV IgM

Table 6 showed that the IgM levels of Rubella in the age group (<20-29) years was higher 18 (18.0%) than other age groups, while Toxoplasma IgM was 2(2.0%)

and rubella IgM was 9 (9.0%) in the age group (30-39) years and Toxoplasma IgM was 4 (4.0%).

### 3.4. Distribution of Toxo, Rubella & CMV IgM Levels Among the Study Groups According to Residence

In Table 7, it is shown that the prevalence of Rubella in the rural patients was 18 (18.0%), and toxoplasmosis was 3 (3.0%), while Rubella 11 (11.0%) and Toxoplasmosis was only 1(1.0%) in the urban areas, but no infections with CMV.

Table 8 and Figure 1 showed that out of 100 patients, gene expression of MicroRNA-184 was done in 33 of them; and 10 (27.2%) of aborted women who were carriers of Toxoplasmosis, CMV, and Rubella IgM, and they had positive MicroRNA-184. Statistically, there was a significant association in the prevalence of MicroRNA-184 among the study groups (P value of 0.005).

## 4. Discussion

The risky infections with TORCH may lead to abortion. The studies showing higher prevalence in women with a history of miscarriage, particularly in the 20-29 age group, aligning with findings linking infections to miscarriages in similar age. These results agreed with ( Mohammad and Salman 2014) and (Al-Alami *et al.* 2024) who reported that women between 20 to 30 of pregnant women are more likely to suffer from diverticulitis and miscarriage (Mohammad and Salman 2014). According to the residency, the rural patients had higher rates of TORCH infections with abortion. These findings matched with (Ahmad and Kareem 2020) who reported that the higher number of aborted women infected with TORCH was rural women than urban women (Ahmad and Kareem 2020). Acute infection of Rubella was more than Toxoplasmosis in abortion with TORCH infections, disagreed (Hussein and Balatay 2019), who found that among infections in aborted positive patients diagnosed with Toxoplasma, CMV, or rubella, and no co-infections (with the Toxoplasma, CMV, and Rubella) and positive Rubella was more infection than Toxoplasmosis (Hussein and Balatay 2019). In the evaluation of Rubella in comparison to CMV infection, there was a highly significant difference, as no CMV was recorded compared to the high rates of Rubella. Our result agreed with (Çetinkaya and Yenilmez 2019), who found in their study that there was more rubella than CMV (Abdul Mohymen

Table 6. Prevalence of infections according to age groups for Toxoplasma Rubella and CMV IgM

Age groups (year)	Age groups (year)		Rubella IgM		CMV IgM		Total	
	Negative	Positive	Negative	Positive	Negative	Positive		
(<20-29)	No.	57	2	41	18	59	0	59
	%	57.0%	2.0%	41.0%	18.0%	59.0%	0.0%	59.0%
(30-39)	No.	34	2	27	9	36	0	36
	%	34.0%	2.0%	27.0%	9.0%	36.0%	0.0%	36.0%
(40-46)	No.	5	0	3	2	5	0	5
	%	5.0%	0.0%	3.0%	2.0%	5.0%	0.0%	5.0%
Total	No.	96	4	71	29	100	0	100
	%	96.0%	4.0%	71.0%	29.0%	100.0%	0.0%	100.0%

Table 7. Distribution of Toxoplasma, Rubella &amp; CMV IgM levels among the study groups according to residence

Residence	Toxo IgM		Rubella IgM		CMV IgM		Total	
	Negative	Positive	Negative	Positive	Negative	Positive		
Urban	No.	38	3	30	11	41	0	41
	%	38.0%	3.0%	30.0%	11.0%	41.0%	0.0%	41.0%
Rural	No.	58	1	41	18	59	0	59
	%	58.0%	1.0%	41.0%	18.0%	59.0%	0.0%	59.0%
Total	No.	96	4	71	29	100	0	100
	%	96.0%	4.0%	71.0%	29.0%	100.0%	0.0%	100.0%

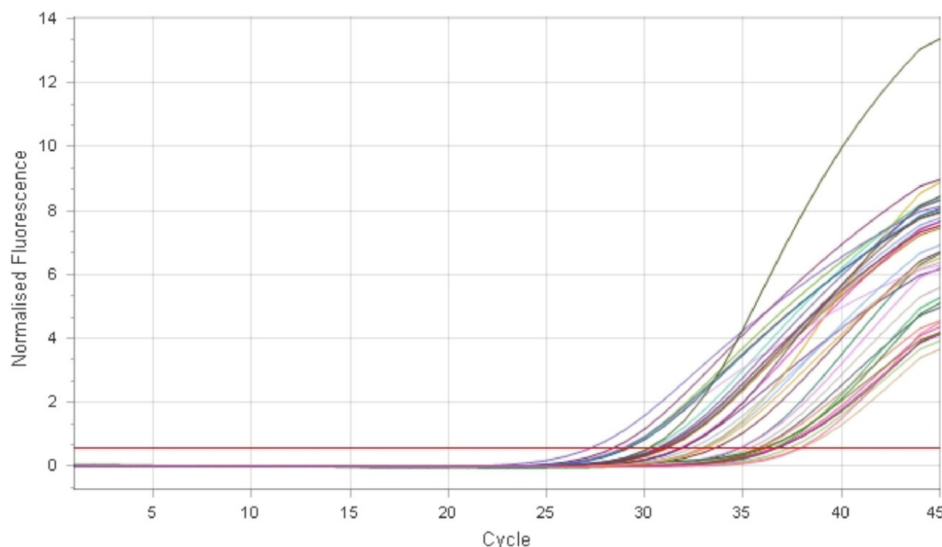


Figure 1. Evaluation of MicroRNA184 in aborted women infected with Toxoplasmosis, CMV, and Rubella

Table 8. The incidence of MicroRNA-184 in the studied group

MicroRNA-184	Cases				P value
	Aborted women		Non-aborted women		
	Count	%	Count	%	
Negative	23	72.7%	33	100.0%	0.005
Positive	10	27.2%	0	0.0%	
Total	33	100.0%	33	100.0%	
Mean $\pm$ SD	8.30	13.34	1.51	1.91	

*et al.* 2009; Çetinkaya and Yenilmez 2019). The IgM levels of Rubella in the age group (<20-29) were higher than in other age groups, and Toxoplasma IgM levels were higher than in other age groups, while the age

group (30-39) had the lowest levels. Rubella infection and Toxoplasmosis were also lowest in this age group. These findings coincided with (Abbas 2018), who found that the ages of rubella were higher than Toxoplasma and CMV infections for pregnant women, and it is the problem that causes miscarriage for those pregnant women (Abbas 2018). The prevalence of Rubella in rural patients was higher than that of toxoplasmosis, but no CMV infection was detected. These results were consistent with those of Hussein and Balatay (2019), who concluded that rubella is more prevalent in rural areas than Toxoplasma and CMV, and that it has caused many miscarriages in the Kurdistan region

of Iraq (Bahia *et al.* 2020). Out of 100 patients, gene expression of MicroRNA-184 was done in 33 patients, and demonstrated that 10 (27.2%) of aborted women who were carriers of Toxoplasmosis, CMV, and Rubella IgM showed positive MicroRNA-184. These results agreed with those of Bahia *et al.* (2020), who found that miR-184 was expressed in decidual stromal cells (DSC) & decidual immune cells (DIC). Also, samples taken from the peripheral blood of RSA patients showed similar results. Furthermore, our study found that miR-184 promotes apoptosis and represses trophoblast cell proliferation. Other studies demonstrated that miR-184 upregulated Fast expression of Fast via WIG1 targeting, leading to cell apoptosis. Finally, following in vivo miR-184 overexpression, there was a significant elevation in embryo desorption rate in pregnant mice. Therefore, our study outlines the pivotal role of miR-184 in maintaining successful pregnancy, providing a new diagnostic and therapeutic target for RSA (Bahia *et al.* 2020).

## Ethical Clearance

This study was approved by the research ethics committee of the faculty of Kamal AL-Samaraee Hospital No. 10.1186/s13104-019-4314-0:2019.

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