

## ORIGINAL

# The Impact of ABO Blood Types on Spontaneous Miscarriage and Cytomegalovirus Infection Iraq

## Efecto de los grupos sanguíneos ABO sobre el aborto espontáneo y la infección por citomegaloviru

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## ABSTRACT

**Introduction:** blood groups are linked to diseases as receptors for pathogens, influencing spontaneous abortion. Understanding these factors is crucial for effective risk factor management.

**Objective:** to elucidate the connection between blood types, Rh factor, and the incidence of spontaneous abortion while also examining its association with the dissemination of antibodies to cytomegalovirus.

**Method:** a cross-sectional study involving 200 pregnant women found that cytomegalovirus seroprevalence was higher in those experiencing spontaneous abortion compared to those not. Rh factor and blood types were also assessed using agglutination.

**Results:** the prevalence of CMV percentage was statistically significant ( $P \le 0,001$ ) in the abortion group CMV IgG (93,0 %, 20,0 %) and CMV IgM (22,0 %,1,0 %). Blood group O+ was the most prevalent among pregnant women who had experienced abortion and CMV IgG latent infection, whereas blood group B+ was the most common in the healthy group. Additionally, the Rh factor did not demonstrate any statistical significance about abortion or the spread of the virus.

**Conclusion:** CMV is widespread among pregnant women in the Diyala Governorate. Also, Blood group O+ is associated with an increased risk of spontaneous abortion and is also linked to a higher likelihood of CMV IgG latent viral infection in aborted women.

Keywords: Blood Groups; CMV Infection; Spontaneous Miscarriage; Agglutination.

### RESUMEN

**Introducción:** los grupos sanguíneos están vinculados a enfermedades como receptores de patógenos, influyendo en el aborto espontáneo. Comprender estos factores es crucial para el manejo eficaz de los factores de riesgo.

**Objetivo:** dilucidar la conexión entre los tipos sanguíneos, el factor Rh y la incidencia del aborto espontáneo, al tiempo que se examina su asociación con la diseminación de anticuerpos contra el citomegalovirus.

**Método:** un estudio transversal que incluyó a 200 mujeres embarazadas encontró que la seroprevalencia del citomegalovirus fue mayor en aquellas que experimentaron un aborto espontáneo en comparación con las que no. El factor Rh y los tipos sanguíneos también se evaluaron mediante aglutinación.

**Resultados:** La prevalencia del porcentaje de CMV fue estadísticamente significativa ( $P \le 0,001$ ) en el grupo de aborto CMV IgG (93,0 %, 20,0 %) y CMV IgM (22,0 %, 1,0 %). El grupo sanguíneo O+ fue el más prevalente entre las mujeres embarazadas que habían experimentado un aborto y una infección latente de CMV IgG,

© 2025; Los autores. Este es un artículo en acceso abierto, distribuido bajo los términos de una licencia Creative Commons (https:// creativecommons.org/licenses/by/4.0) que permite el uso, distribución y reproducción en cualquier medio siempre que la obra original sea correctamente citada mientras que el grupo sanguíneo B+ fue el más común en el grupo sano. Además, el factor Rh no demostró ninguna significación estadística sobre el aborto o la propagación del virus.

**Conclusión:** El CMV está muy extendido entre las mujeres embarazadas en la Gobernación de Diyala. Además, el grupo sanguíneo O+ está asociado con un mayor riesgo de aborto espontáneo y también está vinculado a una mayor probabilidad de infección viral latente de IgG contra CMV en mujeres que han abortado.

Palabras clave: Grupos Sanguíneos; Infección por CMV; Aborto Espontáneo; Aglutinación.

#### **INTRODUCTION**

One of the most common herpesviruses worldwide is the human cytomegalovirus or CMV. The transmission of coronavirus infection occurs in people of all ages, as confirmed by the CDC and the WHO.<sup>(1)</sup> Cytomegalovirus (CMV) is part of the herpes virus family and can induce systemic infections and severe diseases in individuals with compromised immune systems.<sup>(2)</sup> Congenital CMV infection causes microcephaly, seizure disorders, cognitive disabilities, developmental delays, and SNHL, impacting 10 % of infants. Long-term morbidities in neonates with post-natal infection remain unclear.<sup>(3)</sup> It is a significant contributor to prenatal and perinatal infections, potentially resulting in serious complications during pregnancy.<sup>(4)</sup>

An unidentified pathophysiologic mechanism underlies the common pregnancy problem of miscarriage.<sup>(5)</sup> A healthy pregnancy depends on the immune system maintaining homeostasis among several types of immune cells, such as macrophages, dendritic cells, natural killer cells, and T cells.<sup>(6)</sup> It has been found that the ABO blood type could be a biological indicator of disease vulnerability.<sup>(7)</sup> This study investigated the association between the blood group and the risk of spontaneous miscarriages in women infected with CMV.

#### **METHOD**

### Ethical Approval

Was secured by the College of Medicine Council at the University of Babylon on November 20, 2023. Approval to conduct the research was obtained from the Research Committee of the Diyala and Babylon Health Department under order number 8199 dated 12th February 2024. The research involved 200 blood samples collected from 100 pregnant women experiencing spontaneous abortion and 100 healthy pregnant women serving as a control group. A study was conducted in the laboratories of Al-Batoul Teaching Hospital for Obstetrics and Gynecology in Diyala and Babylon Hospital for Maternity and Children, Iraq.

#### Sampling

Samples were collected from 15th February 2024 to 20th September 2024. After getting the pregnant participants' verbal assent to participate in the study, a sterile syringe was used to collect 5 ml of blood after local skin sterilisation with alcohol. To collect the sample, 2 ml of blood was transferred to an anticoagulant tube and placed in an EDTA-coated sample bottle (EDTA bottle). The mixture was gently mixed before being put in a refrigerator bag. The serum was collected by centrifuging 3 ml of blood into a gel tube at 5000 RPM for 5 minutes. The tubes were then kept at 20 °C until they were needed.

#### Principle of ABO Test

The reagents LORNE-labs- UK contain antibodies targeting A and B antigens in human red blood cells, causing agglutination and indicating the absence of the corresponding ABO antigen. Lorne Monoclonal IgM ABO Blood Grouping Reagents are a mixture of mouse monoclonal antibodies diluted in a phosphate buffer, including sodium chloride, EDTA, and bovine albumin.

#### Slide Technique

Mix Lorne Anti-ABO reagent and three drops of whole blood on a slide. Oscillate gently for 30 seconds, then examine under a light source for one minute to distinguish fibrin strands and agglutination. Replicate weak reactions using the tube technique. The presence of the corresponding ABO antigen on red blood cells indicates a positive test result within the established protocol parameters.<sup>(8)</sup>

#### Detection of CMV IgM and IgG

Transfer 100 microliters of standards, controls, and diluted samples into containers and wells. Position the Substrate Blank in slot A1. Incubate in an incubator at  $37 \pm 1$  °C for 1 hour. Rinse wells with washing buffer and aspirate. Dispense Conjugate into each well, incubate for 30 minutes at room temperature, and repeat steps. Introduce TMB Substrate Solution and incubate for 15 minutes without light. Add Stop Solution, resulting in a colour change from blue to yellow. Evaluate absorbance at 450/620 nm within 30 minutes.<sup>(9)</sup>

## 3 Khaleefah NM, et al

## **Statistical Analysis**

The statistical analysis utilised version 28 of the Statistical Package for the Social Sciences (SPSS Inc., Chicago, USA) by chi-square test and logistic regression.

## RESULTS

Seroprevalence of CMV IgG and IgM in Aborted and Pregnant Groups

The logistic regression analysis of the results of the chi-square analysis demonstrated a statistically significant presence of CMV IgM and IgG in both groups, with a particular emphasis on latent CMV infection linked to a risk factor of (OR= 22,000) in women who experienced abortion (figure 1).

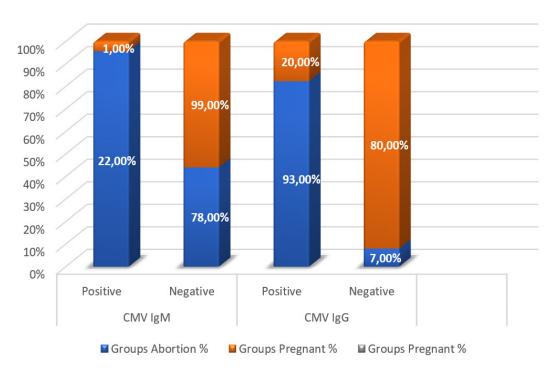


Figure 1. Seroprevalence of CMV IgG and IgM in Aborted and Pregnant Populations

## Analysis of the Abortion and Pregnant Groups Concerning Rh and Blood Groups

The Chi-square and logistics regression analysis results indicated no significant differences in P-value=0,126 between the abortion group and the Rh factor of the blood. However, important differences of P-value $\leq$  0,001\* were observed between the O blood group 66,0 % in the aborted group and the B group 19.0 % with healthy pregnant women, as detailed in table 1.

<b>Variable</b> s			Grou	ps		Measures					
		Abortion		Pre	Pregnant		OR	95 % CI			
		No.	%	No.	%						
Rh	Rh+	88	88,0 %	95	95,0 %	0,605	0,926	0,693-1,238			
	Rh-	12	12,0 %	5	5,0 %	0,100	2,400	0,846-6,812			
	X <sup>2</sup> =3,150		P-value=0,126								
Blood groups	А	26	26,0 %	37	37,0 %	0,168	0,703	(0,426-1,160)			
	В	7	7,0 %	19	19,0 %	≤0,024*	0,368	(0,155-0,876)			
	AB	1	1,0 %	6	6,0 %	0,097	0,167	(0,020-1,384)			
	0	66	66,0 %	38	38,0 %	≤0,007*	0,703	(1,165-2,589)			
	<i>X</i> <sup>2</sup> =18,569		<i>P-value≤ 0,001*</i>								

Analysis of CMV IgG and IgM Concerning Rh and Blood Groups

According to the Chi-square results, table 2 shows significant disparities between the O blood group and CMV IgG but no significant differences between the Rh factor and CMV infection.

Table 2. Association Between CMV IgG and IgM Infection and ABO Groups										
Variable	Scale	CMV IgG				CMV IgM				
		Positive		Negative		Positive		Negative		
		No.	%	No.	%	No.	%	No.	%	
Blood_Groups	А	31	27,4 %	32	36,8 %	7	30,4 %	56	31,6 %	
	В	10	8,8 %	16	18,4 %	0	0,0 %	26	14,7 %	
	AB	2	1,8 %	5	5,7 %	0	0,0 %	7	4,0 %	
	0	70	61,9 %	34	39,1 %	16	<b>69,6</b> %	88	49,7 %	
		<i>X</i> <sup>2</sup> =11,970		P≤0,007*		X <sup>2</sup> =5,840		P≤ 0,120		
Rh	Rh+	102	90,3 %	81	93,1 %	22	95,7 %	161	91,0 %	
	Rh-	11	9,7 %	6	<b>6,9</b> %	1	4,3 %	16	9,0 %	
		X <sup>2</sup> =0,509		P≤0,476		X <sup>2</sup> =0,576		P≤0,448		
* Significant differences at p-value ≤0,05										

### DISCUSSIONS

The most common congenital infection worldwide is cytomegalovirus (CMV), impacting 0,7 % to 1 % of live births, 11 % exhibiting symptoms, and 30 % to 40 % facing developmental delays.<sup>(10)</sup> Statistical analysis indicates a significantly higher seroprevalence of the virus in the group of pregnant women who experienced abortion compared to the group of healthy pregnant women, where it was CMV IgG (93,0 %,20,0 %) and IgM (22,0 %,1,0 %) in Aborted and control groups respectively as in figure 1. That result aligns with a study that found that HCMV IgG and IgM prevalence were (0 %-100 %) and (0 %-93 %), respectively. The results were influenced by various factors, such as the study's design and the sociodemographic and clinical aspects.<sup>(11)</sup> Research has demonstrated that the seroprevalence of cytomegalovirus is elevated among pregnant women in Diyala, particularly those who experience spontaneous abortion. This necessitates a simultaneous serological and molecular investigation to ascertain the virus's true prevalence in the body and its capacity to transmit to the foetus, as in figure 1.

Among the several viral infections, including dengue, hepatitis, Norwalk, and the coronavirus causing the 2003 severe acute respiratory syndrome (SARS), the blood group has been found as a possible risk factor.<sup>(12)</sup> The distribution of ABO and RhD blood group antigens varies according to race and geographic region. Previous studies have revealed that blood group type is associated not just with transfusion-related disorders but also with a range of other conditions, including cancer and infectious diseases.<sup>(13)</sup>

Table 1 shows that blood type O is more common in aborted pregnant who have had miscarriages 66,0 % than in healthy pregnant women 38,0 % and is a risk factor 0,703, more than the rest groups. The blood group test also showed significant differences  $P \le 0,024^*$  in the control group more than in the aborted pregnant women, with a risk factor of 0,368, which is believed to be a protective factor. This result agrees with a study that found a significant prevalence of 18,00 % among women with O+ blood group, indicating a protective effect against infectious diseases, despite previous findings suggesting otherwise. This is due to the significant representation of O+ women in the sample. <sup>(14)</sup> Patients with gestational diabetes are more likely to have a woman of blood type O, which lowers vWF and factor VIII and may increase the risk of postpartum haemorrhage and miscarriage.<sup>(15)</sup> For women with blood type O, the risk profile is complicated by the interplay of environmental factors, such as maternal age and lifestyle, and genetic predispositions.<sup>(16)</sup> The current study disagrees with a study that showed that ABO blood type correlates with abortion. Most women, precisely 92,8 %, and their husbands, at a rate of 77,6 %, exhibited Rh-positive blood groups. Mothers with blood type A negative experienced a 7,35-fold increase in the risk of abortion when compared to those with blood type A positive.<sup>(17)</sup> Also, a study suggests that individuals with blood group O may have a protective advantage. In contrast, those with blood group A may be more susceptible, but these factors do not affect disease progression.<sup>(18)</sup>

Chi-square analysis shows a statistically significant  $P \le 0,007^*$  relationship between blood type 0 61,9 % and CMV IgG as in table 2. These emphasise the results that found that a significant 78,1 % of participants tested positive for anti-CMV-IgG, with the highest infection rates observed in the 26 to 35 age group, and individuals with blood group O+ showed increased susceptibility to infection.<sup>(19)</sup> This study diverges from the seropositivity findings for CMV IgM, noting its presence in blood groups A, B, and AB. In contrast, no infection was detected in females with blood group O. The results regarding anti-CMV IgG antibodies in blood groups revealed 29 seropositive cases. This included 11 individuals in blood group AB with an average of 14,09 AU/ ml, 8 in blood group B, 13,237 AU/ml, and 7 in blood group A with an average of 12,0 AU/ml. In contrast, blood group O had two individuals with a 12,35 IU/ml measurement.<sup>(20)</sup> The current study shows no statistically significant between Rh with both spontaneous miscarriage and CMV infection. Medical services and technology advancements have significantly improved Rh disease management, enabling effective pregnancy monitoring and timely interventions, particularly by detecting elevated antibody titres.<sup>(21)</sup>

### 5 Khaleefah NM, et al

#### CONCLUSIONS

The findings indicated that it has been found that women with blood type O are more likely to have an abortion of their own will and are also more likely to have a latent infection of the CMV IgG virus in Diyala and Babylon Governorates, Iraq.

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## **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

## **AUTHORSHIP CONTRIBUTION**

Data curation: Nedhal Mahmoud Khaleefah. Formal analysis: Nedhal Mahmoud Khaleefah. Methodology: Nedhal Mahmoud Khaleefah. Project management: Nedhal Mahmoud Khaleefah. Resources: Nedhal Mahmoud Khaleefah. Software: Nedhal Mahmoud Khaleefah. Supervision: Bushra Jabbar Al Tamimi, Asmaa Kadhim Gatea. Validation: Bushra Jabbar Al Tamimi, Asmaa Kadhim Gatea. Display: Nedhal Mahmoud Khaleefah. Drafting - original draft: Nedhal Mahmoud Khaleefah. Writing - proofreading and editing: Nedhal Mahmoud Khaleefah.