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Evaluation The Sero-Prevalence of Cytomegalovirus Infection among Abortion Women in Baqubah city

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Abstract

Cytomegalovirus (CMV) consider one of the opportunistic viruses, with a worldwide distribution, that can infect humans at any stage of life. It is a major cause of prenatal and perinatal infections and may lead to important complications in pregnancy. This study aims to assess the serum anti-CMV antibody (IgG and IgM) levels in blood of women who suffered abortion and correlation of aborted women with socio-demographic characteristics such as age, living, academic achievement, number of abortion, and fetus age This research was performed between November 2021 and January 2022 on 80 women suffering from abortion and 20 healthy control women in Al-Batool Teaching Hospital in Baqubah city. ELISA test results showed that the abortion women shown highest percentage of seropositive to CMV for IgG (96.3%) and (40%) IgM with a significant difference as compared to the control group ($P < 0.05$). Anti-CMV IgG and IgM seropositivity were higher in younger women (20-30 years). The current study found that the age group 21-30 years had the highest rate of abortions, with significant differences ($P < 0.05$). The findings of this study also show that uneducated women, and the women living in rural areas have a higher rate of abortion (58.8% and 72.5%) respectively with significant difference ($P < 0.05$). Furthermore, the current study found a

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statistically significant relationship between the number of miscarriages and fetal age, with the highest miscarriage rate for fetuses less than 3 months (98.3%).

Keywords: Cytomegalovirus, Abortion, IgG and IgM.

تقييم الانتشار المصلي لخمج الفيروس المضخم للخلايا (CMV) بين النساء المصابات بالإجهاض في مدينة بعقوبة

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الخلاصة

يعد الفيروس المضخم للخلايا (CMV) أحد الفيروسات الانتهازية، المنتشرة في جميع أنحاء العالم، والتي يمكن أن تصيب البشر في أي مرحلة من مراحل حياته. يعد هذا الفيروس سببا رئيسا للعدوى قبل الولادة وفي فترة الولادة وقد يؤدي إلى مضاعفات مهمة اثناء فترة الحمل. تهدف هذه الدراسه الى تقييم مستويات الاجسام المضاده (IgG وIgM) لcmv في دم النساء اللاتي عانين من الأجهاض وارتباطهن بالخصائص الأجتماعيه والديموغرافيه مثل العمر والمستوى المعيشي والتحصيل الدراسي وعدد مرات الاجهاض وعمر الجنين المجهض. أجري البحث في الفترة ما بين تشرين الثاني 2021 وكانون الثاني 2022 على 80 امرأة عانين من الإجهاض و 20 امرأة صحية في مستشفى البتول التعليمي في مدينة بعقوبة. أظهرت نتائج اختبار الاليزا أن النساء المجهضات أظهرن أعلى نسبة من إيجابيات المصل لـــ CMV بنسبة تقدر بـ (96.3%) لـ IgG و (40%) لـ IgM مع وجود فرق معنوي مقارنة بمجموعة السيطرة (P <0.05). كانت الأجسام المضادة من نوع IgM وIgG للفيروس المضخم للخلايا (HCMV) أعلى في النساء الأصغر سنًا (20-30 عام). كما وجدت الدراسة الحالية أن الفئات العمرية من (21-30) عام لديها أعلى معدل للإجهاض، مع وجود فروق معنويه ذات دلالة إحصائية (P <0.05) تشير نتائج هذه الدراسة أيضًا إلى أن النساء الغير متعلمات والنساء اللواتي يعشن في الريف سجلن معدل اعلى للإجهاض (58.8%, 72.5%) على التوالي مع اختلاف كبير (P <0.05)، وجدت الدراسة الحالية علاقة ذات دلالة إحصائية بين عدد حالات الإجهاض وعمر الجنين، مع أعلى معدل إجهاض للأجنة بعمر اقل من 3 أشهر(98.3%).

الكلمات المفتاحية: الفيروس المضخم للخلايا، الإجهاض، IgM وIgG.

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Introduction

The most common member of the herpes virus family is the cytomegalovirus (CMV). In affluent nations, human cytomegalovirus (HCMV) is the more frequent cause of congenital deformity brought on by viral intrauterine infection [1]. In the majority of the world, HCMV is endemic. In various geographic regions, the prevalence rate of HCMV ranges from (30-100)% [2]. Seroprevalence of HCMV is more common in elderly people, women, and those from lower socioeconomic groups [3].

HCMV infections can be contracted during pregnancy, infancy, or maturity through sexual contact, organ transplantation, or blood transfusions. Viral infection can spread horizontally (via sexual contact or contact with fluids like saliva, breast milk, maternal vaginal secretions, breast milk, and blood) as well as vertically (transplacentally from mother to fetus) [4,5].

The human herpes virus (HSV) with the highest genetic content is cytomegalovirus compared to HSV, it has a genomic DNA that is 240 kbp bigger. Only a few of the many proteins encoded by the virus (~200) have been characterized. One, a cell surface glycoprotein, acts as an Fc receptor that can nonspecifically bind the Fc portion of immunoglobulins. This may help infected cells evade immune elimination by providing a protective coating of irrelevant host immunoglobulins [6]. Because of its extraordinarily wide tissue tropism, HCMV may infect almost all of the body's organ systems [7]. HCMV infection may be either latent (non-productive), lytic (productive), asymptomatic, or symptomatic [8].

When a fetus or embryo naturally dies before it can survive on its own, it is known as a spontaneous abortion or pregnancy loss [9]. Twenty weeks is the threshold after which fetal death is referred to as a stillbirth. Numerous elements, including infections and genetic disorders, are linked to spontaneous abortion [10]. The first trimester accounts for around 80% of spontaneous pregnancies lost, and the rate drops with each additional week of gestation [11]. One of the many causes of prenatal harm that results in abortion is the cytomegalovirus, also known as human herpes virus type 5 [12].

Bacterial infections that rise from the lower genitals through the cervix to the uterus, such as chlamydia, gonorrhea, mycoplasma, and bacterial vaginosis (BV), are frequently to blame for infections connected to abortions. If left untreated, the infection may spread to the fallopian tubes and cause infertility [13]. In 40% of instances, primary CMV infection occurs in (0.15–2.00) % of pregnancies and may be passed on to the fetus [14]. While 0.87% of newborns in a community with high mother seropositivity had congenital CMV infection [15]. Urine testing should be done since saliva samples might yield false-positive findings when used to diagnose congenital CMV. After delivery, CMV-DNA might be found in urine samples for up to two years [16].

The aim of this study is to assess CMV seroprevalence (IgM and IgG) and to evaluate the correlation between viral prevalence and various socio-demographic factors such as age, academic achievement, number of abortions, and fetus age.

Materials and Methods

1. Collection of samples

The study sample was 80 aborted women, their ages ranged between 16-45 years who attended AL-Batool Teching Hospital in Baquba and 20 healthy women (control) with their ages ranged between 18-55 years. Collecting samples were from November 2021 to January 2022. 3 ml of venous blood was from each participant, blood put in gel tubes and left for 15 minutes to clot at room temperature (20-25)°C. Then serum samples were obtained by centrifuging clotted blood at 2500 rpm for 5 minutes. The serum samples were distributed in Eppendorf tubes and stored at (-20°C) until it used for the diagnosis of HCMV for the study groups.

2. Patient characteristics

Patient's information was collected by using questioner, including their name, age, address, academic achievement, number of children, number of abortion, the age of aborted fetus, the vaginal infections and ulcer.

3. Enzyme Linked Immunosorbent Assay (ELISA) for Human anti-cytomegalovirus (CMV) anti HCMV IgM measurement

Serum anti-HCMV antibodies (IgG and IgM) were measured using highly sensitive ELISA kits as directed by the manufacturer.

4. Statistical analysis

The statistical package SPSS version 25.0 and Graph pad prism version 6 were employed to carry out these analyses at significant level $P \leq 0.05$. The parameters (nominal and ordinal) were present as percentage frequencies, and significant differences between frequencies were assessed by Pearson-Chi-square test or two-tailed Fisher exact probability (*P*).

Results and Discussion

1. Immunological detection of cytomegalovirus by ELISA

For the IgM and IgG anti-CMV detection, the ELISA method was utilized. IgG and IgM anti-cytomegalovirus antibodies can be used as a simple method to identify individuals who may be at risk of contracting the virus. The existence of IgG, which was evident in the prior infection, does not stop reinfection or reactivation but may decrease the degree of pathogenicity. While IgM immunoglobulin, which forms right after infection and vanishes within a brief (16–20) weeks, was thought to be a sign of recent or acute infection [17]

According to the current study, 96.3% of women who had abortions were seropositive for HCMV. Numerous investigations were conducted in Iraq to determine the seropositivity of HCMV in women who had abortions, in a study conducted in Baquba showed the seropositivity of HCMV was 16.8% [18], while in Wasit Province had an HCMV seropositivity of 60.2%, Baghdad had a seropositivity of 10%, and Sulimania County had a seropositivity of 9.18% [19]. In Erbil city, Ali and Sharef, [20] found that the serupositivity of HCMV was 30.5%. In Babylon AL-Hajjar and Al-Mousawi, [21] discovered (89%) positive for HCMV IgG. These findings in agreement the findings of the current investigation. In a study conducted show in Basra, in

southern Iraq, has a high seroprevalence of HCMV IgG antibodies (90%) [22]. In Najaf, Hamoud *et al.*[23] discovered a relatively high HCMV seroprevalence (95.09%), with no discernible variations between women who experienced multiple miscarriages and those who experienced a normal pregnancy.

According to Awadh *et al.* [24] the seropositivity of a specific HCMV was found to be 100% in abortions performed during pregnancy in Tanzania City. This seropositivity is higher than the seropositivity rates of (46.8%) and (65.6%) that were previously reported by France and Durango city, respectively. The current study's observed seropositivity is much greater than that of an earlier study among healthy pregnant women conducted in the same environment [25].

The conducted study showed there was significant differences (P <0.05) between IgG and level IgM in pregnant women who had abortions. The positivity of IgG was highest in patients (96.3%) than healthy women (control) (60.0%) with odd ratio (17.11) and relative risk (3.17). In contrast, the positivity of IgM was lower in patients (40.0%)than healthy women (5.0%)with odd ratio (12.67) and relative risk (1.35) as shown in **Table 1**.

Table 1: Comparative of positivity of IgG and IgM for HCMV between research groups calculated using chi-square test.

			GROUPS		TOTAL	STATISTICS
			patients (n=80)	healthy (n=20)		
IgG	Positive	n	77	12	89	*P*<0.001*** OR= 17.11 (4.22-25.11) RR= 3.17 (1.02-8.5)
		%	96.3%	60.0%	89.0%	
	Negative	n	3	8	11	
		%	3.8%	40.0%	11.0%	
IgM	Positive	n	32	1	33	*P*<0.01** OR= 12.67 (1.06-20.12) RR= 1.35 (1.15-1.60)
		%	40.0%	5.0%	33.0%	
	Negative	n	48	19	67	
		%	60.0%	95.0%	67.0%	

The current investigation revealed high HCMV IgG positivity (96.3%) and positive HCMV IgM (40.0%) in pregnant women who had abortions. Anwar and Al-Bayati, [26] in Samara discovered that 30% of patients were positive for HCMV IgM and 70% were positive for HCMV IgG, and these results were virtually comparable with results from previous studies.

Ali and Al-Ahmer, [27] reported significant levels of CMV IgG positivity 72.3% and HCMV IgM positivity 72.2% in pregnant women who underwent abortions were. In Baquba the finding of Hussein *et al*. [28] showed that 36% HCMV positivity in pregnant women with spontaneous abortion. However, the presence of IgM indicates that the women had recently been infected or re-infected with HCMV, and that the IgM was produced shortly after infection and dissipated within a short period of time. The presence of HCMV IgG indicated that the women had previously been infected with HCMV [29].

Our results are in line with the findings of Khudhur *et al.*[30], who identified HCMV in 70% of abortion women using IgG and IgM antibodies. In Baghdad, miscarriage women had the highest proportion of HCMV IgG 40% and IgM 25% seropositives, according to Ramadhan and Jihad, [31]. Using the ELISA technique, Hussein *et al.* [32] in Baghdad found that anti-CMV IgG and IgM antibodies were present in (85%) and (10%) of women who had abortions, respectively.

Studies vary by nation, however Edmunds *et al.* [33] did their research on 130 aborted women and pregnant women, and they found that they could identify positive IgM in 70 cases and positive IgG in 97 infected women. Additionally, a rise in seropositive CMV-IgG in connection to infection and abortion was observed by Mahdi *et al.* [34]. HCMV inhibits cytotrophoblast differentiation and invasiveness as a result of placental infection, might explain why early abortion occurs in women with primary infection [35]. CMV can lead to substantial damage to the fetus and as the damage done in utero cannot be reverted, control of intrauterine CMV infection is important. Hence, prevention of CMV infection is essential by a screening of pregnant women, to reduce the fatal outcome of the pregnancy occurring due to the CMV infection [36].

The fact that the majority of women of reproductive age are HCMV seropositive and get the virus during early infancy, either through prenatal or postnatal transmission, is one of several factors that may be to blame for all samples that tested positive for IgG [37].

According to earlier studies, a first HCMV infection during pregnancy carries a far greater chance of leading to a congenital infection than subsequent infections. Congenital HCMV infection was found in 32.3% of babies delivered of mothers who had primary infections during pregnancy. On the other hand, 1.4% of babies delivered of pregnant women with recurrent HCMV infections had congenital infection. In all of the examinations of pregnant women with suspected primary infection, IgM was used as a marker for main infection in IgG-positive women. Since then, it has been found that women who have had long-term IgG positivity may also have long-term IgM positivity, indicating that IgM is an indication of persistent infection [38]. According to Hamoud et al., 2021, study results revealed high HCMV IgG antibodies titers among study groups which raises a question about these increased levels of prolonged existence antibodies and their impact on pregnancy progression and the probability of recurrent activation of the virus during pregnancy. Study results reported that there was an increment in HCMV IgG titer in the controls and this may be due to the impaired immunity of the pregnant women and the possibility of virus reactivation in this situation since the controls in this study were of pregnant women [23]. The HCMV IgG avidity test is dependent on the sensitivity of the HCMV IgM testing since the rise in IgM titer may happen before the rise in IgG titer following the initial infection. confirming that all HCMV IgM-positive samples have been located and their IgG avidity assessed [21].

The fact that the study cohort was made up of women who said they had signs and symptoms during their current pregnancy may help to explain the study findings that pregnant women with signs and symptoms were much more likely to have HCMV IgG antibodies. Awadh *et al.* [24] findings on IgM seropositivity in abortions pregnant contradict our findings, which showed that (40 %) of abortions during pregnancy were IgM seropositive.

HCMV IgM has been shown to peak in the first (1 – 3) months after primary infections in pregnant women and then persists at low levels for 18 to 39 weeks, with detection depending on both individual patients and IgM antibody assay sensitivity [39]. The highest rate of abortion takes place during the first trimester of pregnancy in women with CMV infection [22]. The study conclusions indicated that *Toxoplasma* and HCMV were more significant causes of

abortion than viruses. Alajeeli and Obaid, in 2022 recommended s more studies to ascertain the relationship between viral infection and abortion as a result [40]. The role of infection in recurrent pregnancy loss (RPL) has been thoroughly investigated in recent years, with viruses HCMV in particular getting more attention because of their propensity to produce intrauterine chronic/recurrent infections. The results show that recurrent pregnancy loss is significantly more common in women who had previously been exposed to CMV, as shown by a positive IgG antibody (RPL) [41].

2. Relation of abortions number with socio-demographic characteristics of patients

The conducted study shown there are significant differences (*P* <0.05) between Age groups, Living, and fetus age and abortions numbers of pregnant women. The abortion pregnant with 1 and 2-3 abortions score the highest percentage was found at groups 21-30 years (50.0% and 60.0%), whereas the pregnant with 4-5 abortions score the highest percentage was found at groups 31-40 years (50.0%) compared to others groups. The abortion pregnant with 1, 2-3, and 4-5 abortions score the highest percentage pregnant living in rural (58.8%, 85.0%, and 66.7%) respectively, than urban. The abortion pregnant with 1, 2-3, and 4-5 abortions score highest percentage in fetus age ≤3 months (58.8%, 85.0%, and 66.7%) respectively, than >3 months. There was no significant differences between bortions numbers and women education and disorders **(Table 2).**

Table 2: Relation of abortions number of pregnant with socio-demographic characteristics calculated by chi-square test.

			Abortion numbers			Total	*P* value
			1 (n=34)	2-3 (n=40)	4-5 (n=6)		
Age groups (years)	≤20	N	10	3	0	13	*P*<0.05*
		%	29.4%	7.5%	0.0%	16.3%	
	21-30	N	17	24	2	43	
		%	50.0%	60.0%	33.3%	53.8%	
	31-40	N	7	11	3	21	
		%	20.6%	27.5%	50.0%	26.3%	
	41-50	N	0	2	1	3	
		%	0.0%	5.0%	16.7%	3.8%	
Life style	Rural	N	20	34	4	58	*P*<0.05*
		%	58.8%	85.0%	66.7%	72.5%	

	Urban	N	14	6	2	22	
		%	41.2%	15.0%	33.3%	27.5%	
Education	Educated	N	19	12	2	33	*P*>0.05
		%	55.9%	30.0%	33.3%	41.3%	
	uneducated	N	15	28	4	47	
		%	44.1%	70.0%	66.7%	58.8%	
Fetus age (months)	≤3	N	20	34	4	58	*P*<0.05*
		%	58.8%	85.0%	66.7%	72.5%	
	>3	N	14	6	2	22	
		%	41.2%	15.0%	33.3%	27.5%	
Disorders	Yes	N	17	19	2	38	*P*>0.05
		%	50.0%	47.5%	33.3%	47.5%	
	No	N	17	21	4	42	
		%	50.0%	52.5%	66.7%	52.5%	

Based on age groups, the present study showed the age groups 21-30years old scored high frequency of abortions with significant different. The drop in the abortion rate was greatest, 46%, among young women aged 15 to 19 years, according to Jones and Jerman, [42]. This is in line with a 23% decline in adolescent birth rates during the same time period [43]. According to new study, the majority of the drop in adolescent fertility between 2007 and 2012 was caused by changes in contraceptive use, particularly an increasing dependence on long-acting reversible contraception (LARC) such the IUD (intrauterine device) and implants [42]. The high prevalence of abortion among sexually active teens emphasizes the urgent need to enhance global adolescent family planning. Unmarried teens may benefit most from interventions aimed at preventing unwanted pregnancy and reducing unsafe abortion. We need to understand how marriage and other social circumstances impact reproductive health outcomes rather than treating teenagers as a homogeneous group [44].

In terms of living, the current study found that women who live in rural areas had a higher rate of abortions than women who live in urban areas. In a previous study, the value of induced abortions in low-income women was poor; the majority had them at health facilities, but 4% had them at home in communities with medical colleges nearby, while no difficulties occurred; there were also misunderstandings and a lack of information [45]. Raza *et al.* [46] found that the age group (15-25) years had the greatest rate of women who had abortions in Kirkuk, Iraq. Mahmood and Kahya, [47] observed that the IgG sero-prevalence rate of HCMV infection was

highest in the second trimester of pregnancy, whereas the highest rate of abortion was found to be 64 percent in the first trimester of pregnancy women in Mousl city, Iraq.

The current study found a substantial difference between the number of abortions and the age of the fetus, with the fetus age of 3 months accounting for a high percentage of abortions. A small amount of evidence suggested that medical abortion in the late first trimester has a wide range of effectiveness, highlighting the necessity for well-designed studies in this gestational age range [48]. Infections with the Cytomegalovirus can cause congenital defects in children, especially if they are contracted during the first trimester of pregnancy. When it comes to HCMV infection during pregnancy, 40% of cases pass through the placenta and infect the fetus, potentially leading to cytomegalovirus syndrome [49]. The research found no correlation between the frequency of abortions with women's education or pregnancy-related diseases. Previous findings imply that more efforts are needed to educate women about early pregnancy detection, particularly younger and less educated women. Furthermore, all women required knowledge on the availability of the legal abortion in the first trimester to facilitate timely access to abortion services [50]. Cytomegalovirus infections have been linked to the cause of abortion in women, with the infection having a stronger link to the presence of candida and a weaker link to other bacterial infections [27].

Relation of age groups pregnant with IgG, IgM of CMV of patients

The conducted study shows there is no significant differences (P >0.05) between IgG, IgM, qPCR and age groups of pregnant women **(Table 3).**

Table 3: relation age groups of pregnant with IgG, IgM of HCMV are calculated by chi-square test.

			Age groups (years)				Total	P value
			≤20	21-30	31-40	41-50		
IgG	positive	n	12	41	21	3	77	P >0.05
		%	92.3%	95.3%	100%	100%	96.3%	
	negative	n	1	2	0	0	3	
		%	7.7%	4.7%	0.0%	0.0%	3.8%	
IgM	positive	n	6	21	5	0	32	P >0.05
		%	46.2%	48.8%	23.8%	0.0%	40.0%	
	negative	n	7	22	16	3	48	
		%	53.8%	51.2%	76.2%	100%	60.0%	

The main and re-infection of latent HCMV infection occurred at the highest incidence in the age range 36-40 years old, according to Naame *et al* .[22] and these findings corroborated our findings, which revealed that the highest rate of HCMV infection was in women aged 21-30 years old. According to our findings, the greatest antibody titer was found in women under the age of 25 years, and women who had never had an abortion had the highest HCMV infection (55%), while there was no significant difference between the IgG and IgM values in the categories of number of pregnancies [30].

Study results confirmed the significant association between CMV infection and (abortion, stillbirths, Age at Menarche, Regularity of Menstrual Cycle, Age at Married, Interval between last pregnancy & present Pregnancy, Causes of Abortion, Number of Para, Type of Previous Delivery to Present Delivery, IgG and IgM Test Results, residence, education level and employment status). It was concluded that socio-economic status of the tested women has no significant effects on the rates of anti-CMV IgG and IgM seropositive results [51].

Study results constitutes the first population-based seroprevalence data based on a large sample representative for the adult population living in Germany. These data indicate that a substantial proportion of women in childbearing age were susceptible to primary CMV infection. Further seroprevalence studies with more recent data are necessary to evaluate CMV seroprevalence in the German population and to better understand the epidemiology of CMV infection. As long as no effective vaccine is commercially available, the primary prevention measure should be educating women about CMV risk reduction measures [52].

Diagnosis of primary maternal CMV in pregnancy should be based on seroconversion in pregnancy (de novo appearance of virus-specific (IgG) in the serum of pregnant women who were previously seronegative) or on detection of specific (IgM) and IgG antibodies in association with low IgG avidity. Prenatal diagnosis of fetal CMV is imperfect and based on amniocentesis performed at least 8 weeks after presumed maternal infection and after 17 weeks

of gestation. Hygiene information and education of pregnant women is currently the most effective strategy for prevention of CMV infection [53].

According to the findings, (50%) of all women of reproductive age (>14 years) are vulnerable to HCMV infection during pregnancy. HCMV testing during pregnancy and alerting seronegative women about HCMV risk reduction strategies might help to avoid congenital HCMV infections and their severe effects [54].

According to a previous study, the number of people who were both IgG and IgM sero positive was 50 (31.1%), whereas the number of people who were both IgG and IgM sero negative (control) was 37. (23%). Young women showed an increase in seropositivity, reaching a peak of (46.6 %) in the 15-19 year age group. In the age range of (30-35) years, however, the proportion was smaller (9.6%) [55].

Conclusions

The serum anti-CMV antibody (IgG and IgM) levels were significantly higher in abortion women as compared to the control group, and the age group 21–30 years old and women living in rural areas had the highest rate of abortion.

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