Iranian Journal of Microbiology

Volume 17 Number 1 (February 2025) 128-136

ORIGINAL ARTICLE

Serological epidemiology analysis of Cytomegalovirus infection in pregnant women in Diwaniyah, Iraq

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Received: October 2024**, Accepted:** January 2025

ABSTRACT

Background and Objectives: Human cytomegalovirus (HCMV) infection is the most common cause of congenital infection during pregnancy. It is a major concern worldwide with a wide range of clinical outcomes in fetuses and newborns due to HCMV reactivation or reinfection during pregnancy. Primary maternal infection is best diagnosed by examining IgM and IgG antibodies. The current study aimed epidemiology survey of congenital HCMV infection in pregnant women in Diwaniyah.

Materials and Methods: 600 blood samples were collected from pregnant women, between 18-45 years old, in Diwaniyah Governorate for 12 months, from January to December 2022, in regards to their place of residence (urban or rural). All samples were monitored for both IgG and IgM antibodies against HCMV using rapid test and ELISA.

Results: Our findings showed a high positive rate for IgG (95.7%) and (96.2%) and a positive rate for IgM (1.5%) and (1.8%) for rapid test and ELISA, respectively. The highest IgG positive rate was in the age group 26-35 years (43.33%), while the lowest rate (13.0%) was in the age group 36-45 years. The HCMV infection rate in rural and urban areas were (96.48%) and (95.26%), respectively, with no significant differences (P value>0.05). Also, the rate of miscarriages among pregnant women infected with HCMV was 28.83%, and the highest infection rate (30.51%) was recorded in the age group 26-35 years.

Conclusion: The prevalence of HCMV infection and its related miscarriage among the studied population is relatively high with the highest rate in the age group of 26-35 years.

Keywords: Human cytomegalovirus (HCMV); Pregnant women; Seroepidemiology

INTRODUCTION

Human cytomegalovirus (HCMV) is a betaherpesvirus belongs to the herpesvirus family (*Herpesviridae*) and is specifically part of the subfamily of *Betaherpesvirinae* and is known as human herpesvirus 5 (HHV5) capable of causing lifelong infection in humans. It is transmitted primarily through body fluids, such as blood, urine, and saliva. In healthy individuals, primary HCMV infection is often asymp-

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tomatic but can lead to persistent or latent infection (1). HCMV is the most prevalent herpes virus worldwide. According to the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO), HCMV infects people of all ages. Serological prevalence refers to the level of presence of a pathogen in the general population, as measured in blood serum. The high seroprevalence of HCMV indicates that a large proportion of the global population has been exposed to this virus. Most people infected with HCMV show no signs or symptoms (2). HCMV, a herpes virus, is endemic worldwide and causes asymptomatic infants (3, 4). With a global distribution, HCMV is a lifelong latent infection in 40 to 90% of recipients. Infection and transmission via breast milk in low birth weight neonates is a worldwide community health problem (5). Seroprevalence of HCMV is highest in South America, Africa, and Asia, while it is lowest in Western European countries and the United States (6). Various published results regarding anti-HCMV immunoglobulin G (IgG) and immunoglobulin M (IgM) levels worldwide vary from 93-97% (7). The impact of HCMV infection is often overlooked because of its asymptomatic nature in infected pregnant women and newborns, difficulty in diagnosis, and the perception that infants born to women with preexisting HCMV antibodies have normal outcomes at birth (8).

The prevalence of HCMV IgG antibodies among women with recurrent miscarriage and normal women was 85.7% and 76.2%, respectively. There was no significant association between HCMV infection and recurrent miscarriage (9). A study conducted in Mazandaran Province, Iran found that the average age of pregnant women was 30 years, ranging from 20 to 42 years. The result showed that 2 (1.6%), 92 (73.6%) and 2 (1.6%) were positive for IgM, IgG and IgM/IgG, respectively. PCR test results indicated the presence of HCMV DNA in 10 (8%) of the pregnant women (10). The study conducted in Najaf Governorate, Iraq, showed that the rate of abortion resulting from bacterial causes varies according to the type of germs, as the rate of infection with HCMV was the highest, followed by rubella and toxoplasmosis (17.6, 15.1, 2.6)%, respectively, and the rates of women with a previous infection (45.8, 16.7, 19.8)%, respectively (11).

The main objective of the study. The present study aims to survey the seroepidemiology of HCMV and its association with miscarriage, understanding that few studies address the epidemiological survey of HCMV (12) in Iraq.

MATERIALS AND METHODS

HCMV detection was performed on women who were married and pregnant in the age of 18 to 45 years old (childbearing age), at the time of sampling in Diwaniyah Governorate by the following methods. As the HCMV infection seems to have a large impact on fetuses and newborns, non-pregnant and single women were excluded.

Sample collection. 5 mL of blood samples were collected from 600 pregnant women who were referred in different hospitals and private clinics of urban and rural areas of Diwaniyah Governorate for 12 months, from January 2022 to December 2022. Information about the pregnant women's ages (18-45 years) and their place of residence were recoded. Collected samples were centrifuged and the serum was collected in Eppendorf tubes and kept at -20°C until rapid and ELISA analysis was performed.

Methods of (HCMV) serological analysis. Rapid Spectrum HCMV IgM/IgG (CTK Biotech, catalog R0224c/USA) and Enzyme-Linked Immunosorbent Assay (ELISA), (Pishtaz Teb diagnostics, catalogue No.PT-HCMV-96/Iran) were used for detecting both IgG and IgM, according to the instructions of the laboratory kit manufacturer. The ELISA test with more sensitivity employed in parallel and as a confirmatory test for rapid test. In normal population, the cut-off value considered as 10 AU/ml. Results lower than 10 AU/ml is considered as negative and those greater than 10 AU/ml considered as positive results. Those results between 9-11 AU/ml are considered as suspected results and should be re-evaluated.

Rapid test principle. HCMV IgM/IgG Rapid Test Strip (Whole Blood/Serum/Plasma), is based on the gold immunochromatography (GICA) test principle. The control line (C) is pre-coated with anti-protein A antibodies and the test line (T) is pre-coated with recombinant human HCMV antigen. Any HCMV IgG/IgM antibodies present in the sample combine with the protein A stained with colloidal gold particles on the sample pad. This complex migrates by capillary

action along the strip. If any HCMV IgG/IgM antibodies are present in the sample, they are captured by the T line and a colour change is observed. The C line confirms whether the test is successful or not (Fig. 1).

ELISA test principle. The HCMV IgM/IgG ELISA Test Kit is a standardized, solid-phase, indirect enzyme immunoassay for the detection of HCMV IgM/IgG antibodies in serum. HCMV antigens are placed on a 96-well (12 × 8) microplate. When a pre-diluted sample is added and then incubated, if the material contains anti-HCMV IgM/IgG antibodies, they will adhere to the antigens coated on the microplate, causing the anti-HCMV IgM/IgG antibodies to precipitate. Complexes will not form if the material lacks anti-HCMV IgM/IgG antibodies. The microplate is washed after the first incubation to remove loose elements. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB Chromogenic Substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM/IgG specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

Serological detection of IgM and IgG to HCMV using ELISA. The concentration of HCMV-specific IgG and IgM antibodies and IgG and IgM markers in all serum samples was estimated following an ELISA kit manufacturer's instruction and the OD was read at 450 nm within 15 minutes using an adsorbent assay technique.

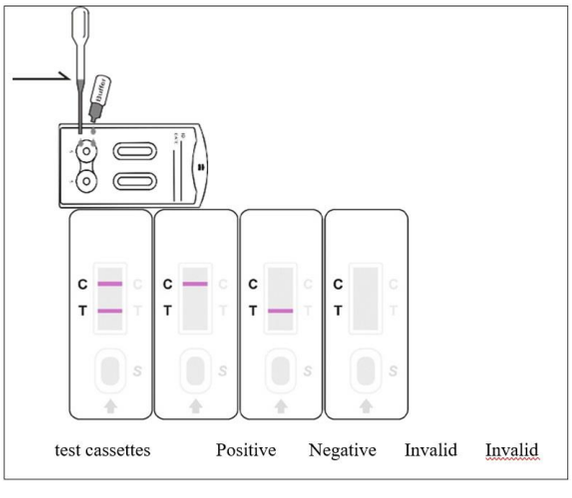

Fig. 1. Rapid test. If there are two lines, one in the test region (T) and the other in the control region (C), the test result is positive. If the line is only in the control region, the test result is negative. If there is no line in the control region, the test result is invalid in any case and must be repeated.

Statistical analysis. Microsoft Office Excel 2010 was used to extract the average and percentage of the study samples in all its categories, as well as the SPSS software version 27 was used to analyze Chi-Squared and Correlation.

RESULTS

Distribution of samples by residential area (urban or rural). The number of those examined from rural areas was 401 (66.8%) and from urban areas 199 (33.2%), (Fig. 2). Noting that the percentage of pregnant women who visited hospitals and private clinics was higher in the countryside than in the city. The residential area is important because the economic and living conditions and attention to cleanliness in the city are greater than in the countryside.

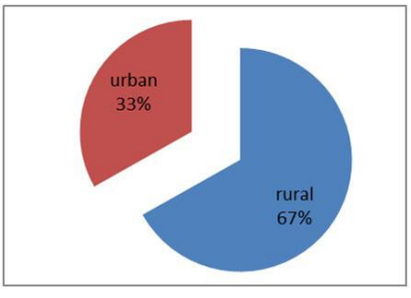

Fig. 2. Distribution of samples according to rural and urban residential areas.

Distribution of samples according to IgG, IgM for (HCMV) by rapid and ELISA test. Assuming that detection of IgM represents new infection of 2-3 weeks, and IgG detection represent of old (more than 3 weeks) or chronic infection, the results of the current study showed 574 (95.7%) IgG positive samples and 26 (4.3%) negative samples by rapid test, while IgM positive samples by rapid test were 9 (1.5%) and 591 (98.5%) samples were negative. While the results show 577 (96.2%) positive samples of IgG and 23 (3.8%) negative samples by ELISA test and the positive samples for IgM by ELISA test were 11 (1.8%) and 589 (98.2%) negative samples (Table 1). The re-

sults of the rapid and ELISA tests showed no significant differences in the value (p-value = 0.05) (Table 1 and Fig. 3). IgG/IgM were measured and their importance in diagnosing human HCMV and knowing whether the infection is acute or chronic. The infection was diagnosed using the rapid method and confirmed by the ELISA method.

Table 1. Summary statistics for IgG and IgM

	Variables	Groups	Frequency	Percentages
Rapid test	HCMV IgG	N	26	4.3%
		P	574	95.7%
	HCMV IgM	N	591	98.5%
		P	9	1.5%
ELISA test	HCMV IgG	N	23	3.8%
		P	577	96.2%
	HCMV IgM	N	589	98.2%
		P	11	1.8%

N: Negative
P: Positive

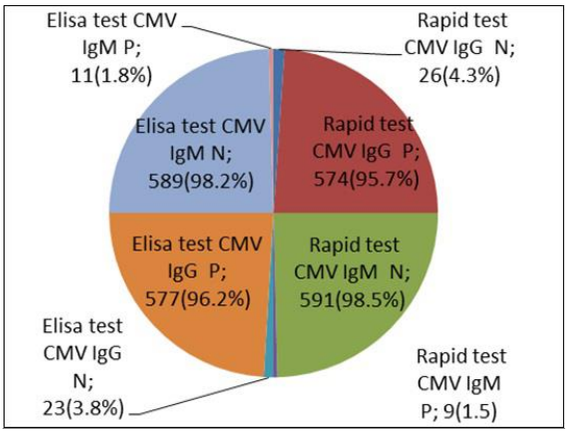

Fig. 3. The comparison between rapid and ELISA results.
N: Negative
P: Positive

The association between IgG, and IgM and the month of sample collection for both rapid and ELISA tests. Table 2 presents the results of a chi-squared test to assess the association between IgG and IgM tests (for both rapid and ELISA tests) and the month of sample collection in a one- year-period. The Table is divided into two sections, one for the rapid test and another for the ELISA test. Each section shows the frequencies of IgG and IgM results for each month, with "N" indicating a negative result and "P" indicating a positive result. The chi-squared test results show that for the rapid test, there is no significant association between IgG and time (p-value = 0.240) and no significant association between IgM and time (p-value = 0.872). However, for the ELISA test, there is a significant association between IgG and time (p-value = 0.046), indicating that the distribution of IgG results varies significantly across different months. For IgM and time in the ELISA test, there is no significant association (p-value = 0.884) The research samples were collected throughout the year, and the current study showed that the virus infects pregnant women at any time of the year and does not have a specific month for infection during the year (Table 2 and Fig. 4).

Distribution of samples according to IgG, IgM for (HCMV) with residential area groups by rapid and ELISA test. Table 3 presents the results of a chi-squared test to examine the association between IgG and IgM tests (for both Rapid and ELISA tests) and the residential area of participants in the city (urban(or out of the city) rural). For the rapid test, there is no significant association between IgG and residential

Table 2. The association between IgG, and IgM and time for both rapid and ELISA tests

Time	Rapid test				ELISA test			
	IgG		IgM		IgG		IgM	
	N	P	N	P	N	P	N	P
January	4	49	52	1	4	49	52	1
February	4	52	55	1	5	51	55	1
March	0	41	41	0	0	41	41	0
April	3	32	35	0	2	33	35	0
May	2	42	43	1	1	43	42	2
June	3	20	22	1	3	20	22	1
July	4	63	65	2	4	63	65	2
August	1	39	39	1	1	39	39	1
September	1	62	62	1	1	62	62	1
October	1	48	48	1	1	48	48	1
November	1	59	60	0	0	60	60	0
December	2	67	69	0	1	68	68	1
Total	26	574	591	9	23	577	589	11
Chi squared test	13.873		6.017		19.247		5.829	
P value	0.240 NS		0.872 NS		0.046 S		0.884 NS	

NS: Non significant association between groups (p value > 0.05)
S: Significant association between groups (p value < 0.05)

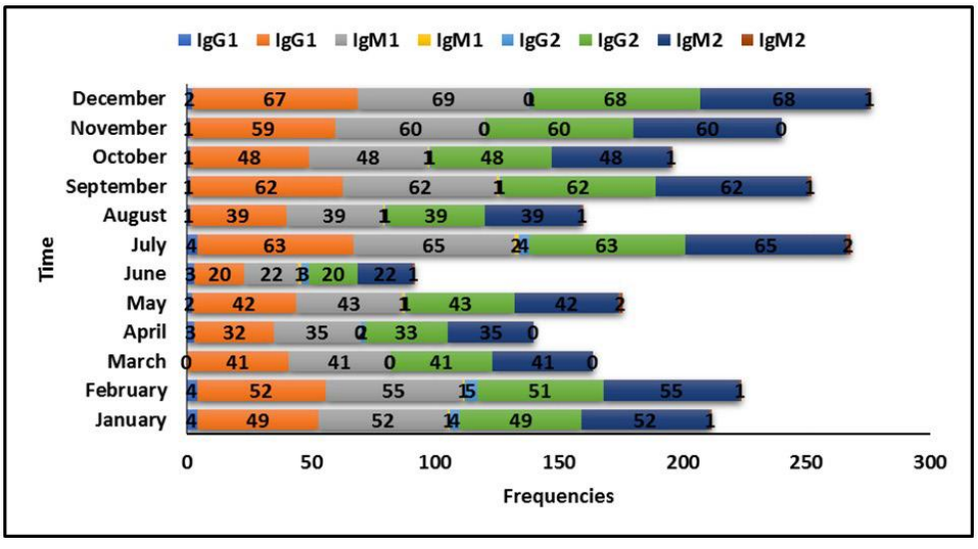

Fig. 4. The results of a chi-squared test to examine the association between IgG and IgM tests with time.

Table 3. Chi-squared test to test the association between IgG, and IgM and residential area for both Rapid and ELISA tests

Tests	Groups		Residential area		Chi squared test P value
			Urban	Rural	
Rapid test	IgG	N	7 (3.51%)	19 (4.73%)	0.478
		P	192 (96.48%)	382 (95.26%)	0.489 NS
	IgM	N	197 (98.99%)	394 (98.25%)	0.494
		P	2 (1.00%)	7 (1.74%)	0.482 NS
Elisa test	IgG	N	6 (3.01%)	17 (4.23%)	0.541
		P	193 (96.98%)	384 (95.76%)	0.462 NS
	IgM	N	196 (98.49%)	393 (98.00%)	0.176
		P	3 (1.50%)	8 (1.99%)	0.675 NS

NS: Nonsignificant association between groups (p value > 0.05)

S: Significant association between groups (p value < 0.05)

area (p-value = 0.489) and no significant association between IgM and residential area (p-value = 0.482). Similarly, for the ELISA test, there is no significant association between IgG and residential area (p-value = 0.462) and no significant association between IgM and residential area (p-value = 0.675). In general, this analysis indicates that there is no significant association between IgG or IgM results and participants in the residential area, suggesting that the distribution of test results does not differ significantly between urban and rural residents, suggesting that rural living is similar to city living.

Distribution of samples according to (IgG, IgM) for (HCMV) and age groups by (rapid and ELISA). Results of a chi-squared test to examine the association between IgG and IgM tests (for both rapid and ELISA tests) and age groups (18-25 years, 26-35 years, and 36-45 years) are shown in Table 4. For the rapid test, there is no significant association between IgG and age (p-value = 0.668) and no significant association between IgM and age (p-value = 0.484). Similarly, for the ELISA test, there is no significant association between IgG and age (p-value = 0.343) and no significant association between IgM and age (p-value = 0.302). Overall, this analysis suggests that there is no significant association between IgG or IgM results and age groups, indicating that the distribution of test results does not vary significantly across different age groups in the study population.

Distribution of samples according to the association of abortion frequency with age. Table 5 shows the relationship between the number of miscarriages and age groups. The results during the research period were as follows: The highest percentage of pregnant women who miscarried once (18%) was in the age group 26-35 years, and the highest percentage of women who miscarried twice (12.5%) was in the age group 36-45 years. As for women who miscarried three times, the percentage was higher in the age group 18-25 years (7.3%), and the percentage of pregnant

Table 4. The association between IgG, IgM, and age for both Rapid and ELISA tests

Tests	Groups		Age			Chi squared test
			18-25 years	26-35 years	36-45 years	P value
Rapid test	IgG	N	12 (2%)	12 (2%)	2 (0.33%)	0.805
		P	236 (39.33%)	260 (43.33%)	78 (13.0%)	0.668 NS
	IgM	N	244 (40.66%)	267 (44.5%)	80 (13.33%)	1.450
		P	4 (0.66%)	5 (0.83%)	0 (00%)	0.484 NS
ELISA test	IgG	N	9 (1.5%)	13 (2.16%)	1 (0.16%)	2.136
		P	239 (39.83%)	259 (43.16%)	79 (13.16%)	0.343 NS
	IgM	N	244 (40.66%)	265 (44.16%)	80 (13.33%)	2.389
		P	4 (0.66%)	7 (1.16%)	0 (00%)	0.302 NS

NS: Non-significant association between groups (p value > 0.05)
S: Significant association between groups (p value < 0.05)

Table 5. The relationship between the number of abortions and age groups.

			No. abortion					Total abortion	Total
			0	1	2	3	4		
Age	18-25 years	Count	181	42	4	18	3	67	248
		% within Age	73.0%	16.9%	1.6%	7.3%	1.2%	27.01%	100.0%
	26-35 years	Count	189	49	12	12	10	83	272
		% within Age	69.5%	18.0%	4.4%	4.4%	3.7%	30.51%	100.0%
	36-45 years	Count	57	7	10	1	5	23	80
		% within Age	71.3%	8.8%	12.5%	1.3%	6.3%	28.75%	100.0%
Total		Count	427	98	26	31	18	173	600
		% within Age	71.2%	16.3%	4.3%	5.2%	3.0%	28.83%	100.0%

women who miscarried four times was higher in the age group 36-45 years (3.6%). The highest percentage of miscarriages occurred in the age group 26-35 years, followed by the age group 36-45 years and then the age group 18-25 years according to the following percentages (30.51%, 28.75%, 27.01%) respectively. The percentages were close in all ages of the current study, which indicates that infection with the virus and the number of miscarriages among pregnant women have nothing to do with age.

DISCUSSION

Human cytomegalovirus (HCMV) is the leading cause of congenital infections worldwide and the most common cause of non-hereditary sensorineural hearing loss. There is no vaccine or other specific intervention to prevent congenital HCMV infection (13). The HCMV infection is affecting approximately 0.5% to 2% of all live births worldwide. Congenital transmission during pregnancy is a major concern, with a wide range of outcomes in fetuses and newborns ranging from asymptomatic infection to severe malformations, including sensorineural hearing loss, visual impairment, various neurological sequelae, developmental delay, and potentially fatal outcomes (14). In high-income countries, 5% to 10% of symptomatic infants with congenital HCMV infection die in early childhood, or 0.4% to 0.8% of all live-born infants with cHCMV. Congenital infection occurs when the virus is transmitted from mother to fetus and causes hearing and mental disability in newborns (15). Although the clinical diagnosis of HCMV during pregnancy has become progressively more accurate, HCMV counseling remains a challenge. Although universal prenatal serology is possible, its introduction into prenatal diagnosis still raises concerns about its true cost-effectiveness (16). HCMV infection occurs commonly in humans and is usually

characterized by a mild or asymptomatic condition with self-limited latent infection associated with mononucleosis syndrome (17).

This study aimed to determine the seroprevalence of anti-HCMV IgG and IgM antibodies among pregnant women in Diwaniyah Governorate – Iraq, for the period of one year.

Our results revealed that there were no significant differences (P value = 0.05) depending on residence location, as the positive percentage for IgG in urban and rural areas was close for both the rapid test and the ELISA test, respectively 192 (96.48%) and 382 (95.26%). The results also showed a difference with the study conducted in Iraq by Sarah Khayoun Shaker et al., which also revealed the presence of statistically significant differences according to the residence location variable, The rural infection percentage (15.8%) was highest than the urban infection rate(3.33%) (18). In the study conducted in Romania in 2024, by Cristiana Luiza Radoi et al. showed a higher seroprevalence of anti-HCMV IgG in rural areas (93.97% to 95.52%) compared to urban areas (93.52% to 94.27%), seroprevalence was higher in rural areas compared to urban regions, suggesting a relationship between HCMV infection and region of residence (19).

The results of current study demonstrated that out of the total research samples (600), 574 (95.7%) were found positive for IgG by the rapid test and 577 (96.2%) by the ELISA test, and the negative results were 26 (4.3%) and 23 (3.8%), respectively. It was also found that the positive IgM test results for the rapid test and ELISA were 9 (1.5%) and 11 (1.8%), and the negative results were 591 (98.5%) and 589 (98.2%), respectively, indicating that there were no statistically significant differences. The results are comparable with a study conducted in Bangladesh by Jahan Munira et al. in 2017 which reported that 100% of pregnant women in Bangladesh had HCMV IgG positivity, with 60% showing HCMV IgM positivity during pregnancy, suggesting a high incidence of HCMV infection among pregnant women (12). These results are also consistent with MK Murshid, WS Abood 2020 who found that the number and percentage of anti-HCMV-IgG antibodies was 180 (98.9%) (20). The majority of women were positive for HHCMV IgG antibodies, a result similar to what Naqid Ibrahim et al , reported in Iraqi In Kurdistan (2019), 95% of the pregnant women showed seropositivity for HCMV IgG antibodies (21). The results of the current study also showed a difference with the study conducted in 2023 in Iraq by Sarah Khayoon Shakir et al. on 76 positive samples (63.3%), while the negative samples for IgG through the rapid test were 44 samples (36.7%) (18).

The results of this study showed that the highest rate of HCMV infection was in the age group 26-35 years, where 260 (43.33%) and 259 (43.16%) samples were positive for IgG, while the negative samples were 5 (0.83%) and 7 (1.16%) for the IgM rapid test and ELISA, respectively, followed by the age group 18-25 years, and the lowest rate was in the age group 36-45 years. A study conducted by Partana et al. (2024) in Singapore found that the incidence of HCMV increases with age. They found that the incidence rate among pregnant women aged 20-29 years was 3.68%, among those aged 30-39 years it was 72.5%, and among women over 40 years it was 79.0%. These results are not consistent with our study (23).

In the current study, high rates of abortion were found at all ages, and the highest rate (30.51%) was found in the age group 26-35 years, followed by (28.75%) in the age group 36-45 years, while the age group 18-25 years was (27.01%), and the rate was (28.83%) in the total samples of the current study, which is a high rate due to the presence of a high rate of infection with IgM and IgG for HCMV. In a 2003 study conducted in Maysan Governorate, Iraq by Hassan S. et al., the results showed that women in the age group 20-24 years had a high rate of infection with HCMV (25%) and a high rate of abortion (27.5%) with high levels of IgG. The abortion rate (26.66%) was observed in patients aged 15-19 and 40-45 years, with a rate (33.33%) of IgM and IgG. Also, the results of this study are comparable with the study conducted by Khikani et al., (2024) that the abortion rate among women living in rural areas is higher compared to urban areas, indicating a relationship between HCMV infection, residential area, and socio-economic factors (22).

A study by Pourroostaei Ardakani et al. in Iran (2022), found that pregnant women with HCMV were more likely to miscarry than uninfected women. This suggests that HCMV plays an important role in recurrent pregnancy loss, as infection with the virus can lead to health problems that affect fetal growth and pregnancy continuation (24). In the 2017 study conducted by Farshidi, F. et al., 16 research papers were reviewed, providing 20 evidences on

the prevalence of HCMV in Iran. The prevalence of HCMV IgG and IgM as well as the primary infection rate among pregnant women were (90.6-94.9), (2.8-9.9), and (0.7-1.5), respectively. The prevalence of HCMV IgM among newborns was (0.09-1.2), while the prevalence of HCMV IgG and HCMV IgM among non-pregnant women was (70 - 86.8) and (1.5-7.6), respectively (25).

CONCLUSION

This study showed that the prevalence of HCMV infection among the studied population is relatively high. Therefore, deaths, complications, malformations, and injuries among fetuses and newborns (25). These findings also provide clear evidence to have an active compulsory screening program for women before marriage to prevent the consequences of congenital HCMV infection on fetuses and newborns. Moreover, the nationwide HCMV monitoring program would be justified as a part of marriage certificate issuing, assuming that only negative results of rapid test employed for ELISA test.

ACKNOWLEDGEMENTS

The authors extend our thanks and gratitude to Diwaniyah Governorate hospitals and private clinics for their assistance during the course of this study.

Ethics statement. The current study protocol was reviewed and approved by the Ethics Committee of the Shahid Beheshti University, Tehran, Iran (IR. SBU.RE C.1403.030). Also, informed consent was obtained from all participants in our study.

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