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Molecular and Immunological Study for The Relationship of CMV in Miscarriage in Women with a History of Repeated Abortion in Najaf Governorate

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Abstract

The study was conducted in Al-Zahra Hospital for Maternity and Children in Najaf Governorate for the period from 1/8/2024 to 1/1/2025., The stream study directed to establish the occurrence of CMV infectivity in women who experienced of continual miscarriage and perform a analyze of the amount of miscarriage situations arising in the Najaf governorate. The study was divided into two parts. The first part was aiming to determine the frequency of CMV (IgM), CMV (IgG) antibodies, using ELISA technique among miscarriage pregnant women with a history of repeated abortion was attended to the hospital from August to January (2024-2025). The first part of study indicated that there were significant differences in favor of detection of CMV-IgM antibody by 76% compared to 24% detection of CMV-IgG antibody, the results of the study showed that the highest rate of miscarriage in women was within the age group (20-25) years by 39%, while the age group (43-38) years gave the lowest percentage where it was 8%. also, this study showed that 27% of Women had miscarriages in the fifth month of pregnancy, while 25% in the fourth month. The lowest rate of miscarriage was recorded in the first month of pregnancy, at 9%. The fallouts confirmed that there stayed considerable alterations in the quantity of terminated women in advance of urban expanses than in rustic ranges. The second part of the study we have (100) placental soft tissue was the application of localized hybridization technique using ISH technique. the tissue sample examined by histopathology's in training Labs form the hospital of AL-Sader teaching hospital in Al Najaf Al Ashraf / Iraq.

Keywords: CMV, antibody**,** pregnancy, ELISA, herpes virus

INTRODUCTION

CMV virus is a virus from the herpes virus (these herpes viruses tend to remain dormant in the body of the infected person and outbreak in various causes related to immunosuppression. Most people in the community develop CMV in their childhood (between 80-85%) and develop antibiotics that provide protection Partially against the virus. Those who have never been infected with the virus remain vulnerable to the initial infection with the virus, which often passes without symptoms but is sometimes cold-like or infectious mononucleosis accompanied by fever for a long period of time, muscle pain and weakness. [1–3]. If infection occurs during pregnancy, there is a possibility that the fetus will become infected through parts of the virus transmitted from the placenta to it. In contrast to the adult, the fetus is difficult to deal with the virus that attacks many devices causing significant damage As a result of the primary infection of the mother during pregnancy, about 40% of fetuses become infected with the virus through the placenta while they are still in the womb, meaning that in 60% of cases there is no infection of the fetus with the virus despite the mother's disease[4–6].

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Regularly, this septicity arises before the woman recognizes she is pregnant. Essential contamination is probable to have a more main influence on the fetus than repeated viruses **[7,8].** CMV affects a high proportion of people during their lifetime and after recovery from disease, Hiding in a group of leukocytes. Although this virus is not considered a serious health factor, it is a major factor that threatens the health of fetuses. Its clinical manifestations range from asymptomatic (90% of cases) to severe killer Damages, and rare deaths after late abortion The primary infection can be permeated by periodic re-activations that lead to repeated infection [9].

Abortion is defined as the loss of a fetus before reaching the twentieth pregnancy, and according to statistics, one out of every 5 confirmed pregnancies end in abortion and there are many women who undergo an abortion without realizing the presence of pregnancy. Basically, CMV infection during pregnancy is much more complicated than other infections, due to the ability of the virus to be revitalized repeatedly during the reproductive age and then transmitted to the fetus despite the mother's immunity [10].

The current study aimed to determine the presence of CMV infection in women who suffered of repeated miscarriage and conduct a survey of the number of miscarriage cases occurring in the Najaf governorate during the study period by conducting it in a specialized hospital for childbirth and children to determine the extent of its association with CMV infection by tow technique : serological detecting IgG and IgM

Most of the symptoms associated with miscarriage are bloody bleeding, ranging from mild bleeding to drops to severe bleeding, and the bleeding may contain blood clotting or tissue belonging to the fetus. Especially if it is small and lasts for a short period, but the length of the bleeding period and being very severe, it gives a sure indication that the fetus has been lost and abortion is often accompanied by other symptoms or other signs, including severe contraction of the lining of the uterus as well as severe pain interrupted period and then return as those that are Accompanying labor (delivery), and this may be accompanied by fluid flow, especially if the pregnancy is advanced and in special cases women suffer from loss of appetite, chest pain and extreme fatigue [11–14]]. These disorders occur in some factors responsible for the process of blood clotting, especially factors (x11) [15].

Endocrine Disorders

Abortions occur in women who suffer from disorders in the effectiveness of the thyroid gland or who have diabetes, in which case these women need normal care and in the absence of such care the pregnancy becomes threatened [16–18].

Materials and Methods

The study was conducted at Al-Zahraa Hospital for childbirth and children in AL- Najaf Governorate / Iraq for the period from 1/8/2024 to 1/1/2025, which included:

- collected of (140) cases of repeated abortion women referred to the hospital. They were examined by drawing 5 ml of blood and then was transferred blood samples to sterile test tubes were left at room temperature for 30 minutes to coagulate and then the serum was isolated using a centrifuge at a speed of 1500 r / min and for 5 minutes the serums were kept at a temperature of 20 ° C until use.
- The second part of the work was taken of 100 a placenta tissue for aborted women and was genetically studied using CISH technique to detect the presence of CMV. CISH Employment AP-NBT kit for recognition of CMV (DNA) for In Situ Interbreeding /Revealing Kit (ZytoVision GmbH. Bremerhaven. Germany) Zyto Fast PLUS CISH Implementation Kit HRP-AEC aimed at Chromogenic In Situ Hybridization (CISH) Using Any Digoxigenic-Labeled Zyto Fast CISH Probe. The query comprises digoxigenin stamped oligonucleotides which point CMV - DNA. Reconnaissance of statistics was succeeded by means of the accessible statistical set of (SPSS-22) at an importance erect of $p< 0.05$.

RESULTS AND DISCUSSION

CMV infection is endemic in Iraq and intra-uterine infection has been associated with birth defects, intra-uterine growth retardation, intrauterine death of the fetus, and other squeal such as developmental delay, blindness, and deafness for the affected child (13). CMV infection during pregnancy is more dangerous than other infections, due to the reactivation of the virus during childbearing age and then transmitted to the fetus despite maternal immunity. (14)

- *Detecting of CMV in aborted women:* Out of the 140 blood samples examined serologically using the ELISA technique, only 100 samples showed positive results on the presence of immune antibodies to CMV. The current study showed that 24% of aborted women who were subjected to a serological examination were carrying immunoglobulin CMV IgG, while 76% were carrying immunoglobulin CMVIgM. Figure 1a, 1b.

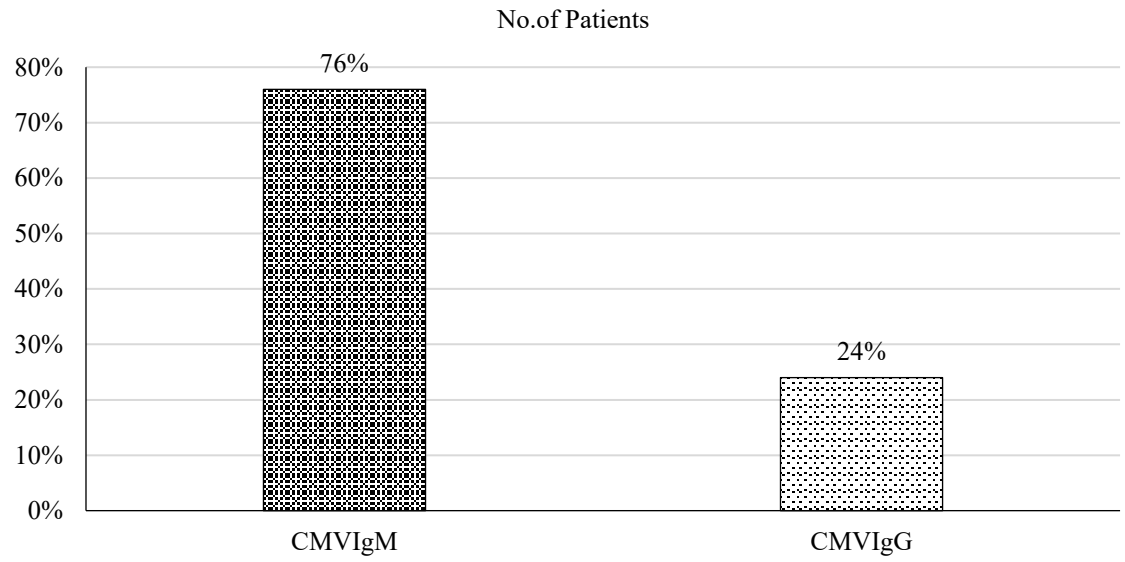

Figure 1a. Carrying immunoglobulin.

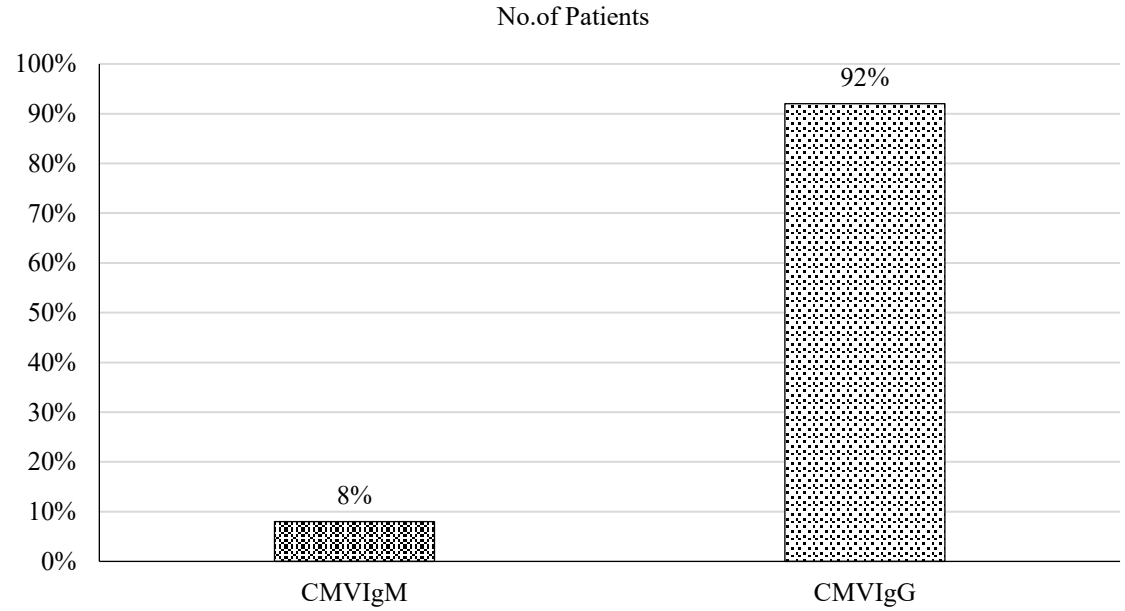

Figure 1b. Confirmatory test after 10 days showed the percentage of the IgG became 92% while the percentage of IgM decreased to 8%.

The chromo-genic in situ interbreeding (CISH) performance, the entire part of confident CISH of CMV concerning eutherian mammal lenient tissue in the loss cluster existed 65%, although not a soul of the 35% eutherian tissues consume exposed confident CISH- gestures for CMV marker (label.1). Regarding the indication counting, the maximum often noticed CMV – constructive eutherian tissue in the loss cluster were experiential to consume the (groove I), (61.5%), and 53.8% publicized scrawny emblem métier with substantial variance experiential ($p < 0.05$) as in (Table 1). Figure 2a, 2b, 2c.

Table 1. Dissemination of HCMV DNA CISH result of the examined placental tissues of miscarriage.

CMV signal Score & Signal intensity		**Miscarriage Group**		**P value**
CMV CISH		Number	%	P=0.005 P<0.05 SIG.
Negative		35	35%	
Positive		65	65%	
Positive Score	Score I	40	61.5%	
	Score II	13	20%	
	Score III	12	18.5%	
Positive Intensity	weak / I	35	53.8%	
	Moderate /II	18	27.7%	
	strong / III	12	18.5%	

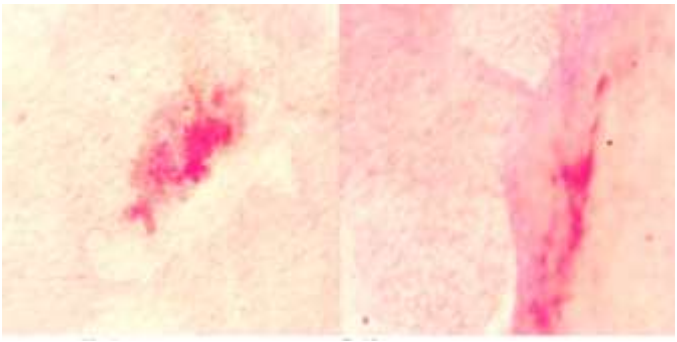

Figure 2. (a) & (b) Intense chromogenic signals indicating positive CMV marker expression in mammalian soft tissue.

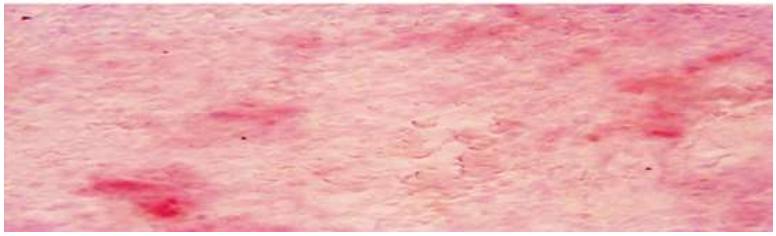

Figure 2. (c) Representative section showing weak staining intensity (métiers) as observed in the study group.

Figure 2. Histopathological sections showing PAS-positive staining.

The results of the current study showed that out of (100) samples that gave positive results in the serological examination by the ELISA technique only (65) samples showed the presence of DNA of CMV and this indicates that the localized in situ hybridization technique is more accurate by molecular detecting the presence of the virus from the immunological examination

THE RELATIONSHIP BETWEEN THE NUMBER OF MISCARRIAGES AND THE AGE

The current study indicated that most abortions were within the age group (20-25) 39%, while the age group (38-43) years recorded the lowest number of repeated miscarriages with significant differences (0.05P <) No. (1) (Figure 3 and Table 2)

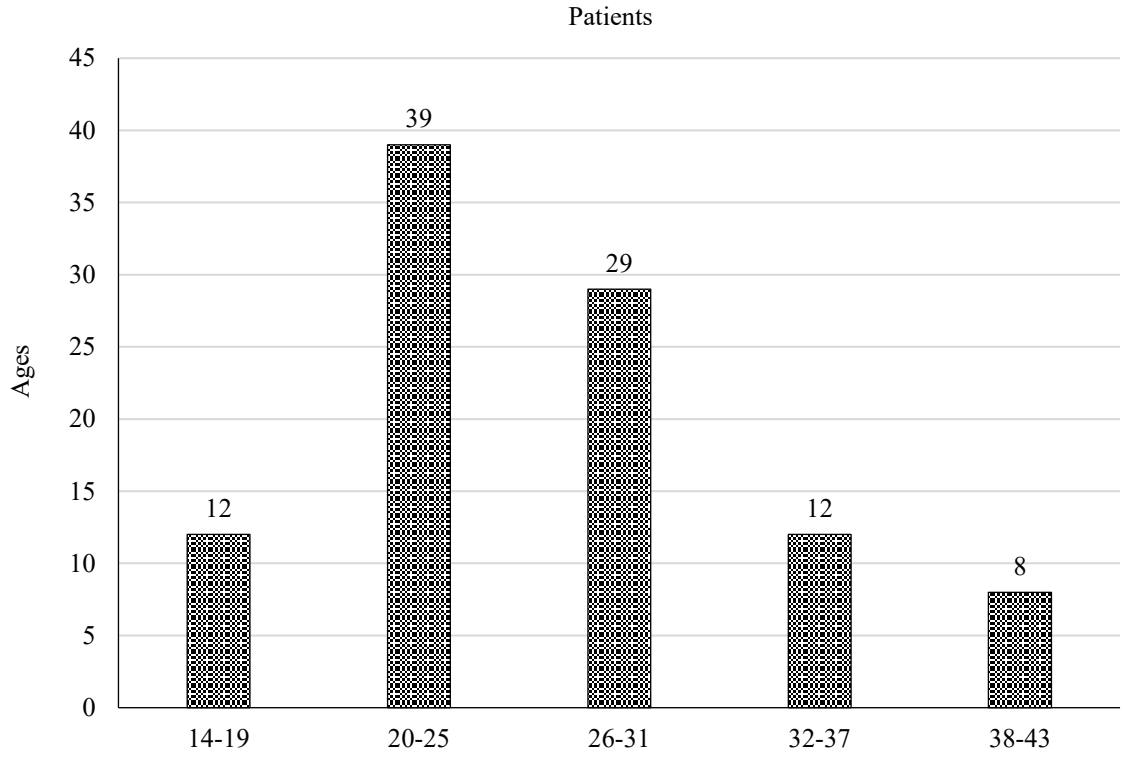

Figure 3. Number of abortion cases by age groups.

Table 2. Number of abortion cases by age groups.

Age	Patients
14 – 19	12
20 – 25	39
26 – 31	29
32 – 37	12
38 – 43	8
Total	100
X^2	8.9 Sign 20 – 25

The current study showed that the number of miscarriages was dependent on age, as the age-related distribution showed a significant difference in the number of miscarriages between the age groups (20-25) years and the age group (38-43) years at a significant level (0.05 <<). It is in line with the results of previous studies. In a study by Yasir 2012 that indicated that the highest rate of miscarriage was within the age group (27-32) years, also [19, 20] indicated that the positive detection rate was 94% within the age group (25-34) This may be due to the higher chance of getting pregnant in younger ages (20-30) years than in older ages (31-40) years. The fallouts of the training were besides consistent with another research guided via who implied that infectivity using CMV raises rapidly in the mature gather relating to 15-30 duration where erotic interaction is extremely efficient [21, 22].

Practicing Miscarriages Via Month

The fallouts of the existing study displayed a momentous variance (P <0.05) with regard to the month of miscarriage, as most abortions were in the fifth and fourth months and the lowest in the first month, and this may be due to several reasons, including infection with viruses such as CMV and rubella or parasites as a parasite Toxoplasma Gondii, where infection with CMV can cause fetal deformity, which leads to its fall before the completion of the pregnancy(Figure 4 and Table 3)

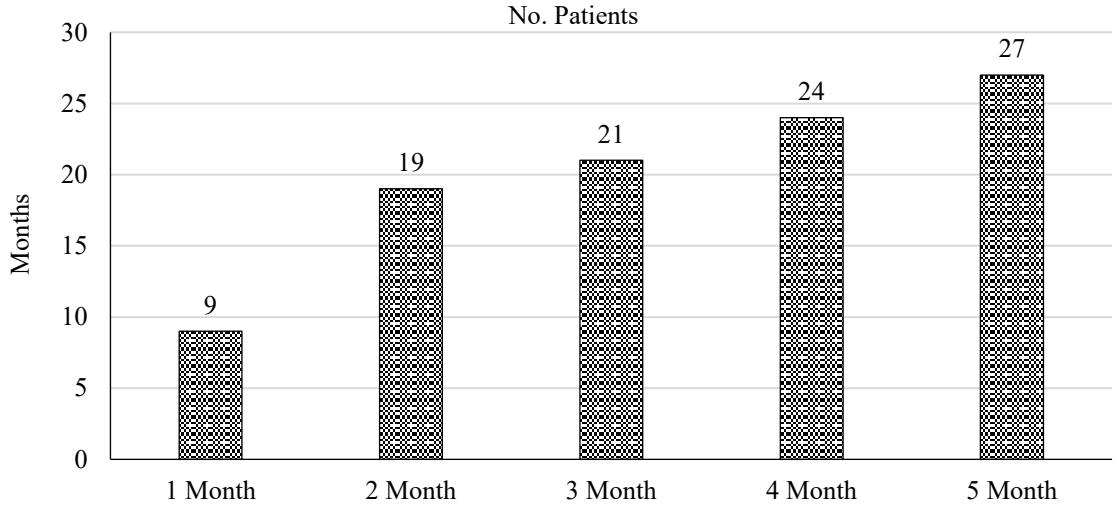

Figure 4. Numbers of aborted women according to months.

Table 3. Number of cases of abortion by month.

Month	No. Patients
1 month	9
2 month	19
3 month	21
4 month	24
5 month	27
Total	100
X^2	11.4 Sign 5 month

The Number of Miscarriages According to the Nature of Housing

The current study indicated that there are significant differences in favor of the city from it in the countryside, as the number of registered cases for cities was 67 while the number of registered cases from rural areas was only 33 cases, and this matter may be due to women's awareness in urban areas and the large dependence of rural women on the day More than visiting hospitals, which results in not recording a large percentage of miscarriages that occur in these areas.(Figure 5 and Table 4)

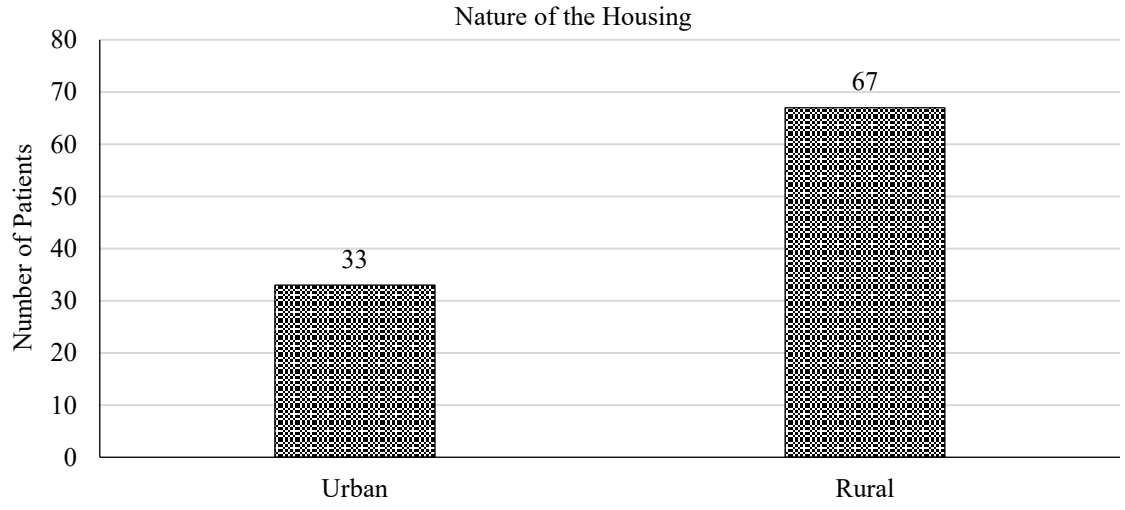

Figure 5. Number of cases of abortion according to the nature of housing.

Table 4. Number of abortion cases by nature of housing.

	T P<0.05	T Calculation	Number of infected	Nature of the housing
Differences in favor of the city	1.96	5.2	33	rural
			67	urban

CONCLUSIONS

The results of the study were also compatible with previously studies which indicated that infection with CMV increases rapidly in the age group between 15-30 years where sexual contact is very effective., CMV infection during pregnancy is more dangerous than other infections, due to the reactivation of the virus during childbearing age and then transmitted to the fetus despite maternal immunity.

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