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Immunological Indicators in Aborted Women Infected with Human Cytomegalovirus in Karbala City, Iraq

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ABSTRACT

Introduction: In affluent countries, human cytomegalovirus (CMV) is the most common cause of congenital malformations caused by viral intrauterine infection. HCMV infection is the infectious cause of deafness, visual loss, mental retardation, and other neurodevelopmental disorders in children, causing significant morbidity and mortality in infants.

Objective: The work was conducted to identify the rates of spontaneous abortion occurrence among the age groups, stages of pregnancy, and detection of the type of abortion that most frequently occurred in the pregnant women community.

Patients and Methods: 65 Blood samples were collected from abortifacients women at the moment of miscarriage. Each pregnant woman had five milliliters of blood collected to separate the sera. The CMV-IgM and CMV-IgG antibody levels in the patient's serum were determined using an ELISA assay.

Results: The outcome of antibodies against CMV were found in 50 of 65 (76.92%) aborted mothers, while 15 (23.07%) of 65 women had abortions for unknown reasons. Meanwhile, most pregnant women who had abortions tested positive for CMV-IgG, with 36 (72%) of them being positive, the number of people who tested positive for CMV-IgM was 14 (28%). The age group 25–34 years old was the most infected with CMV infection, accounting for 25 (50%) of the cases. 96% and 4% of patients were infected in urban and the countryside regions, respectively.

Conclusion: Based on this research, spontaneous abortion rates are increasing within young age groups, particularly for missed abortions during the first trimester of pregnancy.

Keywords: Human Cytomegalovirus, CMV, Congenital infection, HCMV

INTRODUCTION

The prototypical member of the Betaherpesvirinae subfamily Herpesviridae is Cytomegalovirus (CMV) [(1)]. In most parts of the world, CMV is endemic. The seroprevalence of HCMV varies by geographic region, ranging from 30 to 100 percent[(2)].

This virus is transmitted by sexual intercourse, organ transplantation, or blood transfusion in the perinatal period and infancy, as well as in adults [(3)]. HCMV, like other herpesviruses, causes a long-term infection, and its reservoir is primarily monocytes and polymorphonuclear leukocytes, from which the virus is shed many years later (present in the throat and urine). The appearance of so-called "Owl-eyes" is caused by the presence of distinctive intracellular inclusions surrounded by a halo of poor reflection. The virus infects the salivary gland, the breast epithelium, the prostate, the endometrium, the kidney tubules, and other organs such as the bone marrow and the lungs.

Milk, sperm, cervical secretions, blood products, and urine can all be used to isolate it. HCMV infection can be latent (non-productive), lytic (productive), asymptomatic (non-symptomatic), or symptomatic [(4)].

PATIENTS AND METHODS

1. Patients:

The research included 50 pregnant women who had an abortion and were between the ages of 15 and 45. Age, parity, gynecologic and medical history of abortion, and domicile were all covered in a structured interview utilizing a standard mother questionnaire. The research participants had a clinical examination and laboratory tests to rule out other reasons for fetal loss, such as hypertension, diabetes mellitus, syphilis, Rh (rhesus) incompatibility, and physical causes of abortion.

2. Immunological Assays:

Immunological Assays: The ELISA method was used to detect CMV-IgM and CMV-IgG utilizing kits. Sigma Diagnostics USA provided the kits, and the methods were done according to the manufacturer's instructions.

Principle of the assay

The surface of tiny wells is covered with purified CMV antigen. The wells are filled with diluted patient serum, and the Cytomegalovirus IgG specific-antibody, if present, attaches to the antigen. Everything that isn't bound is washed away. The HRP-conjugate is introduced to the Ab-Ag complex and binds to it. The remaining HRP-conjugate is rinsed away, and a TMB reagent solution was then added.

The catalytic process of the enzyme conjugate is halted at a particular time. The quantity of CMV IgG-specific antibodies in the sample determines the intensity of the color generated. The findings are read using a small well reader in tandem with calibrators and controls.

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Ethical Consideration:

The study was approved by the Ethics Board of AlSafwa University College, All participants in the research agreed, and the work was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

RESULTS

The present study looked for HCMV IgG and IgM antibodies in 65 pregnant women who had abortions and found that 50 of them 76.92% were seropositive, whereas 15 (23.07%) were seronegative, as shown in **Figure 1**.

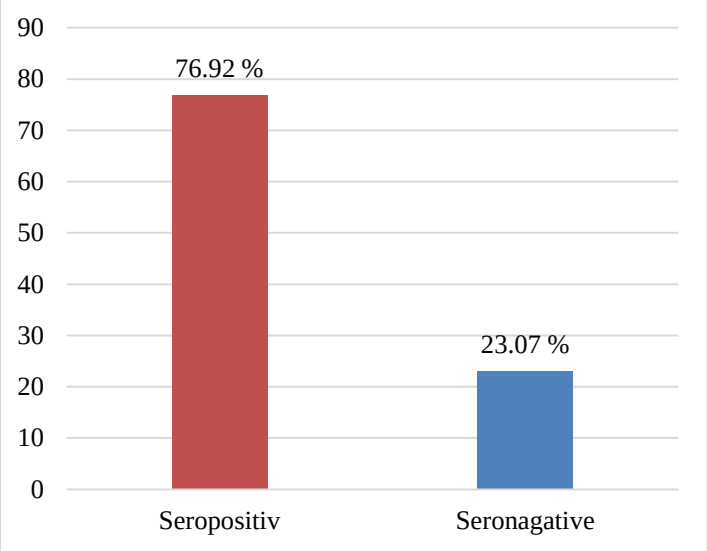

Figure (1): HCMV antibody seropositive and seronegative women that were aborted.

Table (1): describes the serological status of pregnant mothers, including the frequency of IgG Ab 36 (72%) cases per 50 seropositive HCMV cases and IgM Ab 14 (28%) cases per 50 seropositive HCMV cases.

Seropositive	NO.	%
	50	76.92
IgG	36	72
IgM	14	28
seronegative	15	23.07

The average age of pregnant mothers who had abortions induced by CMV, with the more affected age group being between 25 and 34 years old.

Table 2 shows that the age group between 15 and 24 years had 25 (50%) instances, the age group between 35 and 44 years had 13 (26%) cases, and the age group between 35 and 44 years had 12 (24%) cases.

Table (2): displays the average age of pregnant women who had abortions owing to CMV infection.

Age group (year)	No.	%
15-24	13	26
25-34	25	50
35-44	12	24
Total No.	50	100

Figure 2 shows the most infected pregnant mothers with abortion had Cytomegalovirus infection in the urban region 48% and the rural area 4%, respectively.

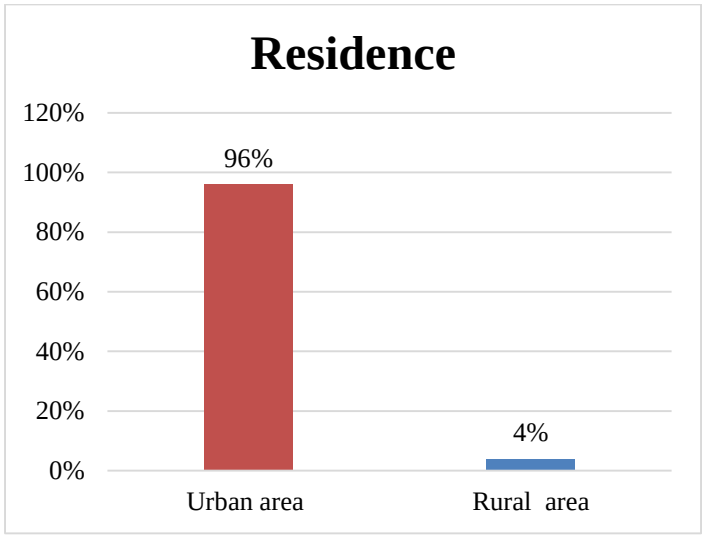

Figure (2): Prevalence of HCMV infection in aborted mothers by residence.

Figure 3 depicts the prevalence of Cytomegalovirus infection in pregnant women based on the timing of abortion. The largest risk of infection occurred in the first trimester 78 %, followed by the second trimester 8 %, and finally the third trimester 3 %, 6 %.

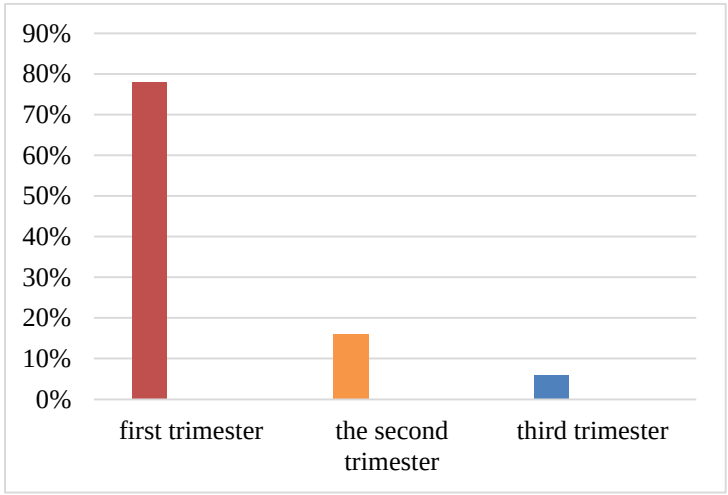

Figure (3): Pregnant mothers with CMV infection are distributed according to the amount of time their fetus spends in the womb.

Figure 4 illustrates the prevalence of Cytomegalovirus infection in pregnant women based on the type of abortion. Missed abortion has the highest risk of infection 66%, followed by incomplete abortion 12%, and inevitable abortion 6%, although threatened abortion has the lowest prevalence, 2 (4 %).

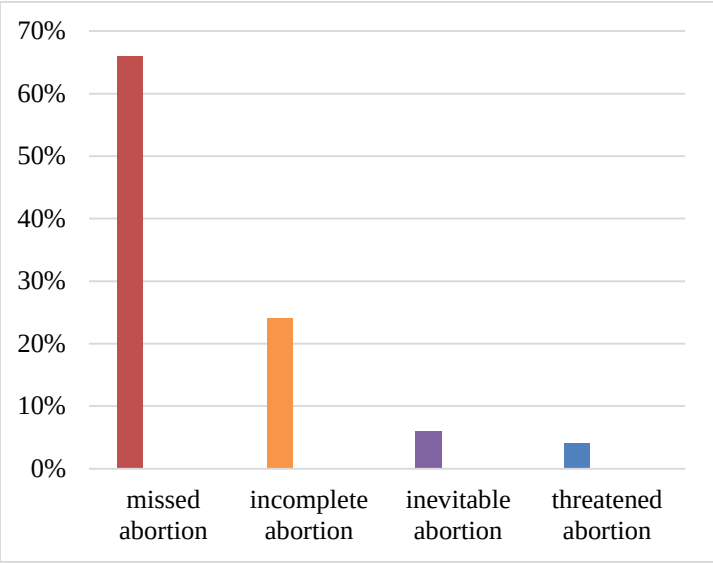

Figure (4): Pregnant women infected with CMV are distributed according to the type of abortion they had.

Figure 5 depicts the prevalence of Cytomegalovirus infection in pregnant women based on the timing of abortion. First-time abortion has the highest incidence of infection 54 %, whereas recurrent abortion has the lowest rate 23 %, 46 %.

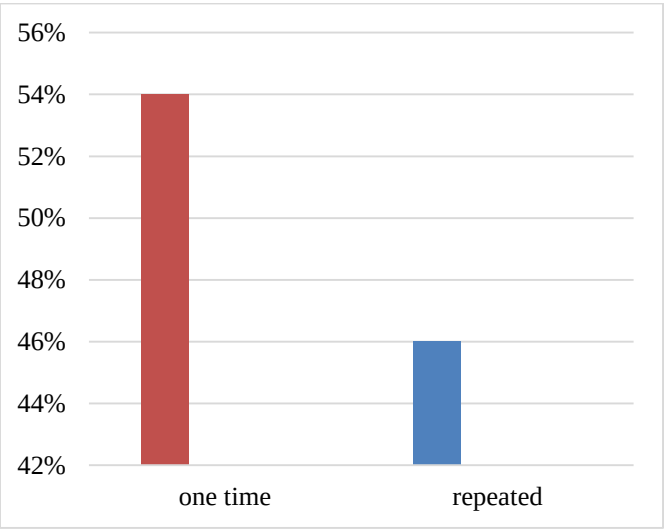

Figure (5): Pregnant mothers with CMV infection are distributed depending on the number of abortions that occur.

DISCUSSION

We attempted to establish the percentage levels of CMV-IgG and IgM seropositivity among pregnant mothers who had abortions in the completed study. This test revealed that 50 (76.92%) of the 65 instances were seropositive, with 15 (23.07%) having a condemnatory serotype or having additional reasons for abortion.

These results are consistent with the findings of the researcher Salih in 2013 [5], in Sulaymaniyah governorate, where he found that the IgG percentage was 90.2% and the IgM percentage was 9.18%. The results are also consistent with a study conducted in the province of Mosul, the prevalence of CMV-IgG and IgM viral infection among pregnant mothers was 90 % and 2.5 %, respectively, according to the study [6]. In addition, a study done in the Baghdad province in 2014 revealed that the proportion of IgG was \85 % greater than the exhibited IgM, respectively, among abortive women [7]. CMV-IgG positive was found in 92.8 % of pregnant women's serum, whereas IgM positivity was found in 5.8% [8].

In addition, additional investigations in Iran found that CMV-IgG was more prevalent than CMV-IgM, with 93 % of patients seropositive for HCMV-IgG and only 5.4 % seropositive for HCMV-IgM. Another research in Iran found that 94 % of the women had CMV-IgG, whereas only 5.2 % had CMV-IgM [9,10].

The researchers found a rise in the serum prevalence of CMV-IgG antibodies in this and other studies, and the explanation for this is due to past exposure to pregnant mothers, who now have immunity to CMV, especially when they have been exposed to CMV (negative IgM). These ladies might be considered immune, as their initial CMV infection happened before they were pregnant.

Furthermore, pre-pregnancy immunity to Cytomegalovirus provides only protective effects against intrauterine transmission, and infected children delivered to mothers who were allergic before pregnancy may suffer severe effects [11,12]. Previous Cytomegalovirus protection isn't 100% effective against re-infection or direct transfer from the mother to the fetus [13,14]. According to other studies, the incidence of neonatal CMV infection rises as maternal CMV seroprevalence rises. CMV transmission through the placenta in immunocompromised women may be due to viral reactivation [15], or during pregnancy, infection with a new strain of CMV (re-infection) [16].

Table 2 illustrates the age distribution of mothers who have miscarried. With 25 (50 %) cases, the age group 25-34 years was the most afflicted, followed by 15-24 years with 13 cases 26 %. With 12 instances, the lowest age group affected was 35-44 years old 24 %. These results are consistent with the findings of the

researcher in the governorate of Tikrit [17], which showed a higher rate of positivity in the age group 27-32 years. Another study also showed a positive rate of 94% at the age of 25-34 years [10]. According to the study, women who had abortions were on average 25.6 ±7.6 years old. Arabpour [9]. The 20-24 age group was shown to be 55 % more impacted, whereas the 25-26 age range was 30 %.

In comparison to another study, it indicated that IgG seropositivity was 75 percent in women under the age of 20, The serum prevalence of CMV-IgM was lower in women aged 26-30 years of 8%, and higher at 17% in women aged 16-20 years [18]. It was also shown that the age range 41-45 years old had the highest prevalence of HCMV antibody, with 100percent IgG for IgM and 11 % for IgM, whereas the younger group 16-20 years of age had the lowest prevalence of HCMV-IgG antibody, with 75%. However, the IgM with the greatest percentage is 17%. The cause may be indicated by many factors such as miscarriage and multiple pregnancies, as well as physiological and hormonal changes, and increased access to the virus with age [7]. Figure 2 showed an increase in the prevalence of aborted women with high serum positivity for CMV in urban areas by 96% compared to 4% in rural areas.

These findings opposed those of researchers in Tikrit Province [8], who found that women in rural regions were 92 % seropositive for CMV-IgG, whereas seropositivity in urban areas was 90.9 %. The results of the current study are in agreement with the findings of researchers in Sulaymaniyah [5], which reported the presence of cytomegalovirus IgG and IgM among laboratory women according to their area of residence. They discovered a 91 % rise in the frequency of aborted women with high CMV serum positive in metropolitan settings. CMV-IgG antibody positivity was 8 %, while CMV-IgM antibody positivity was 8%. CMV-IgG antibodies were found in 89 % of rural residents, while CMV-IgM antibodies were found in 11 %. Furthermore, the present research is backed by Arabpour's claims [9]. The seroprevalence of HCMV was found to be greater in rural women than in urban women, according to the study.

Although hypothesizing the probable function of regional influence on HCMV seroprevalence may be the infection route, identical effects were found between locations of residency. In the countryside. After delivery, saliva may be the most common way for the virus to spread. Inadequate sanitation is most likely the mechanism through which the virus is spread early in life among babies and young children [19]. On the other hand, it appears that later in life, at reproductive age, urban sexual transmission is the major route of infection.

Figure 3 shows that 78% suffered from a miscarriage during the first stage of pregnancy, while the percentages decreased with the second 16% and third 6% stages of pregnancy. The reason may be that the majority of miscarriages occurred in the first 12 weeks of pregnancy because the first stage of pregnancy is considered a period fraught with many complications, which is characterized by poor implantation of the fetus, and incomplete growth of the placenta, which constitutes an important barrier to prevent some (Impaired implantation). The pathogens that infect the mother from reaching the fetus, and an invasion occurs Spontaneous abortion occurs early, while invasive trophoblasts. Premature labor in the later period (preeclampsia) preeclampsia occurs. This result agreed with [20]. Which was shown through Hassan's study. The percentages of women who had abortions during the first trimester of pregnancy were higher than two-thirds of the second pregnancy and the third.

Figure 4 indicates that missed abortion is the most common type of miscarriage among the community of women who have abortions in Karbala Governorate during the study period, at a rate of 66%, followed by incomplete abortion at 24%, then inevitable abortion at 6%, followed by threatened abortion and who scored the lowest percentages 4%. This result is a close approximation of what was reported by [21] who showed that the incidence of forgotten abortion scored second place after threatened abortion, followed by incomplete and inevitable miscarriage, the reasons for the occurrence of forgotten abortion.

In general, the same causes of spontaneous miscarriage or early pregnancy failure are represented by various factors such as the mother's age, Chromosoma abnormalities, endocrine disorders, and thrombophilia. In addition to environmental factors and bacterial infections [22], viral infections during pregnancy are dangerous for the fetus, such as being infected with rubella virus, Cytomegalovirus, and herpes simplex virus, as they are among the known causes of congenital anomalies and morbidity, and a recent study conducted in Korea by researchers [23] Coxsackievirus B is an important cause of forgotten miscarriage.

It is noticed in **Figure 5** that there was no significant difference in the rates of one-time and repeated-time abortions, but the highest rates were recorded with cases that had first-time abortions 54%. These results agree with the Al-dorri study showed in Tikrit governorate / Iraq that there was no significant difference between the incidence of one-time and repeated time abortions [17].

CONCLUSION

1. Seropositive of pregnant women with CMV high at 25-34 years of age from urban areas especially in the first trimester.

2. Missed abortion is the most common type of miscarriage.

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Author contribution: Each of the researchers has a contribution rate equal to the rest of the researchers in the study.

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