Serological and Molecular Detection of Cytomegalovirus in Aborted Women in Diyala Province, Iraq

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KEYWORDS

CMV, ELISA, IgG, IgM, UL123, PCR, abortion women

ABSTRACT

Background: Cytomegalovirus (CMV) is a common virus that can infect individuals of all ages. In pregnant women, CMV infection can lead to complications such as congenital CMV infection, which can cause miscarriage, stillbirth, or significant fetal abnormalities. **Objective**: To evaluate the prevalence of CMV infection in women with and without a history of miscarriage in Diyala Province, Iraq. **Methods**: A total of 120 women were recruited and divided into three groups: 40 pregnant women with a history of miscarriage, 40 non-pregnant women with a history of miscarriage, and 40 healthy women without a history of miscarriage (control group). Blood samples were collected from each participant and analyzed for the presence of CMV-specific IgG and IgM antibodies using enzyme-linked immunosorbent assay (ELISA). Some positive results were retested to detect the MIE gene region IE-2 (UL122) by real-time PCR and the IE1 (UL123) gene by conventional PCR. Demographic data of study groups were recorded, and statistical analysis was performed using SPSS Version 29. **Results**: The serological analysis revealed the following prevalence of CMV IgG antibodies: 100% in both pregnant and non-pregnant women with a history of miscarriage, and 95% in the control group of healthy women without a history of miscarriage. For CMV IgM antibodies, the prevalence was 47.5% in pregnant women with a history of miscarriage, 40% in non-pregnant women with a history of miscarriage, and 7.5% in the control group of healthy women without a history of miscarriage. The PCR results showed that 9 of the samples were positive for both the UL122 and UL123 genes. **Conclusion**: The study indicates a high prevalence of CMV infection among women with a history of miscarriage in Diyala Province, Iraq. Routine screening for CMV in pregnant women, especially those with a history of miscarriage, is recommended to reduce potential risks and improve pregnancy outcomes.

1. Introduction

Cytomegalovirus (CMV), also known as human herpesvirus 5 and beta herpesvirus, is a globally prevalent double-stranded DNA virus [1]. It is one of nine herpesviruses that infect humans and is the most common congenital infection worldwide [2]. CMV virions are the largest among herpesviruses, featuring a linear genome of approximately 235 kb that encodes over 160 gene products [1]. A significant portion of these genes is crucial for the virus's ability to evade the host immune system, establish latency, and exhibit broad cellular tropism [3].

CMV infection can be transmitted from one person to another through direct contact with bodily fluids such as tears, saliva, urine, genital secretions, organ transplants, or blood transfusions ("horizontal transmission"). It can also be passed from mother to child ("congenital CMV (cCMV) infection") during childbirth or through breast milk ("postnatal CMV infection") [4, 5]. In healthy individuals, CMV infection is typically asymptomatic or presents as mononucleosis syndrome; however, in immunocompromised patients, it can be life-threatening [4]. Both primary and non-primary infections can result in active CMV infection. Non-primary infection occurs due to reinfection with exogenous CMV strains or reactivation of latent endogenous CMV, whereas primary infection occurs when an individual without prior CMV immunity contracts the virus for the first time [6].

In women of childbearing age, the estimated seroprevalence of CMV is 86% [2]. The prevalence of congenital CMV (cCMV) in newborns ranges from 0.3% to 2.4% in industrialized nations, although it is likely higher in developing nations [7]. Young children are the primary source of CMV transmission to pregnant women due to their prolonged high viral load shedding of CMV in urine and saliva [8]. Women with preconception immunity are expected to develop a non-primary infection at a rate of 10-30%, whereas 1-4% of CMV seronegative mothers acquire the virus during pregnancy [9]. While the risk of severe fetal harm decreases with advancing gestational age, the likelihood of vertical

transmission following primary maternal CMV infection increases with gestational age, ranging from 20% in the first trimester to 75% in the third trimester [5].

In general, the likelihood of vertical transmission following a non-primary CMV infection is significantly lower, around 1-3% [9]. Approximately 10% of neonates with congenital CMV (cCMV) infection exhibit symptoms at birth [10]. The disease can present with a range of symptoms, from mild non-specific findings such as petechiae, thrombocytopenia, anemia, leukopenia, conjugated hyperbilirubinemia, mild hepatosplenomegaly, or being small for gestational age (SGA), to more severe cases. Moderately and severely symptomatic infections are characterized by multiple manifestations, such as widespread petechiae, jaundice, marked hepatosplenomegaly, and/or central nervous system involvement, which may include microcephaly, ventriculomegaly, periventricular cysts, cerebral or cerebellar hypoplasia, or life-threatening disease [11].

Newborns with congenital CMV (cCMV), whether asymptomatic or symptomatic, are at risk for long-term neurodevelopmental issues, including intellectual disability, cerebral palsy, autism spectrum disorder, sensorineural hearing loss (SNHL), and visual impairment [12]. Specifically, 10-15% of newborns with asymptomatic cCMV infection and 40-60% of infants with symptomatic cCMV infection experience persistent sequelae [13]. Current estimates indicate that congenital CMV infection is the primary non-genetic cause of SNHL [14]. However, it is unclear whether postnatal CMV infection leads to SNHL in children [15-16].

2. Methodology

The design and context of the research

A total of 120 women were included in this cross-sectional study: 40 pregnant women who have previously had an abortion, 40 non-pregnant women who have previously experienced a miscarriage, and 40 healthy women who have never had one of these events. From October 2023 to May 2024, those ladies were enrolled in the Al-Batool Teaching Hospital in Diyala.

Data gathering

Every woman was given the option to sign an oral or written permission form outlining the details of the research. Through direct interviews, information on age, place of residence, education, and number of prior abortions was gathered and documented in a carefully thought-out questionnaire.

Blood sample collecting For the serological test,

three milliliters (3 ml) of venous blood were obtained by venipuncture fromall patients and healthy participants involved in this investigation. The blood was placed in a vacuum-sealed gel tube (without anticoagulant) and left at room temperature until it clotted. The serum was then separated by centrifugation at 2500 rpm for 10 minutes and stored in a deep freezer at -20°C.

For the molecular test, 2 mL EDTA tubes were used to collect plasma samples for CMV PCR assays. The samples were centrifuged at 1,300 g for 15 minutes to separate the plasma, then stored at -70°C until nucleic acid extraction was performed.

Serological diagnosis of CMV

A commercial enzyme-linked immunosorbent assay (ELISA) kit (NovaLisa®, Germany) was used to measure the titers of IgG and IgM antibodies against cytomegalovirus. According to the manufacturer's instructions, IgG and IgM levels greater than 11 NTU were considered positive for CMV. The results were read by a microwell reader and compared in parallel with controls, with the optical density measured at 450 nm on an ELISA reader.

Molecular diagnosis of Cytomegalovirus by PCR

All samples in the study had their genomic DNA successfully extracted using a commercial viral Genomic DNA/RNA Kit (AddBio Inc, Korea) following the manufacturer's instructions. CMV was detected by real-time PCR using TaqMan master mix. Additionally, the extracted DNA was confirmed

and analyzed for the UL123 gene using horizontal gel electrophoresis in 1.5% agarose for 30 minutes at 75 volts, then exposed to UV light where the DNA appeared as compact bands.

The primers used in this study are listed in Table 1.

Table I: Sequences of oligonucleotide primers for PCR-based CMV detection.

Target	Amplifie region	5' → 3' Primers	Position	Product size (bp)
MIE gene	1 (UL-123)	CCAAGCGGCCTCTGATAACCAAGCC	-755	435
		CAGCACCATCCTCCTCTTCCTCTGG	50-1165	

Statistical Analysis

A statistical analysis was conducted on the seroprevalence of antibodies against Cytomegalovirus infection in both the control group and the patients. SPSS version 29 was used to analyze the data. Quantitative data were reported as mean and standard deviation, while qualitative data were expressed as numbers and percentages. A P-value of less than 0.05 was considered statistically significa

3. Result and Discussion

Anti-CMV IgG association:

Table (2) and Figure (1), showed the results of the Enzyme-linked immunoassays applied for all study groups. All participated women (100%); the pregnant and non-pregnant groups were positive for anti-CMV IgG, while the positivity rate among healthy women was 38 (95%). Therefore, the difference among the study groups was statistically insignificant (P= 0.131).

Table (2): Distribution of immunological markers among study groups

Immunological markers		**Pregnant with abortion**	**Non-pregnant with abortion**	**Healthy women**	**P value**
		No. (%)	**No. (%)**	**No. (%)**	
CMV IgG	Positive	40 (100)	40 (100)	38 (95.0)	0.131
	Negative	0 (00)	0 (00)	2 (5.0)	

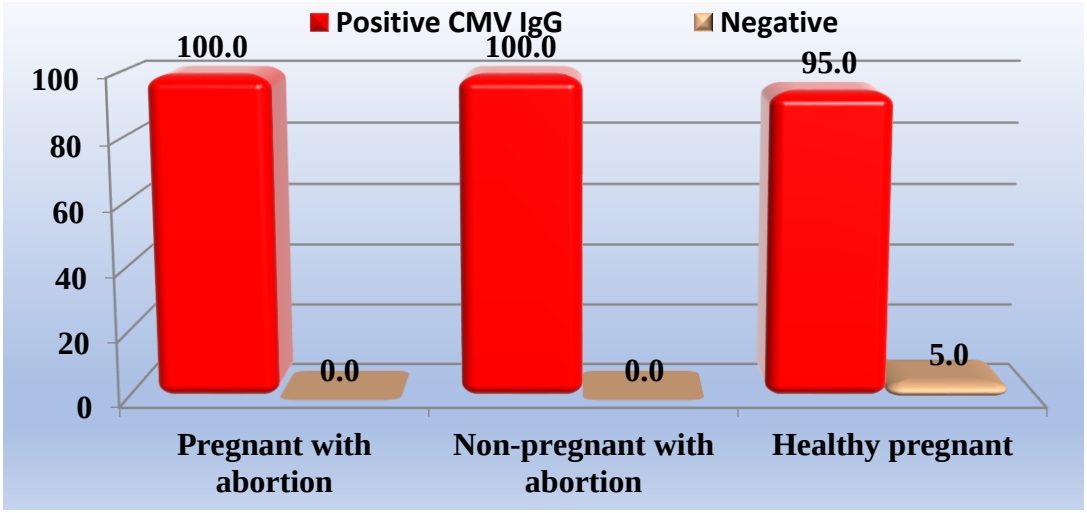


Figure (1): Bar diagram of CMV IgG positivity among study groups

Anti-CMV IgM association:

The anti-CMV IgM positivity rate among pregnant women was 19 (47.5%) versus 21(52.5%) the negativity rate. In the non-pregnant women, the anti-CMV IgM positivity rate was 16 (40%) versus 24

(60%) negativity rate. While 3(7.5%) of the healthy women were positive versus 37 (92.5%) were negative. Thus, the anti-CMV IgM positivity rate in pregnant and non-pregnant women was significantly higher compared to the control (P= 0.0001). Table (3) and figure (2).

Table (3): Distribution of immunological markers among study groups.

Immunological markers		Pregnant with abortion	Non-pregnant with abortion	Healthy women	P value
		No. (%)	No. (%)	No. (%)	
CMV IgM	Positive	19 (47.5)	16 (40.0)	3 (7.5)	0.0001*
	Negative	21(52.5)	24 (60.0)	37 (92.5)	

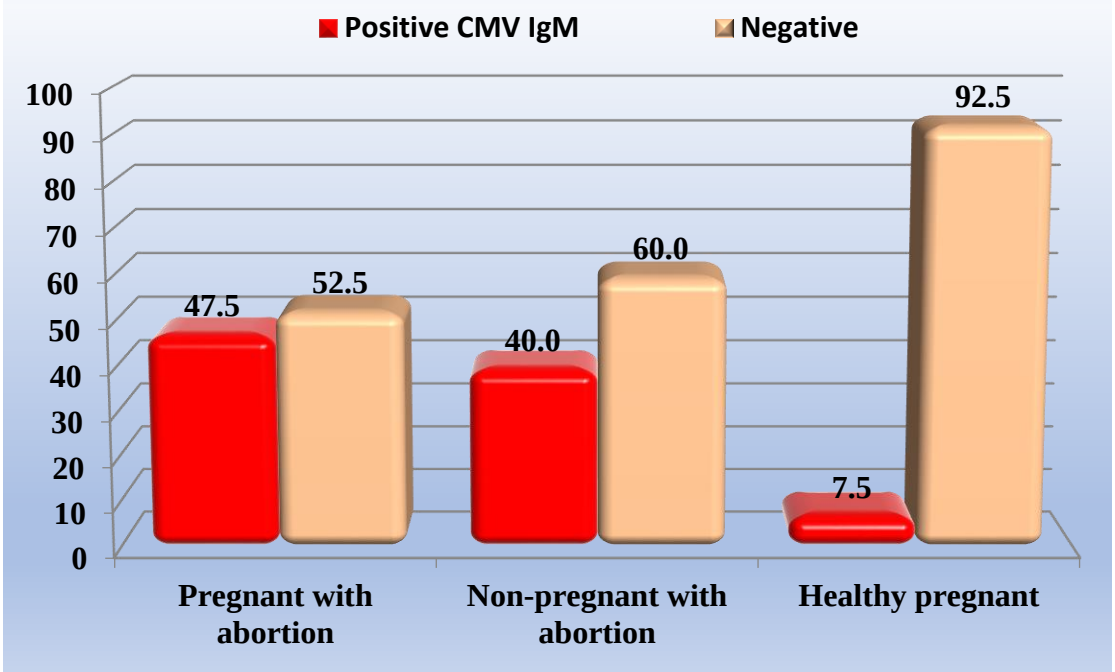


Figure (2): Bar diagram of CMV IgM positivity among study groups.

Association of CMV IgM with age categories:

Table (4) showed that in the pregnant women group, the highest anti-CMV IgM positivity rate (61.5%) was among those 25-29 years old. In the non-pregnant women group, the highest positivity rate (100) was among those less than 20 years old. While in healthy women, three women were positive for anti-CMV IgM; one (14.3%) was her age was 20.24 years, the second was in the 30–34-year category and the third was in the ≥ 35 years category. In all three study groups, the difference was statistically significant (p= 0.802), (P= 0,212), and (P= 0.725) respectively.

Table (4): Association of anti-CMV IgM with age categories of study groups.

Age categories	Anti-CMV IgM					
	Pregnant with abortion		Non-pregnant with abortion		Healthy pregnant	
	Positive	Negative	Positive	Negative	Positive	Negative
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
< 20 years	1 (50)	1 (50)	2 (100)	0 (00)	0 (00)	3 (100)
20---24	4 (40)	6 (60)	2 (50)	2 (50)	1 (14.3)	6 (85.7)
25---29	8 (61.5)	5 (38.5)	7 (50)	7 (50)	0 (00)	12 (100)
30---34	3 (37.5)	5 (63.5)	3 (30)	7 (70)	1 (10)	9 (90)
≥ 35years	3 (42.5)	4 (57.1)	2 (20)	8 (80)	1 (12.5)	7 (87.5)

P value	**0.802**	**0.212**	**0.725**

*** A significant difference between percentages was determined using the Pearson Chi-square test (χ^2-test) at the 0.05 level.**

Association with education and residence:

The results in Table (5) found that the higher anti-CMV IgM positivity rate (54.5%) in the pregnant women group was among those with secondary levels of education. Similarly, in the non-pregnant women group, the highest positivity rate (47.6%) was among those with secondary levels of education. Whereas, in healthy women, the highest positivity rate (11.1%) was among those with a university level of education. However, in the three study groups the difference was statistically insignificant (P= 0.481), (P= 0.517), and (P= 0.717) respectively.

Regarding the residence, the results found that in all study groups; pregnant, non-pregnant, and healthy women, the highest anti-CMV IgM (48.0%), (43.5%) and (9.5%) respectively were among those from urban areas. Nevertheless, there was an insignificant difference in all these groups (P= 0.935), (P= 0.601), and (P= 0.609) respectively.

Table (5): Association of anti-CMV IgM with education and residence of study groups.

Education	**Anti-CMV IgM**					
	Pregnant with abortion		**Non-pregnant with abortion**		**Healthy pregnant**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
	No. (%)	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**
Primary	5 (45.5)	6 (54.5)	2 (25.0)	6 (75.0)	0 (00)	6 (100)
Secondary	12 (54.5)	10 (45.5)	10 (47.6)	11 (52.4)	2 (8.0)	23 (92.0)
College	2 (28.6)	5 (71.4)	4 (36.4)	7 (63.6)	1 (11.1)	8 (88.9)
P value	**0.481**		**0.517**		**0.717**	
Residence						
Rural	7 (46.7)	8 (53.3)	6 (35.3)	11 (64.7)	1 (5.3)	18 (94.7)
Urban	12 (48.0)	13 (52)	10 (43.5)	13 (56.5)	2 (9.5)	19 (90.5)
P value	**0.935**		**0.601**		**0.609**	

*** A significant difference between percentages was determined using the Pearson Chi-square test (χ^2-test) at the 0.05 level.**

Association of CMV IgM with occupation and animal contact:

Regarding the occupation, the higher anti-CMV IgM positivity rate among pregnant (48.4%) and non-pregnant (40.6%) women were among housewives. While, in healthy women, the highest positivity rate (8.3%) was among employers. However, none of these showed significant differences (P= 0.835), (P= 0.872) and (P= 0.896) respectively.

Speaking of animal contact, in the pregnant women group, the highest (48.6%) anti-CMV IgM positivity rate had no animal contact. On the other hand, in non-pregnant and healthy women, the higher positivity rates (55.6%) and (25%) respectively had animal contact. However, in all three groups, there was no significant difference (P= 0.720), (P= 0. 279), and (P= 0.161) respectively. All data are seen in Table (6).

Table (6): Association of anti-CMV IgM with occupation, endogamy, and animal contact of study groups.

Occupation	**Anti-CMV IgM**		
	Pregnant with abortion	**Non-pregnant with abortion**	**Healthy pregnant**

	Positive	Negative	Positive	Negative	Positive	Negative
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Housewife	15 (48.4)	16 (51.6)	13 (40.6)	19 (59.4)	2 (7.1)	26 (92.9)
Employer	4 (44.4)	5 (55.6)	3 (37.5)	5 (62.5)	1 (8.3)	11 (91.7)
P value	**0.835**		**0.872**		**0.896**	
Animal contact						
Yes	2 (40.0)	3 (60.0)	5 (55.6)	4 (44.4)	1 (25.0)	3 (75.0)
No	17 (48.6)	18 (51.4)	11 (35.5)	20 (64.5)	2 (5.6)	34 (94.4)
P value	**0.720**		**0.279**		**0.161**	

*** A significant difference between percentages was determined using the Pearson Chi-square test (χ^2-test) at the 0.05 level.**

Association of CMV IgM with chronic diseases and number of abortions:

The results in Table (7) revealed that the highest anti-CMV IgM positivity rate (60%) among pregnant women had chronic disease, but the difference was statistically insignificant (P=0.550). On the other hand, in the non-pregnant and healthy women, all positive (43.2%) and (7.9%) women respectively had no chronic diseases. Again, there were no significant differences in these groups (P= 0.141) and (P= 0.679) respectively.

Talking about the number of abortions and its association with anti-CMV IgM positivity rate, the results found in pregnant and non-pregnant women, the higher positivity rates (55.6%) and (60 %) respectively were among women who had one previous abortion, with no statistical significant differences (P= 0.648) and (P=0. 125) respectively. Of course, all positive women (7.5%) in the healthy group had no abortion.

Table (7): Association of anti-CMV IgM with chronic diseases and number of abortions in study groups.

Chronic diseases	Anti-CMV IgM					
	Pregnant with abortion		Non-pregnant with abortion		Healthy pregnant	
	Positive	Negative	Positive	Negative	Positive	Negative
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Yes	3 (60.0)	2 (40.0)	0 (00)	3 (100)	0 (00)	2 (100)
No	16 (45.7)	19 (54.3)	16 (43.2)	21 (56.8)	3 (7.9)	35 (92.1)
P value	**0.550**		**0.141**		**0.679**	
Number of abortions						
None	0 (00)	0 (00)	0 (00)	0 (00)	3 (7.5)	37 (92.5)
One	10 (55.6)	8 (44.4)	9 (60.0)	6 (40.0)	0 (00)	0 (00)
Two	6 (40.0)	9 (60.0)	4 (25.0)	12 (75.0)	0 (00)	0 (00)
3 & more	3 (42.9)	4 (57.1)	3 (33.3)	6 (66.7)	0 (00)	0 (00)
P value	**0.648**		**0.125**		**--**	

*** A significant difference between percentages was determined using the Pearson Chi-square test (χ^2-test) at the 0.05 level.**

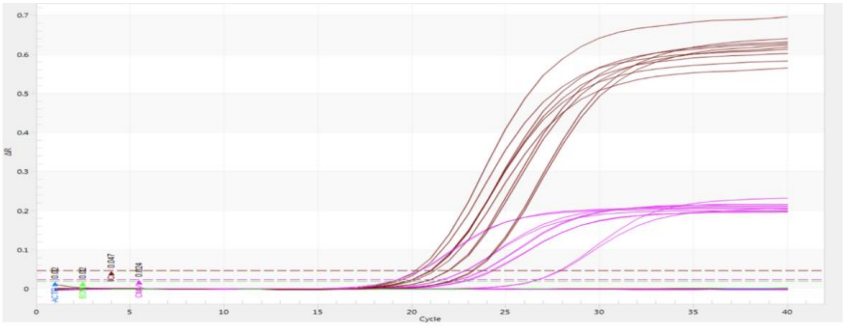
Molecular detection of CMV

For the detection of Cytomegalovirus nucleic acid**,** table (8) shows the detection rates of the gene of HCMV among the study group. Among the 12 participants tested for the UL122 gene, 9 were positive.

Table (8): Detection of IE-2 (UL122) Gene among study groups.

PCR	Pregnant women with an abortion	Non-pregnant women with an abortion	Healthy women
	No. (%)	No. (%)	No. (%)
IE-2 (UL122) Gene			
Detect	6(85.7%)	3(100%)	0(0%)
None detect	1(14.3%)	0(0%)	2(100%)
Total	7(100%)	3(100%)	2(100%)

Figure (3): Cytomegalovirus detection by Real-time PCR



The current study was conducted for amplification of the HCMV UL123 gene and the results of PCR showed the detection of the UL123 gene in 9 (100%) of specimens out of 9 of the total specimens infected with HCMV that were collected from aborted women and then detected by real-time PCR technique (figure 4).

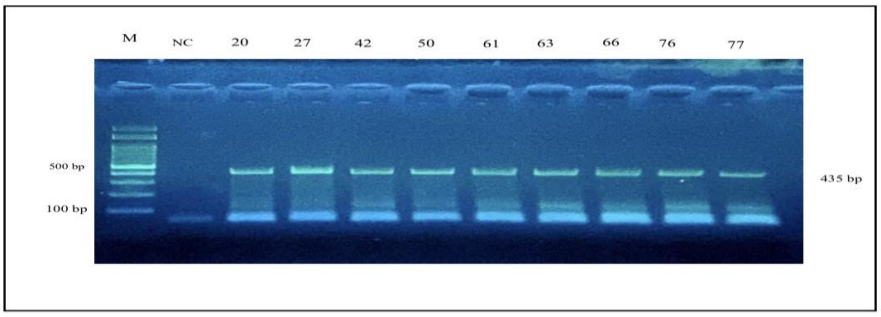


Figure (4): Agarose gel electrophoresis of PCR products was performed using 1.5% agarose at 75 volts for 90 minutes. Lane M represents the DNA Ladder, and Lane NC is the negative control. All lanes (1-9) of CMV samples showed positive results.

Discussions

Detecting specific infectious agents, such as CMV infection during pregnancy, is crucial for preventing the associated abnormalities. The value of screening varies across countries due to the influence of geographical, social, economic, and cultural factors on its prevalence. CMV is a widespread virus with a global seroprevalence ranging from 45% to 100% [17]. After initial infection, CMV can remain dormant and reactivate during pregnancy or be transmitted through contact with body fluids [18]. Globally, congenital CMV infection is the leading cause of neurological damage in children, associated with growth retardation, hearing loss, permanent disabilities, and microcephaly [19-20]. We aimed to screen pregnant and non-pregnant women for acute CMV infection by assessing IgG and IgM antibodies in women attending the hospital. In our study, anti-CMV IgG positivity was 100% in both pregnant and non-pregnant women with previous miscarriages and 95% in the control group, while anti-CMV IgM positivity was 47.5% in pregnant women, 40% in non-pregnant women, and 7.5% in healthy women. These findings align with other studies conducted in our country. In Iraq,

studies have reported anti-CMV IgG positivity rates ranging from 37% to 100% [21-24], and anti-CMV IgM positivity rates between 2.27% and 53% [25-27]. Globally, CMV seropositivity in pregnant women ranges from 40% to 90% in the USA [28], 56.8% in Australia [29], and 100% in Thailand [30]. In Turkish women, CMV seropositivity rates range from 84.5% to 97.3% [31-33]. In developing countries, individuals often acquire the virus during early childhood.

4. Conclusion and future scope

early diagnosis is crucial due to the elevated risk of congenital infections during pregnancy. Serological tests, which are affordable and straightforward, should be conducted in pregnant women and those planning pregnancy, tailored to the seroprevalence rates and regional risk levels.

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