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Review Article

Correlation of Human Cytomegalovirus Prevalence With Repeated Miscarriages In The City of Karbala, Iraq

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Abstract:

Cytomegalovirus (CMV), also recognized as human herpesvirus 5 (HHV-5), is prevalent within the herpesvirus family. The majority of CMV infections exhibit either no symptoms or mild flu-like manifestations, complicating its detection and often resulting in undetected infections. CMV infections can have serious consequences for certain populations, including newborns and individuals with weakened immune systems.

This study sought to elucidate the associations between human cytomegalovirus (HCMV) and the frequency of miscarriages as well as the gestational age at which pregnancy loss occurs among expectant mothers.

In this study, 150 samples were analyzed, comprising women who had experienced recurrent miscarriage (100 samples) and a control group (50 samples), recruited from the Obstetrics and Gynecology Hospital in Karbala, Iraq. The age range of participants varied from 15 to 50 years old. Sample collection occurred between December 2022 and February 2023, focusing on placental specimens.In this investigation, scholars employed the Statistical Package for the Social Sciences (SPSS) software, version 28.0 developed by IBM in Chicago, Illinois, USA.

A study was revealed HCMV DNA presence in 28 samples (18.67%), including both miscarriage patients and healthy pregnant women.A statistically significant correlation was observed between HCMV and various factors: the number of abortions, the week of abortion, and maternal age categories (35-44), (25-34), (15-24) ($P < 0.01$, $P < 0.05$) respectively. However, no significant correlation was found between recurrent miscarriage and the number of abortions, the week of abortion, and maternal age (45-54 years).Furthermore, there was no significant correlation between the number of participants and the control maternal age.

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1.Introduction:

In the absence of detected pregnancy, the dynamic relationship between the mother and the semi-allogeneic fetus is characterized by a state of harmonious coexistence.

This coexistence is facilitated by various maternal and placental mechanisms that enable bidirectional communication, establishing an "immune-diplomatic" dialogue between trophoblast and maternal immune cells[1]. This dialogue serves to protect the fetus from rejection and creates a privileged environment in the uterus. Several factors, including changes in the immune system, hormonal fluctuations, stress, and alterations in the microbiome, influence susceptibility to infections during pregnancy[2]. Infections contracted during pregnancy can have significant consequences for the developing fetus if left untreated, potentially resulting in maternal, fetal, or neonatal mortality, as well as other adverse outcomes.Viral infections during pregnancy can spread through different pathways, with vertical transmission being the most common. Other routes of transmission include ascending transmission and, less frequently, transmission through the fallopian tube from the peritoneal cavity or via invasive medical procedures[3,4]. Abortion, which involves the medical termination of a pregnancy, is a commonly sought-after medical service among women at some point in their lives. It is classified into two types: spontaneous abortion, also known as miscarriage, occurring before 20 weeks gestation; and induced abortion, which is a conventional method of birth control often indicating unmet maternal reproductive needs[5,6,7]. Research indicates that over 10% of married women resort to induced abortion for birth control purposes.Human cytomegalovirus (HCMV), also known as human herpesvirus 5 (HHV-5), belongs to the Beta-Herpesviridae family, a subgroup of the Herpesviridae family[8]. This family includes herpes simplex virus types 1 and 2, varicella-zoster virus, Epstein–Barr virus, and human herpesviruses 6 and 7. Herpesviruses, including HCMV, are classified into major subfamilies (Alpha, Beta, and Gamma herpesvirinae) based on genetic organization, growth characteristics, and cell tropism. The entry of HCMV into host cells is a complex process that relies on sophisticated interactions between viral glycoproteins and host receptors. Key glycoproteins such as gB and gH/gL play crucial roles in facilitating HCMV entry into various cell types. Additionally, the pentameric complex gH/gL/UL128-131, formed by UL128, UL130, and UL131 proteins, facilitates infection of epithelial and endothelial cells. The glycoprotein gO aids in the intracellular transportation of gH/gL to the viral assembly site. Importantly, the absence of HCMV gO results in a significant reduction in gH/gL content within virions, impairing the virus's ability to infect fibroblasts, epithelial, and endothelial cells[9]. Furthermore, the HCMV genome consists of double-stranded DNA enclosed within a capsid, composed of 162 capsomeres. The capsid of human cytomegalovirus (HCMV) is surrounded by an amorphous protein layer known as the tegument, and it is further encased within a lipid bilayer envelope containing various glycoproteins. This intricate structure plays a crucial role in the virus's ability to infect host cells and evade immune detection[10]. By categorizing herpesviruses into subfamilies based on genetic organization and growth characteristics, researchers can gain insights into their distinct behaviors and pathogenic mechanisms. This classification is invaluable for understanding how these viruses interact with host cells and cause disease, which in turn informs the development of therapeutic strategies.Understanding the complex process by which HCMV enters host cells is essential for identifying potential targets for intervention and the development of antiviral therapies. Key viral glycoproteins such as gB, gH/gL, and the pentameric complex gH/gL/UL128-131 play critical roles in mediating viral entry into various cell types. By elucidating the mechanisms involved in this entry process, researchers can identify vulnerabilities in the virus's lifecycle that can be targeted with antiviral drugs, ultimately leading to more effective treatments for HCMV infections.Insights into the structure

and composition of the HCMV genome and virion also provide valuable information for vaccine development and the design of preventive measures against HCMV infection[11]. Vaccines that elicit an immune response against specific viral proteins or components can help prevent HCMV infection in vulnerable populations, such as pregnant women and immunocompromised individuals. By understanding the molecular makeup of the virus and how it interacts with the host immune system, researchers can design vaccines that effectively stimulate protective immunity without causing harm.Preventive measures against HCMV infection are particularly crucial in pregnant women, as the virus can have serious consequences for both the mother and the developing fetus[12]. Vertical transmission of HCMV from mother to fetus can lead to congenital infection, which may result in a range of developmental abnormalities and long-term health problems. By implementing strategies to prevent HCMV transmission during pregnancy, such as promoting hand hygiene and avoiding contact with bodily fluids from infected individuals, healthcare providers can help reduce the risk of congenital infection and improve maternal and infant health outcomes.A comprehensive understanding of the structure, entry mechanisms, and pathogenesis of HCMV is essential for developing effective therapies and preventive measures against HCMV infection. By leveraging this knowledge, researchers can continue to make strides in the development of vaccines and antiviral drugs that offer hope for the prevention and treatment of HCMV-related diseases[13].

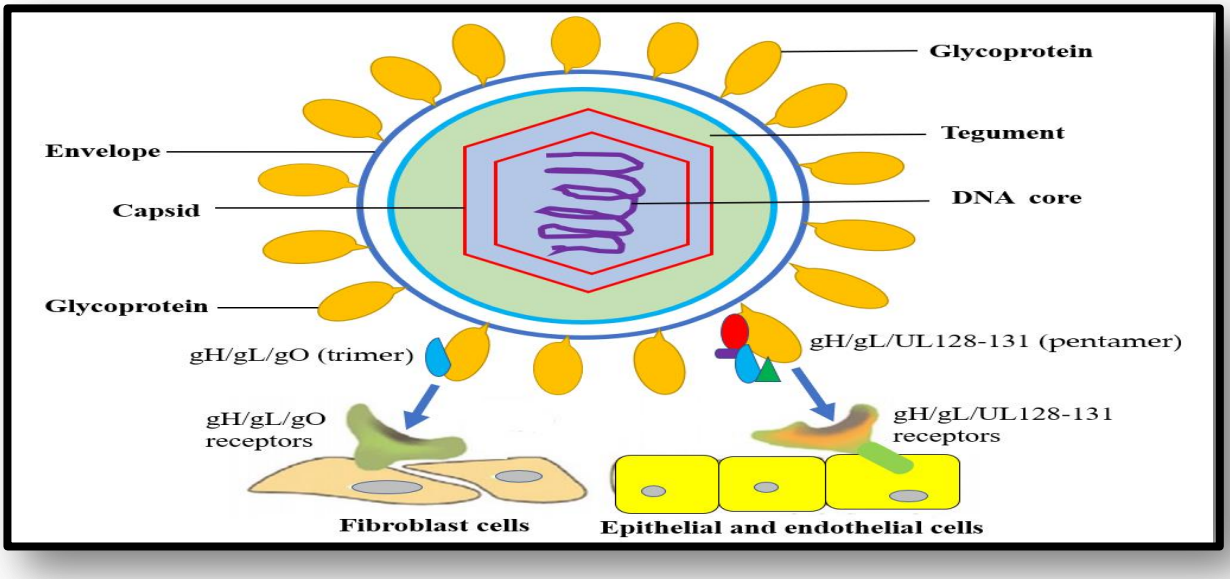

Figure 1 : HCMV structure and entry into host cells

Human cytomegalovirus (HCMV) is highly prevalent globally, affecting over 90% of the general population. Transmission of the virus can occur through various routes, including sexual contact, exposure to bodily fluids like saliva, breast milk, maternal vaginal secretions, or blood, and vertical transmission from mother to fetus during pregnancy[14]. Additionally, solid organ and stem cell transplantation can lead to infection. While HCMV infection is widespread, with 40-100% of the global population affected, most cases are asymptomatic, with severe illness primarily seen in immunocompromised individuals[15]. According to the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO), HCMV infects people of all ages, with around one-third of children in the United States acquiring the infection by age five. Notably,

vertical transmission occurs in 30-40% of cases during the first and second trimesters of pregnancy, increasing to up to 70% in the third trimester.[16].

The increased vulnerability to vertical transmission of CMV during advancing pregnancy likely arises from a combination of factors[17,18,19]. One contributing factor is the reduction in the number of uterine natural killer (uNK) cells. uNK cells, which constitute the majority of leukocytes in early pregnancy and account for about 70% of leukocytes in utero, have origins that are still debated [20]. Furthermore, CMV can evade recognition by NK cells by downregulating MHC class I complexes and upregulating MHC decoy UL18 on infected NK cells, thereby making the placenta more susceptible to pathogenic invasion [21]. Various factors have been linked to patterns of HCMV acquisition, including geographic and socioeconomic backgrounds, as well as age, ethnicity, and sex [22].

2.Material and Methods:

2.1 The clinical specimens

A total of 150 samples were gathered from women aged 17 to 50 who underwent abortion and received treatment at the Obstetrics and Gynecology Hospital in Karbala, Iraq. Furthermore, 50 samples were obtained from healthy control women aged 15 to 38 during the period spanning from December 2022 to February 2023. Following sample collection, extraction of total DNA viral genomes was conducted on placental tissue samples. Subsequently, the identification of human cytomegalovirus (HCMV) was carried out using RT-qPCR on samples obtained from both women who had experienced abortion and those who appeared to be healthy.

2.2 DNA Extraction of CMV

By using specific viral DNA/RNA extraction kit (Intron/Korea); the viral genome was extracted from the placenta as a first step to amplify the target Human Cytomegalovirus DNA.

2.3 Real Time PCR (qPCR)

Detection of CMV DNA relies on amplifying a segment of the pathogen's genome using specialized primers, as depicted in Table 1. This process, known as PCR (Polymerase Chain Reaction), specifically targets and replicates the DNA sequence unique to CMV. By utilizing primers designed to bind to specific regions of the CMV genome, PCR enables selective amplification of CMV DNA from samples. Subsequent analysis of the amplified DNA provides a reliable method for identifying the presence of CMV in biological specimens.

Table 1: Primers sets that used for Detection of Human Cytomegalovirus .

Human CMV	Sequence (5′-3′)	Reference	Origin
Forward	TGGTTTGGTAGTGTTCTGGC	Designed by this study	IDT/ USA
Reverse	GTAAGATTCAGACTGGAGCGG		
Probe	CTGGTGGAACTCGAACGTTTGGC		

Polymerase chain reaction amplification was done using RT-qPCR System (Promega-USA).),after preparing the reaction mix and adding it to each sample ,and according to the instructions of the kit used ,the samples to be examined are entered into the device with the addition of a standard or negative control and also rely on the Internal Control (CT) , analyze the data after the run is complete.

3. Results:

3.1.Study groups according to their age:

Significant disparities were observed between the mean age of patients experiencing miscarriage (28.07 ± 5.85 years) and apparently healthy controls (AHC) (23.69 ± 5.19 years), representing a statistically significant distinction. Analysis revealed highly significant statistical differences (p=0.001) among the study population, as detailed in Table 2. These findings underscore notable variations in age distribution between individuals who underwent miscarriage and those in apparent good health, highlighting potential age-related factors influencing pregnancy outcomes and warranting further investigation.

Table(2):Distribution of women patients with(AHC) according to their age

Study groups	No.	Mean of ageYears	SD^	SE*
Patients	100	28.07	5.85	0.58
AHC**	50	23.69	5.19	0.74
Total	150	(P value =0.001) Significant differences		

**: Apparently Healthy Controls.

3.3.Detection of Cytomegalovirus by quantitative PCR (Real-time PCR):

Real-time PCR (polymerase chain reaction) stands as the gold standard for molecular DNA detection due to its remarkable specificity, sensitivity, and immunity to common diagnostic pitfalls like sample contamination. Diagnostic kits for real-time PCR are easily accessible in laboratories, cost-effective, and require no special storage or transportation conditions, making them highly practical [23,24].In this particular investigation, the focus was on detecting HCMV (Human Cytomegalovirus) DNA extracted from placental tissue. The results unveiled that 28% (28 out of 100) of patients previously identified as CMV-positive showcased the presence of HCMV DNA. The odds ratio was computed at 20.6, indicating a significant association, with a p-value of 0.001. Conversely, the control group exhibited no viral presence (0.0%), as depicted in Table (3).Quantitative PCR (qPCR) emerges as a highly sensitive and specific technique for CMV detection, furnishing valuable insights for diagnosing and monitoring CMV infections, particularly in immunocompromised individuals. Its widespread adoption in clinical laboratories underscores its accuracy and efficacy, rendering it the preferred method for precise detection and quantification of CMV. Consequently, it plays a crucial role in effective disease management.Overall, the study underscores the reliability and efficacy of real-time PCR, especially in the context of detecting HCMV DNA from placental tissue. With its ability to provide precise results swiftly and with high sensitivity, real-time

PCR contributes significantly to the understanding and management of CMV infections, offering clinicians a powerful tool for diagnosis and monitoring in both research and clinical settings.

Table (3): Detection of HCMV by RT- qPCR between patients and control.

qPCR		Study Groups		Pearson Chi-Square X^2 (P value)
		Patients (N=100)	Control (N=50)	
Positive	N	28	0	X^2= 17.23 P= 0..001 Highly significant
	%	28%	0	
Negative	N	72	50	
	%	72%	100%	

3.4 Relation of age groups of pregnant patients with qPCR of CMV of patients:

The current findings indicated that there were no significant differences observed in HCMV infection across different age ranges (p=0.68). Among women experiencing miscarriage, the age group most commonly affected by DNA-HCMV was 25-34 years, accounting for 57.14% (16 out of 28 cases). Following this, the age range of 15-24 years constituted 28.57% (8 out of 28 cases), and the age group of 35-44 years accounted for 12.29% (4 out of 28 cases). Notably, there were no recorded instances of HCMV infection in the age group of 45-45 years, as outlined in Table (4).

Table 4 : Relation of the Age Stratum of pregnant patients with qPCR of HCMV as calculated by chi-squaretest.

	Years	HCMV			P value
		No. %	Positive	Negative	
Age Stratum	15-24	22	8	14	X^2 1.48 P= 0.68 NS
	%	22%	28.57%	19.45%	
	25- 34	64	16	48	
	%	64%	57.14%	66.66%	
	35- 44	13	4	9	
	%	13%	12.29%	12.5%	
	45- 54	1	0	1	
	%	1%	0	1.39%	
Total	-----	100	28	72	
%	----	100	28%	72%	

X^2 : chi-square test, **NS:** No significant.

The study results revealed no discernible differences between qPCR results and age groups among pregnant women (P= 0.68), as depicted in Table (4). This finding is consistent with a study by Hussein et al. (2017) in Iraq, which similarly observed that the highest incidence of viral infection occurred in women aged 26-35 years. Furthermore, the Iraqi study found no significant correlation between HCMV infection in pregnant women experiencing miscarriage and various other factors, including education level, occupation, economic status, place of residence, gestational age, history of previous abortions, and parity. Consequently, these factors cannot be regarded as risk factors for infection [25].

3.5 **Correlations among HCMV, number of abortion and week of abortion according to the age stratum of study population:**

Table (5), illustrated the relationship (with statistically significant correlation) was found between HCMV and participants; number abortion ; week of abortion and maternal age (35-44)(25-34)(15-24)($P < 0.01$, $P < 0.05$) respectively. However, there are no significant correlation among recurrent miscarriage with participants number abortion ; week of abortion and maternal age(45-54years),also there are no significant correlation between number of participants with control maternal age.

Table 5:The correlations among HCMV ,number abortion and week of child loss according to the age stratum of study population.

Variables	Study groups (+ CMV)cases (N=28) No. %				Study groups (control) (N=50) No. %			
Maternal age (Years) Number	15-24	25-34	35-44	45-54	15-24	25-34	35-44	45-54
	8	16	4	NF	13 %48.67	29 %58	8 %16	0 0.0%
Participants Mean ±SD	16.8 ± 4.5	28.5 ± 2.9	29.5 ±3.3	NF	19.46 ±3.5	28.2 ± 3.8	42.8 ± 4.9	0
Number of abortion Mean ±SD	1.15 ± 0.34*	1.01 ±0.22*	2.16 ±0.49**	NF	NF	NF	NF	NF
Week of abortion Mean ±SD	13.6 ±4.01 *	18.48 ±4.21**	17.26 ±2.38 **	NF	NF	NF	NF	NF

* P< 0.05 , ** P< 0.01.

In the present study shows, that the age stratum (35-44 years) of study population was recorded highly statistically significant correlation(P< 0.01) between infection of HCMV and number abortion and week of abortion, while the age group(25-34) was recorded statistically significant correlation(P< 0.05) with number abortion and highly statistically significant correlation(P< 0.01) with number abortion and week of abortion. According the age stratum (15- 24 years) of study population was recorded statistically significant correlation(P< 0.05) with number abortion and week of abortion. The age stratum (45-45) years ,although was found participants in this age but not found any relationship with number abortion ; week of abortion and maternal age. Table(5).

4-Discussion:

Quantitative reverse transcription polymerase chain reaction (RT-qPCR), an essential method for early and accurate infection diagnosis, employs a molecular approach that enables the detection of infections even prior to symptom onset. This capability is pivotal in curtailing disease transmission and progression [26]. Contrary to the findings of a Brazilian study, which diagnosed cytomegalovirus (CMV) by extracting it from placental tissues, this study revealed that 19% of parturients with a history of spontaneous abortion tested positive for CMV in the placenta. However, no correlation was found between the detection of CMV DNA in cord blood and the presence of the virus in the placenta, suggesting that vertical transmission may not occur through ascending infection from the genital tract [27].Various sociodemographic factors have been identified as influential in maternal CMV infection, including skin color, educational attainment, and duration of virus exposure, particularly in cases of reactivation. Early onset of sexual activity in women, particularly before age 15, has been identified as a risk factor for the presence of the virus in umbilical cord blood. Additionally, income levels have shown a significant association with CMV molecular prevalence in placentas, indicating a link between poor knowledge about the disease, inadequate sanitary conditions, and socioeconomic status [27].The current study's results may be attributed to differences in geographical region, sample composition, and study design. The age of women included in each study may have played a role in the distribution of infection [28]. In an American study by Jerman et al. (2017), it was found that the lowest cases of miscarriage occurred in women above 30 years old [29]. Other factors influencing HCMV infection include a woman's immune system, deficiencies in trophoblast progenitor stem cell differentiation and function, dysregulation of Wnt signaling pathways, tumor necrosis factor-mediated trophoblast apoptosis, HCMV-induced cytokine changes in the placenta, and inhibition of indoleamine 2,3-dioxygenase [30,31].The herpes simplex virus (HSV) family, including HCMV, exhibits a strong affinity for humans. Following primary infection, the virus undergoes phases of replication, shedding in body fluids, viremia, and possibly an infectious mononucleosis phase before entering a latent phase [32]. Numerous studies have identified a significant relationship between HCMV infection and frequent abortion among Iraqi females. Challenges such as a lack of management and diagnostic policies on HCMV contribute to the high prevalence of infection in the country. Factors such as suboptimal antenatal and postnatal care, inadequate laboratory facilities, poverty, low awareness, literacy, sexually transmitted diseases, and teenage pregnancies exacerbate health issues among Iraqi women, underscoring the urgent need for enhanced knowledge and international recommendations on managing HCMV [28].

Conclusion:

Recent research highlights a significant disparity (p=0.001) in detecting HCMV in placental tissue between women who had abortions and those in the control group. Noteworthy correlations emerged between HCMV presence and various factors including the frequency and timing of abortions, as well as maternal age, spanning from 15 to 44 years. However, within the age bracket of 45-54 years, no significant correlation was found.This discrepancy underscores potential implications for understanding HCMV's role in pregnancy outcomes and maternal health. The observed correlations with abortion frequency and timing suggest a complex interplay between HCMV infection and reproductive events. Additionally, the association with maternal age indicates varying susceptibility across different age groups, with notable trends observed up to 44 years old but not beyond.The absence of significant correlation in the 45-54 age range prompts further inquiry into potential factors influencing immunity or viral susceptibility within this demographic subset. Understanding such nuances is crucial for devising targeted prevention and intervention strategies tailored to vulnerable populations.these findings illuminate the multifaceted nature of HCMV infection in pregnancy and underscore the importance of comprehensive research to elucidate its mechanisms and implications for maternal and fetal health across different demographic groups.

Conflicts of interest:

The authors report no conflict of interest.

Declarations of interest:

None.

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