Title: Silent Threat: Investigating the Prevalence of Cytomegalovirus in Expectant Mothers in Northern Iran, Gorgan

Running title: HCMV in pregnant women

Abstract

Cytomegalovirus (CMV) infection during pregnancy is the leading cause of congenital infections globally, often resulting in significant health issues in newborns. These issues include sensorineural hearing loss, which can affect communication and language development, and neurodevelopmental delays that manifest as cognitive impairments, motor dysfunction, and behavioral challenges. The virus can be transmitted from the mother to the fetus, particularly if the mother experiences a primary infection during pregnancy. Early detection through maternal screening and fetal diagnostic tests, such as polymerase chain reaction (PCR) analysis of amniotic fluid, is crucial. Prompt management strategies, including antiviral therapies and immunoglobulin treatments, are essential to reduce viral load and mitigate these risks, thereby improving outcomes for affected infants. Vaginal secretions and blood specimens of 315 pregnant women referred to an educational hospital in the North east of Iran were tested for HCMV using PCR and ELISA assays. Chi-Square test was utilized to evaluate the association of qualitative variables and the level of significance was set at $p \leq 0.05$. Moreover, statistical analysis was performed using SPSS Statistics V.26.0. The findings of the molecular and serological investigation of cytomegalovirus (CMV) in the current population revealed that 16.2% (51/315) of the individuals tested positive for DNA-CMV, 87.6% (276/315) displayed IgG antibodies, and 3.2% (10/315) had IgM antibodies. Studying the prevalence of CMV in pregnant women is crucial to understand the extent of maternal and fetal exposure to this virus, which can lead to significant congenital disabilities and developmental issues in newborns. This data is essential for developing effective screening protocols and preventive measures to mitigate the health risks associated with CMV infections during pregnancy.

Keywords:

Human Cytomegalovirus, HHV-5, Pregnant women, Iran.

1. Introduction

Human herpes virus 5 (HHV-5) or Cytomegalovirus (CMV) has emerged as the foremost agent leading to such calamitous sequelae as non-genetic sensorineural hearing loss (SNHL), neurodevelopmental disability, and visual impairment (5-15% of neonates). Additionally, 10-15 percent of congenitally infected infants show symptoms including intrauterine growth restriction, microcephaly, hepatosplenomegaly, petechiae, jaundice, chorioretinitis, thrombocytopenia, and anemia; however, 85-90% of them are asymptomatic at the time of birth. The diagnosis of asymptomatic infants with CMV is crucial as early intervention and follow-up can mitigate the complications and disabilities caused by this virus (1, 2).

The virus can be transmitted through such various ways as direct contact with body fluids (such as saliva, urine, blood, cervical secretions, and semen), sexual intercourse, and breastfeeding. Similar to other herpesviruses, CMV has the ability to establish latent infection and can reactivate after primary infection (3). Congenital CMV infection typically arises from primary maternal infection during pregnancy, particularly in the first trimester, with around 50% of infants born to mothers with primary CMV infection acquiring intrauterine infection. However, less than 5% of pregnant women with primary CMV infection exhibit symptoms, which are mostly non-specific and mild. Routine laboratory tests in pregnancy reveal only a rise in atypical lymphocytes and a slight increase in liver transaminases (4).

The global prevalence of CMV seropositivity ranges from 40-100 percent (5). The overall risk of primary CMV infection during pregnancy is estimated to be between 0.15% and 2%, with a 40% probability of fetal transmission. (5) The probability of postnatal infection via breastfeeding in premature infants is calculated to be 16.5%. The shedding rate of the virus in breast milk among CMV-seropositive mothers is estimated at 80.5%. Furthermore, the rate of infection in infants after consuming CMV-positive breast milk is estimated at 20.7% (6). In Iran, reproductive-age women are reported to have a 90% immunity rate (7). Additionally, 81.27% of pregnant women in Golestan province show positive total antibodies against CMV (5).

Given the absence of standard pregnancy screening protocols to identify congenital CMV infection and its serious complications for the fetus, as well as limited studies in Iran assessing the epidemiology of this virus in pregnant women, we decided to conduct a study to determine the

molecular and serological epidemiology of CMV in pregnant women residing in Golestan province. The results of this research can be utilized to devise preventative measures, mitigate potential risks, and establish protocols for screening and prompt interventions (8).

2. Materials and methods

2.1.Study population and clinical specimens

This research project was granted approval by the Ethics Committee for Human Medical Experimentation at Golestan University of Medical Sciences, Gorgan, Iran (Ethics code: IR.GOUMS.REC.1401.043). In accordance with the previously mentioned description (9), the subjects of this study obtained from pregnant women who admitted to Sayyad Hospital in Northern Iran between May and September of 2018. Prior to commencing the study, approval was obtained from the participants and an explanation of the project was provided. Moreover, they were required to fill out a questionnaire covering a list of clinical, behavioral, and sociodemographic factors. In total, 315 sera and cervicovaginal lavage specimens were collected and then transported on ice to the department of microbiology at Golestan University of Medical Sciences, Gorgan, Iran. Following cervicovaginal lavage specimen's centrifugation (1000 × g for 10 min), the supernatant discarded. Cellular materials were re-suspended in 1 ml PBS and stored at -20^{o}C for short term storage or at –70^{o}C for the longer term. Peripheral blood specimens were taken, and aliquots of serums were obtained by centrifugation at 2000g for about 10 minutes and then stored at −20°C until the serological analyses.

2.2.Viral DNA extraction and polymerase chain reaction

Viral DNA extraction method is the one comprehensively explained in our previous study (9). The presence of UL54 (HHV-5) gene was evaluated via PCR assay using a master mix PCR kit (Amplicon, USA). The aforementioned gene was amplified under the condition explained by Yasaghi et al. (2022) (10).

2.3.Detection of CMV-specific antibodies

Serum IgG and IgM antibodies against CMV were detected by competitive type-specific enzyme-linked immunosorbent assay (PT-CMV.G-96 and PT-CMV IgM-96). Results were recorded in Microsoft Excel 2019, and classified as either seropositive or seronegative based on the interpretation of the specimens.

2.4.Statistical analysis

The analysis of clinical, behavioral, sociodemographic factors, and laboratory results was conducted using SPSS Statistics V.26.0. The Chi-Square test was employed to assess the qualitative variables, with a significance level of P values ≤0.05.

3. Results

From May to September 2018, 315 pregnant women aged 24-33 years (Table 1) admitted to Sayyad hospital in northern Iran. Detailed demographic, behavioral as well as clinical data have been previously described (9).

Table1. Demographic data of Pregnant Women, Gorgan, Iran, May 2018 to September 2018.

	Sample Size	CMV Positive		IgG Positive		IgM Positive	
Item	N (%)	N (%)	P.value χ^2	N (%)	P.value χ^2	N (%)	P.value χ^2
Demographic factors							
Age, y			0.9		0.4		0.6
14-23	78 (24.8%)	12 (15.4%)		69 (88.5%)		2 (2.6%)	
24-33	167 (53.0%)	27 (16.2%)		143 (85.6%)		7 (4.2%)	
34-43	70 (22.2%)	12 (17.1%)		64 (91.4%)		1 (1.4%)	
Occupation:			0.5		0.8		0.5
Employee	19 (6.0%)	4 (21.1%)		17 (89.5%)		0	
Housewife	296 (94.0%)	47 (15.9%)		259 (87.5%)		10 (3.4%)	
Accommodation:			0.2		0.6		0.7
Urban	172 (54.6%)	32 (18.5%)		153 (88.4%)		6 (3.5%)	
Rural	142 (45.1)	19 (13.4%)		123 (86.6%)		4 (2.8%)	
Educational Level:			0.8		0.7		0.4
Illiterate	60 (19.0%)	11 (18.3%)		51 (85.0%)		2 (3.3%)	
Diploma or less	194 (61.6%)	30 (15.3%)		173 (88.3%)		8 (4.1%)	
Higher levels	59 (18.7%)	10 (16.9%)		52 (88.1%)		0	

The results obtained from the molecular and serological analysis of CMV in the current population revealed 16.2% (51/315) DNA-CMV, 87.6% (276/315) IgG, and 3.2% (10/315) IgM. Table 2 provides the summary statistics for further behavioral and clinical data. Accordingly, in participants who reported the experience of anal intercourse 19.6% (10/51, P= 0.4), 84.3% (43/51,

P= 0.4), 2.0% (1/51, P= 0.5) CMV-DNA, IgG and IgM was detected, respectively. Additionally, only 15.6% (49/315) of the current population reported condom use during their sexual behavior in which 24.5% (12/49, P=0.08) CMV-DNA and 85.7% (42/49, P=0.6) CMV-IgG observed. Almost half of the female participants in this study reported having had their first sexual experience before the age of 20 (50.4%, 159/315), during which CMV-DNA, IgG, and IgM were detected at the following rates: 15.1% (24/159, P=0.5), 88.1% (140/159, P=0.8), and 2.5% (4/159, P=0.5), respectively.

The next section of the survey was concerned with the association of clinical factors and CMV virus which the results obtained from the preliminary analysis are set out in table 2. As indicated in this table, 9.2% (29/315), 3.5% (11/315), and 87.3% (275/315) of the attendants are in their first, second and third trimester of pregnancy, respectively. Closer inspection of the table shows that the distribution of vaginal delivery (46.9%, 148/315) and cesarean section (48.2%, 152/315) is relatively even. However, it is worth noting that 4.8% (15/315) of women were referred to the hospital due to various medical conditions, leading to termination of pregnancy. The most noteworthy aspect of these findings is the proportion of IgM, which is particularly striking at 4.7% (7/148), 1.3% (2/152), and 6.7% (1/15) in women who underwent vaginal delivery, cesarean section, and abortion, respectively. This result is significant at the $P \leq 0.05$ level. From the data in table 2, it is apparent that a significant proportion of the attendants, amounting to 29.8% (315/1052), have had an abortion. Among these individuals, 13.8% (13/94) tested positive for CMV-DNA, while 89.4% (84/94) and 4.3% (4/94) had detectable levels of IgG and IgM, respectively. The P-values associated with these findings are 0.4, 0.5, and 0.4, respectively. Upon further examination of the clinical variables, it was discovered that 39% (123/315) of the participants exhibited unusual discharge, with the majority of these individuals testing positive for CMV-IgG (86.2%, 106/123, P=0.5). Additionally, 28.9% (91/315) of the population of this study complained from pain during their sexual activity, molecular and serological analysis revealed 15.4% (14/91, P=0.8) CMV-DNA and 87.6% (77/91, P=0.3), 2.2% (2/91, P=0.5) IgG and IgM in this group. In the final part of the survey, respondents were asked whether they've undergone a pap smear or not. Among the 315 respondents, 24.1% (76 individuals) indicated that they have got a pap smear, while 75.8% (239 individuals) reported that they have never been tested. It is worth noting that there was no discernible difference between the two groups.

Table2. Cytomegalovirus Molecular & serological Prevalence by Respondent Characteristics Among Pregnant Women, Gorgan, Iran, May 2018 to September 2018.

	Sample Size	CMV Positive		IgG Positive		IgM Positive	
Item	N (%)	N (%)	P.value χ^2	N (%)	P.value χ^2	N (%)	P.value χ^2
Behavioral Factors							
Anal intercourse:			0.4		0.4		0.5
Yes	51 (16.1%)	10 (19.6%)		43 (84.3%)		1 (2.0%)	
No	264(83.2%)	41 (15.5%)		233 (88.3%)		9 (3.4%)	
Condom use:			0.08		0.6		0.1
Yes	49 (15.6%)	12 (24.5%)		42 (85.7%)		0	
No	264(83.8%)	39 (14.7%)		234(88.0%)		10 (3.8%)	
Age at first sexual intercourse:			0.5		0.8		0.5
<20	159 (50.4%)	24 (15.1%)		140 (88.1%)		4 (2.5%)	
≥20	156 (49.5%)	27 (17.3%)		136 (87.2%)		6 (3.8%)	
Clinical Factors							
Trimester of pregnancy:			0.1		0.3		0.1
1st	29 (9.2%)	1 (3.4%)		23 (79.3%)		2 (6.9%)	
2nd	11 (3.5%)	2 (18.2%)		10 (90.9%)		0	
3rd	275 (87.3%)	48 (17.5%)		243 (88.4%)		8 (2.9%)	
Mode of delivery:			0.2		0.6		**0.04**
Vaginal	148 (46.9%)	25 (16.9%)		131 (88.5%)		7 (4.7%)	
Cesarean	152 (48.2%)	26 (17.1%)		133 (87.5%)		2 (1.3%)	
Abortion	15 (4.8%)	0		12 (80.0%)		1 (6.7%)	
History of abortion:			0.4		0.5		0.4
Yes	94 (29.8%)	13 (13.8%)		84 (89.4%)		4 (4.3%)	
No	221(70.1%)	38 (17.2%)		192 (86.9%)		6 (2.7%)	
Unusual discharge:			0.5		0.5		0.9
Yes	123 (39.0%)	22 (17.9%)		106 (86.2%)		4 (3.3%)	
No	192 (60.9%)	29 (15.1%)		170 (88.5%)		6 (3.1%)	
Sex pain:			0.8		0.3		0.5
Yes	91 (28.9%)	14 (15.4%)		77 (84.6%)		2 (2.2%)	
No	224 (71.1%)	37 (16.5%)		199 (88.8%)		8 (3.6%)	
Pop smear:			0.8		0.1		0.2
Yes	76 (24.1%)	13 (17.1%)		63 (82.9%)		4 (5.3%)	
No	239 (75.8%)	38 (15.9%)		213 (89.1%)		6 (2.5%)	

Abbreviations: CMV: Cytomegalovirus.

4. Discussion

Human cytomegalovirus (HCMV) infection during pregnancy poses considerable risks to both the mother and developing fetus. HCMV is the most prevalent congenital viral infection worldwide and can lead to severe neurological, sensory, and cognitive impairments in infants, including hearing loss and developmental delay(11). Pregnant women who acquire a primary HCMV infection, particularly during the first trimester, are at the greatest risk of transmitting the virus to the fetus. However, reactivation or reinfection with HCMV during pregnancy can result in adverse outcomes(12). The precise mechanisms of vertical transmission and fetal damage are not entirely understood, but likely involve the placenta, with HCMV capable of crossing the placental barrier and infecting fetal tissues directly(13). Early detection of HCMV infection in pregnant women through serological screening and symptom monitoring is crucial for timely intervention and management strategies. These may include antiviral therapy or supportive care to mitigate the risk of transmission and minimize its impact on fetal development. Furthermore, educating pregnant women about preventive measures such as maintaining good hand hygiene and avoiding contact with bodily fluids from young children can help reduce the incidence of HCMV infection during pregnancy(14, 15).

The current investigation furnishes valuable information on the molecular and serological prevalence of HCMV infection in women from northeast Iran, considering that a significant percentage of individuals are unacquainted with this viral infection due to its inadequate screening approach. The prevalence of these viruses is influenced by several factors, such as sample size, demographic variables (e.g., age, gender), age at coitarche, the number of lifetime sexual partners, and the diagnostic tests' specificity and sensitivity. Analysis of CMV in the current population using molecular and serological methods yielded 16.2% (51/315) of individuals testing positive for DNA-CMV, while 87.6% (276/315) had IgG antibodies, and only 3.2% (10/315) had IgM antibodies. The molecular data reported in this study vary from the figure reported by Saravi in 2023(16), which stands at 8%. Nevertheless, the serological data corresponded with the data documented in several publications in Iran and other countries (17, 18). These results can be attributed to the nature of the CMV infection and immunity. CMV-IgG antibodies indicate previous exposure to the virus and long-term immunity, which is common among adults owing to the widespread prevalence of CMV. Most women of childbearing age are exposed to CMV earlier in life, leading to a high seroprevalence of CMV-IgG (19). Conversely, CMV-IgM antibodies are

markers of recent primary infection or reactivation and tend to be transient and usually present for only a few months. The detection of CMV DNA in the blood or bodily fluids signifies active viral replication, which is less frequent in pregnant women because their immune systems often control the virus, leading to low viral loads detectable by DNA assays. Thus, while most pregnant women show evidence of past infection (CMV-IgG), only a small percentage have recent or active infection (CMV-IgM and CMV DNA), reflecting the dynamics of CMV immunity and reactivation (20, 21).

Turning to the demographic factors considering in this study, although there were not significant associations between these factors and CMV prevalence in pregnant women, it is worth mentioning that the incidence of CMV infection in pregnant women is influenced by various sociodemographic factors, including age, education, and accommodation(22). Research has indicated that older women tend to have higher CMV seroprevalence rates than younger women, mainly because of the increased cumulative exposure to the virus over time. Moreover, educational attainment plays a significant role; women with lower levels of education often have a higher CMV prevalence, which may be related to socioeconomic factors that impact hygiene practices and living conditions. Furthermore, the type and quality of accommodation are critical; those living in overcrowded or substandard housing conditions are at a greater risk of CMV transmission due to closer contact with young children, who are common reservoirs of the virus. These children frequently shed the virus in their saliva and urine, facilitating household transmission. Therefore, addressing these sociodemographic factors is essential for understanding and managing CMV infection risk among pregnant women (23).

Regarding sexual behaviors, although these results found no association between engaging in anal sex, condom use and age at first sexual intercourse with having CMV, studies suggest that anal sex may be associated with a higher risk of CMV transmission due to the potential for mucosal damage and higher viral shedding in genital secretions, creating a more efficient route for the virus to spread(24). Condom use, on the other hand, has been shown to reduce the risk of CMV and other sexually transmitted infections by providing a barrier that limits the exchange of bodily fluids that can carry the virus(25). Additionally, an earlier age at first sexual intercourse is linked to a longer cumulative period of sexual activity and potentially more sexual partners, increasing the likelihood of CMV exposure and infection (26). Understanding these associations helps in

developing targeted interventions to reduce CMV prevalence among pregnant women through sexual health education and promotion of safe sex practices.

The prevalence and impact of CMV infection in pregnant women during different stages of pregnancy and modes of delivery can vary greatly. In particular, primary maternal infection during the first trimester poses a high risk of transmission to the fetus, with severe consequences for the baby's development due to the critical fetal development stages. Although infections during the second and third trimesters are still a concern, they generally result in less severe fetal outcomes (27). The mode of delivery can also affect CMV transmission, with cesarean delivery potentially reducing the risk of neonatal CMV infection compared with vaginal delivery, as it limits the newborn's exposure to maternal genital secretions that may contain the virus (28). Here, we did not find any evidence to support a relationship between the two factors and CMV prevalence in our target population.

The association between abortion, history of abortion, and the prevalence of Cytomegalovirus infection in pregnant women is an important area of study. Women with a history of abortion may exhibit higher CMV seroprevalence due to increased exposure to the virus during previous pregnancies or medical procedures, which can facilitate CMV transmission. Furthermore, CMV infection during pregnancy is a known risk factor for adverse pregnancy outcomes, including spontaneous abortion, due to the virus's ability to cause placental inflammation and compromise fetal development. Studies have shown that primary CMV infection or reactivation of latent CMV can lead to higher rates of abortion, underscoring the importance of CMV screening and management in prenatal care (29).

Studying the prevalence of cytomegalovirus (CMV) in pregnant women is crucial because of its significant implications for maternal and neonatal health. CMV is the most common congenital infection worldwide, often leading to serious outcomes, such as hearing loss, developmental delays, and neurodevelopmental disabilities in affected newborns. Understanding the prevalence of CMV in pregnant women helps to identify the proportion of the population at risk and inform public health strategies for screening and intervention. Early detection of CMV infection during pregnancy can facilitate timely medical interventions, such as antiviral treatment or enhanced prenatal monitoring, to mitigate adverse outcomes. Furthermore, prevalence studies can guide the development of educational programs aimed at reducing transmission risks, such as promoting good hygiene practices and increasing awareness of CMV transmission routes. Given the

substantial health burden and economic costs associated with congenital CMV infection, ongoing research is essential to improve preventive measures and optimize healthcare resources for managing this infection.

DECLARATIONS

Abbreviations

WHO: World Health Organization, dsDNA: double stranded DNA, CMV: Cytomegalovirus, ELISA: Enzyme-Linked Immunosorbent Assay, PCR: Polymerase Chain Reaction.

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Authors' contributions

SDH, MY and AT designed the study. SDH, EM and AT collected specimens, SDH, AT, EM, HS, AV and MY analyzed and interpreted the data and drafted the manuscript. SDH, MY and AT was involved in reviewing the article. All authors critically revised the manuscript and approved the final version.

Ethics

This research project was granted approval by the Ethics Committee for Human Medical Experimentation at Golestan University of Medical Sciences, Gorgan, Iran (Ethics code: IR.GOUMS.REC.1401.043).

Conflict of interests

The authors report no conflict of interest in this work.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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