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ORIGINAL ARTICLE

A seromolecular study to determine the prevalence of cytomegalovirus in pregnant women referred to health centers in the north of Iran

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ABSTRACT

Background and Objectives: Because of the controversial aspects of the CMV virus during pregnancy, it should be considered a serious health threat, especially in developing countries. The present seromolecular study aimed to determine cytomegalovirus prevalence in pregnant women referred to health centers in the north of Iran.
Materials and Methods: One hundred and twenty-five pregnant women who were referred to health centers in Mazandaran province for regular health checks were randomly selected from Jan 2022 to Oct 2022. To detect the presence of the CMV genome and specific IgM and IgG antibodies against cytomegalovirus, the conventional PCR and ELISA tests were applied respectively.
Results: All 125 pregnant women that attended the study were from Mazandaran province with a mean age of 30 years ranging from 20 to 42 years. The result showed that 2 (1.6%), 92 (73.6%), and 2 (1.6%) of the cases were positive for IgM, IgG, and IgM/IgG, respectively. The PCR test results indicated that the CMV DNA was present in 10 (8%) pregnant women. Our study shows that all PCR-positive cases were negative for the IgM test. Of the 10 PCR-positive samples 3 were positive and 1 was suspicious for the IgG test.
Conclusion: Our study revealed that there is an urgent need for vaccination or other strategies to prevent and treat congenital CMV infection. Reducing the burden of congenital CMV infection requires global awareness. Further studies are recommended to obtain accurate estimates of the risk of congenital CMV infection.

Keywords: Seromolecular; Cytomegalovirus; Pregnant women; Iran

INTRODUCTION

Cytomegalovirus (CMV) which belongs to the herpes virus family is the largest human herpes virus with a double-stranded DNA genome of 150-200 nm in diameter (1). CMV is considered the most common cause of congenital viral infection in humans. The CMV infection is transmitted through several

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ways including horizontal transmission, contaminated fluids (urine, saliva), transplacental transmission in a fetus, and postnatally through breast milk (2). Hearing loss, neurological disorders, and mental retardation are the most common clinical manifestations of CMV infection in children (3, 4). The frequency of CMV infection is 1 to 5% in all live births in developed countries (5).

Similar to other herpes viruses, CMV resides latently in cells and does not usually lead to any symptoms (6). Approximately 25% of the human fetuses get infected with primary CMV infection in the first trimester of the pregnancy and on the other hand, mothers can transfer antibodies (IgG) to the fetus during pregnancy (7). There is a 30% to 40% risk of vertical transmission of the virus to the fetus in primary maternal CMV infection. Among all pregnancies with confirmed vertical transmission of CMV, just 10% to 20% of the newborns demonstrated evidence of clinical infection (8, 9). In comparison to the women who are infected in late pregnancy, women who develop primary CMV infection in the first trimester of pregnancy are more susceptible to having a child with sensorineural hearing loss (24% vs 2.5%) or other CNS sequelae, such as mental retardation, cerebral palsy, seizures, or chorioretinitis (32% vs 15%) (10, 11).

Laboratory tests (Molecular and Serology) are usually used to detect the presence of viruses in pregnant women (12). The CMV IgM antibody can be evaluated following primary infection in pregnant women (13) and the presence of congenital CMV infection in the blood can be detected by polymerase chain reaction (PCR). The CMV-PCR test that has high specificity and sensitivity is usually used for monitoring the presence of this infectious agent's DNA. It is one of the most sensitive methods that have been introduced for CMV infection diagnosis (14).

Although CMV infection is found in different geographical locations and among people with different social and economic backgrounds, it is more common in developing countries and societies with lower economic status (15). The overall prevalence of CMV infection in Africa, South America, Asia, North America, Australia, and Europe are > 95%, > 95%, 81-95%, ≤ 70%, ≤65%, and ≤60 %, respectively (16). About 40,000 children are born annually in the United States with a congenital CMV infection (3). In Australia and other developed countries, the prevalence of congenital CMV infection is estimated to be 0.64% (2). Sharghi et al., reported that among women of reproductive age, the highest rate of CMV IgG was detected in Tehran, Mashhad, Rasht, and Yasoj with a prevalence of 100% (95% CI: 100-100%) and the lowest rate of CMV IgG positive samples was observed in Jahrom with the frequency of 0.62% (95% CI: 53-71%) (17). Despite the use of general treatment against this virus, it can be noted that there is currently no antiviral therapy for CMV in asymptomatic infants (18). For the treatment of symptomatic CMV, oral Valganciclovir is prescribed for 12 months. Valganciclovir can ameliorate the clinical symptoms of congenital CMV in neonates (e.g., microcephaly, intracranial calcification, abnormal cerebrospinal fluid index, chorioretinitis, or sensor neural hearing loss) and because of insufficient evidence, treatment of mild CMV infection is not recommended in newborns under 32 weeks or more than 30 days (19).

Since CMV infection and re-infection or reactivation is an important factor in newborn malformations and due to the difficulties of its treatment, the prevention of maternal infection during pregnancy is an important strategy to inhibit the adverse effects of CMV on the fetus and neonates. Investigation of the prevalence of CMV among pregnant women could help the decision-making centers be aware of the risk of CMV infection and choose appropriate strategies for confronting CMV spread. In general, because of the controversial aspects of the virus during pregnancy, it should be considered a serious health problem, especially in developing countries. The present seromolecular study aimed to investigate cytomegalovirus prevalence in pregnant women referred to health centers in the north of Iran.

MATERIALS AND METHODS

Sample collection. In this cross-sectional descriptive study, 125 women who were referred to health centers for regular health checks in Mazandaran province were randomly selected from Jan 2022 to Oct 2022. Five milliliters of whole blood were taken from each volunteer, 2.5 ml of which were collected for serum separation by a centrifuge at 5000 RPM for 5 minutes, and the remaining blood was poured into tubes containing EDTA and stored at -20ºC for further DNA Extraction and PCR tests.

Preparation of reagents for DNA extraction.

Lysis buffer 1 consisted of 0.1 mM of EDTA Merck (Germany), 155 mM of NH_4CL Merck,

10 mM of N_a4HCO_3 Merck. Lysis buffer 2 contains 400 mM of NaCl Merck (Germany), 10 mM of Tris-HCl Merck (Germany), 2 mM of EDTA Merck (Germany), and 2% of SDS Merck (Germany). Lysis buffer 3 consisted of 5M of NaCL Merck.

DNA extraction using salting out method. The genomic DNA was extracted by the salting out method. In brief, 5 ml of red blood cell Lysis buffer 1 (0.1 mM of EDTA, 155 mM of NH_4CL, and 10 mM of Na_4HCO_3) was added to the 1 ml of whole blood and incubated on ice for 15 min. Then, it was centrifuged at 6000 rpm for 4 min, and two-thirds of the supernatant was discarded. This step was repeated 3 times until a white-pink pellet was observed. Next, the supernatant was poured away and the precipitate was mixed with 400 µL of Lysis buffer 2 (400 mM of NaCl, 10 mM of Tris-HCl, 2 mM of EDTA, and 2% of SDS). The mixed solution was incubated for 2 hours at 55°C. Then 300 microliters of 5 M NaCl were added to the solution and centrifuged for 10 minutes at 10000 RPM. The supernatant was transferred to a new microtube, mixed with absolute ethanol, and centrifuged at 10,000 rpm for 10 min. Then, the precipitated DNA was washed twice with 70% ethanol at 13,000 rpm for 3 min. Finally, 30 to 60 microliters of distilled water were to the DNA and stored at -20ºC.

PCR reaction. The conventional PCR method using 5´- GGTCACTAGTGACGCTTGTATGATGA-3´ and 5´-GATAGTCGCGGGTACAGGGGACTCT -3´ primers was applied as published before for CMV DNA detection (20). The PCR reaction was performed in a total volume of 15 µL containing 0.5 µL of each primer (10 pmol/ml), 2 µL of extracted DNA 4.5 µL of distilled water, and 7.5 µL of Mastermix (AMPLICQON, Denmark).

PCR-reaction conditions included an initial denaturation step of 94°C for 3 min, followed by 35 cycles of 94°C for 45 sec, 64°C for 45 sec, and 72°C for 1 min, followed by a terminal extension of 72°C for 10 min. Finally, the amplification of CMV was checked using the electrophoresis of PCR products on 1% agarose gel.

CMV IgG measurement. The serum CMV IgG Level was determined using a commercial CMV IgG kit (Pishtazteb; Iran) and according to the manufacturer's protocol. The Cobas e 801 analyzers (Roche Diagnostics) were used for performing the test. Samples were classified into three different categories based on the serum IgG level. Subjects with >1.9, <1.1, and 0.9-1.1 AU/ml serum IgG were considered positive, negative, and suspicious, respectively.

CMV IgM measurement. For the measurement of the serum Anti-CMV IgM level, the commercial kit (Pishtazteb; Iran) using the Cobas e 801 analyzers (Roche Diagnostics) was applied. Measurements were performed according to the manufacturer's instructions. The positive, suspicious, and negative cases were categorized according to the manufacturer's protocol; >1.1, 0.9-1.1, and <0.9 AU/ml, respectively.

Ethical statement. This study was approved by the ethical review committee of Mazandaran University of Medical Sciences (MAZUMS) (Ethical code: IR.MAZUMS.REC.1400.11760). The informed consent form was filled out by all the participants and their medical records remained confidential and used exclusively for research purposes.

RESULTS

All of the 125 pregnant participants were from Mazandaran province with a mean age of 30 years ranging from 20 to 42 years. The result showed that 2 (1.6%), 92 (73.6%), and 2 (1.6%) of the cases were positive for IgM, IgG, and IgM/IgG, respectively (Table 1). PCR test detected the CMV DNA in 10 (8%) pregnant women (Table 2). Table 3 indicates the seroprevalence rate of anti-CMV IgG/IgM among individuals with a positive PCR test. All of the PCR-positive cases were negative for the IgM test. Of the 10 PCR-positive samples 3 were positive and 1 was suspicious for the IgG test. The results of the amplification of virulence genes in CMV are shown in Fig. 1.

DISCUSSION

Cytomegalovirus (CMV) is the largest opportunistic human herpes virus that belongs to the Herpes Viridae family, from the Beta-Herpes family. HCMV has a double-stranded DNA genome with a size of 150-200 nm in diameter. The HHV-5type usually infects humans which is also called human

Table 1. CMV seroprevalence among pregnant women

Subjects characteristic	CMV					
	IgG (%)			IgM (%)		IgM/IgG (%)
Pregnant women	+	-	S	+	-	+
	92 (73.6)	26 (2.8)	7 (5.6)	2 (1.6)	123 (98.4)	2 (1.6)
Total sample	125			125		125

(+, positive; -, negative; S, suspicious)

Table 2. CMV-PCR test results among pregnant women

	CMV	
Pregnant women	HCMV-PCR (+) (%)	HCMV-PCR (-) (%)
	10 (8)	115 (92)
Total sample	125	125

(+, positive; -, negative)

Table 3. Seroprevalence rate of anti-CMV IgG/IgM among individuals with the positive PCR test

Subjects' characteristic	CMV				
	IgG (%)			IgM (%)	
Pregnant women	+	-	S	+	-
	3 (30)	6 (60)	1 (10)	0 (0)	10 (100)
Total sample	10			10	

(+, positive; -, negative; suspicious)

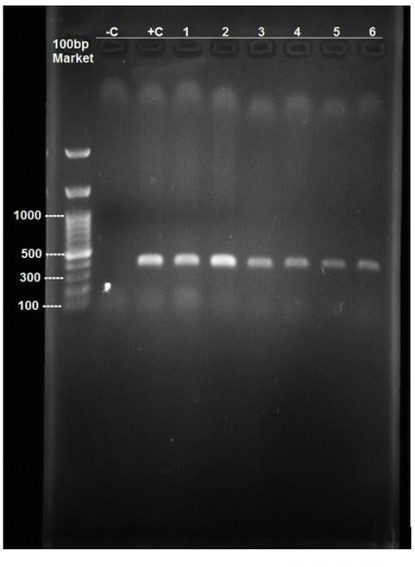

Fig. 1. Agarose gel electrophoresis of CMV-PCR test. lanes 1 to 6 display positive HCMV; Lane +C represents HCMV positive control (373 bp); lane -C represents HCMV negative control.

CMV (21). Horizontal transmission (birth), cervical shedding, contaminated fluid (urine, saliva) injection, mucosal contact, milk, vaginal secretions, and semen are the main ways of CMV transmission. In the condition of immunodeficiency, the activation of latent infection may lead to a wide range of clinical manifestations (22). The largest and most complex member of the herpes family is the virus that infects humans (23). Due to its cytopathic effect (structural changes in host cells) that results in enlarged cells that have intranuclear and cytoplasmic inclusions, it is named CMV. CMV can be found in almost every human cell type (24, 25). Like other herpes viruses, CMV creates a latent and persistent infection (in leukocytes) after the initial infection subsides that often lasts until the end of life and it has the reactivation potential due to various factors such as pregnancy (26). Primary infection occurs after transmission of the virus from an infected person or a carrier to a person who is seronegative for CMV. Reactivated infection occurs following the reactivation of a latent virus in seropositive individuals. Reinfection is the reacquisition of a virus from an external source in the mother (27). Considering the importance of this infection, the present study was conducted to investigate the seroepidemiology and prevalence of cytomegalovirus DNA in pregnant women referring to health centers in Mazandaran province.

The present study was conducted on 125 blood samples to determine the prevalence of cytomegalovirus infection by PCR and ELISA methods. The results indicated that only two subjects were positive for anti-CMV IgM antibody which shows primary and recurrent infection while 97 samples were positive for anti-CMV IgG antibody which indicates latent and previous infections of the participants. In terms of PCR test, 10 cases were positive which shows the presence of CMV DNA in investigated subjects. Bagheri et al. reported the seroepidemiology of cytomegalovirus infection during pregnancy in eastern

Iran, Gonabad and the results indicated that among 240 women, 69.6% had previous CMV infection, 27.9% had never been infected with CMV, and 2.5% and 72.1%were positive for CMV IgM and CMV IgG antibody respectively that were close to the results of our study (1). The results of the present study and similar studies in Iran are presented in Table 4.

In a cross-sectional study Ahmadpour et al. have also evaluated the seroprevalence, transmission, and factors related to specific antibodies against cytomegalovirus in pregnant women and their newborns in Mashhad. All of the 225 pregnant women were positive for the anti-CMV IgG antibody (100%), and 6 mothers (2.6%) were reported to have positive anti-CMV IgM antibody but the antibody was not detected in their babies (7). Tabatabai et al. have also conducted the same study on pregnant women at Valiasr Hospital in Kazeroon, Fars province on 1472 mothers, and based on the results 1438 participants (97.69%) were positive for anti-CMV IgG antibody while only 64 women (4.35%) were positive for anti-CMV IgM antibody (6).

As shown in our study, the seroprevalence of anti-CMV IgG was 73.6% which is higher than the United States (50.4%) (28). Different from other industrialized regions in the world, China has revealed a striking increase in CMV IgG seropositive rate among pregnant women. Higher IgG seroprevalence rate likely leads to an increased chance of reactivation within the hosts. Japan has successfully decreased the CMV IgG seroprevalence rate which possibly correlates with wealth, socioeconomic status, and hygiene. In the present study, the CMV IgM seroprevalence rate was lower (1.6%) than what was reported in previous studies (29-32).

Maternal serologic screening for the detection of primary CMV infection during pregnancy is insufficient to effectively identify all infants with congenital CMV infection and a universal PCR screening test is recommended for the detection of CMV-DNA in vaginal fluids during the first trimester of pregnancy.

In conclusion, there is an urgent need for vaccine research and other strategies to prevent and treat congenital CMV infection. Reducing the burden of congenital CMV infection requires global awareness. Further studies are recommended to obtain accurate estimates of the risk of congenital CMV infection.

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Table 4. Comparison of the results of the present study with similar studies in Iran

Reference	Author	Age (mean age)	CMV PCR (%)	Result
(6)	Tabatabaee (2009)	26.1	NA	Among 1472 pregnant women, the rate of seropositivity was found as 97.69% and the rate of positive CMV-IgM was 4.35%.
(1)	Bagheri (2012)	28.72	NA	Among 240 pregnant women, 72.1% were positive for CMV-IgG during pregnancy. The rate of positive CMV-IgM was 2.5%.
(33)	Nabizadeh (2020)	14-45 (30 ± 7)	NA	Among 2726 pregnant women, the rates of anti-Cytomegalovirus IgG were found 99.7%. The rates of anti-Cytomegalovirus IgM were discovered in 16 cases (0.6%).
(34)	Rahimzadeh (2022)	17-51 (30.31±5.30)	NA	Among 1092 pregnant women, the percentage of participants with CMV IgM and IgG antibody titers above normal was 0.2% and 91.8%, respectively.
(35)	Sherkat (2014)	18-45	NA	Among 43 pregnant women, one case (2.3%) of positive anti-CMV IgM was detected in each group. Anti-CMV IgG positivity was more frequent in patients (90.6%).

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