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Cytomegalovirus Infection among Pregnant Women in Beijing: Seroepidemiological Survey and Intrauterine Transmissions

Qing'e Jin*, Jianrong Su, and Shanna Wu

Department of Clinical Laboratory, Capital Medical University Affiliated Beijing Friendship Hospital, Beijing 100050, P.R. China

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**Corresponding author*
Phone: +86-10-63138577;
Fax: +86-10-63138550;
E-mail: fldfhmypaper@163.com

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Primary cytomegalovirus (CMV) infection during pregnancy can cause congenital defects. Available data for CMV infection during pregnancy in north China are inadequate. The aim of this study was to evaluate the epidemiology of maternal CMV infection and explore the incidence of congenital infection. In this prospective study, serum CMV IgG and IgM antibodies were measured in 2,887 pregnant women using ELISA, and the IgG avidity test was performed on all IgM-positive subjects. The seroprevalence of anti-CMV IgG was 94.70%, and of anti-CMV IgM was 1.28%. CMV IgG prevalence increased significantly with age ($p < 0.01$). Women living in downtown areas showed higher IgG prevalence than those residing in urban areas ($p = 0.023$). CMV-IgM seroprevalence was highest in autumn ($p = 0.021$). There was no difference in IgM seroprevalence by age, socioeconomic status, geographical area, or gravida. The rate of primary CMV infection was 0.45% (13/2,887) at the first trimester. The seroconversion rate during pregnancy was 0.76% (22/2,887). One woman underwent seroconversion during pregnancy and gave birth to an infant with asymptomatic CMV infection. Congenital CMV infection was diagnosed in five of the 14 infants from 14 mothers with active infection, for a vertical transmission rate of 35.71% (5/14). Three infants were asymptomatic, whereas two infants presented symptomatic infection with hearing deficits. Although CMV IgG prevalence is relatively high in north China, significant attention to primary CMV infection during pregnancy is still needed.

Keywords: Cytomegalovirus, epidemiology, congenital infection

Introduction

Human cytomegalovirus (CMV) infection is a major cause of congenital malformation worldwide, which can lead especially to childhood hearing loss and CNS disorders [1, 2]. Epidemiology of CMV infection varies broadly depending on many factors. It is well known that the seroprevalence of CMV in pregnant women is less than 50% in developed countries [3, 4]. However, both the seroprevalence of CMV in women and the incidence of congenital CMV infection are high in developing countries (1%–5% of births) [5, 6]. Both primary and recurrent CMV infections are usually asymptomatic in healthy individuals, whereas they can cause severe outcomes in immunocompromised individuals or infants [6]. Moreover, the vertical transmission rate is higher in women with primary infection than in women with reactivated or recurrent CMV infection. Primary CMV infection during the third trimester of pregnancy carries a higher risk of congenital transmission than those acquired during the first trimester [7].

Seroepidemiological data are important for estimating the risk of primary CMV infection. However, reliable estimates of prevalence and outcomes of CMV infection from north China are not readily available. CMV seroprevalence in infants still needs to be clarified. In this study, we collected serum samples from women at the first and third trimesters over a 5-year period, from 2010 to 2015, in Beijing. We surveyed the epidemiological state of CMV infection and observed the seroconversion at the third trimester among pregnant women. As for IgM positivity (IgM+) with low IgG avidity of women, we explored the congenital CMV infection rate and performed

effective treatment. CMV IgG seroprevalence in Beijing remained as high as 94.70%. However, a higher seroprevalence among pregnant women may not guarantee a lower incidence of congenital CMV infection. Extensive and more precise epidemiological surveys are needed to implement preventive strategies against in utero transmissions.

Materials and Methods

Samples

During a 5-year period from July 2010 to June 2015, a total of 2,887 women aged 22–44 years at the first trimester of pregnancy were enrolled in this prospective study. Blood samples were collected and centrifuged at 1,200 ×*g* for 10 min to detect CMV antibody levels at the first trimester, and the antibody levels were reassessed during the last stage of pregnancy to identify seroconversion. All subjects gave written consent to participate in the study. Guardians also signed the written informed consent on the behalf of their children. Capital Medical University Affiliated Beijing Friendship Hospital granted ethics approval (review code 2010/017). Two specialists performed the clinical assessments and obtained epidemiological data, including gestational age, educational level, gravidity, parity, and economic status.

Serology

Serological tests for CMV IgG and IgM were performed using quantitative ELISA kits (Virion/Serion, Germany), according to the manufacturer protocol. The women with positive anti-CMV IgM were further tested for CMV IgG avidity index (AI), since anti-CMV IgG avidity was a reliable tool for distinguishing primary infection from recurrent infection (EUROIMMUN anti-CMV ELISA) [8–10]. Serological testing for CMV IgM levels by ELISA was performed in the neonates of mothers with positive anti-CMV IgM and low IgG avidity.

Quantitative PCR

Blood samples from the infants who were CMV IgM positive were also evaluated for CMV DNA by PCR. DNA was extracted from the blood samples on the day of collection and stored at −20°C until use (QIAamp DNA minikit; Qiagen, Germany). The PCR primers and reaction conditions were as described previously [7].

Table 1. Serological data of 2,887 pregnant women at the first trimester in north China[a].

CMV Ig status		Pregnant women
IgG	IgM	
-	-	149
+	-	2,701
+	+	33
-	+	4

[a]+, positive; -, negative.

Statistical Analysis

Statistical analyses were performed using SPSS software (ver. 17.0; SPSS Inc., USA). A value of $p < 0.05$ was considered statistically significant.

Results

CMV IgG and IgM Seroprevalence among Pregnant Women in North China

We measured CMV IgG and IgM antibody levels in 2,887 pregnant women. Those who had an equivocal serology result or an IgM-positive result were screened 1 week later to confirm their serostatus. The majority (94.70%) of women were CMV IgG seropositive, with 1.28% being IgM positive at the first trimester. Of 37 IgM+ women, 33 were CMV IgG positive and four were CMV IgG negative (Table 1). Low IgG avidity was demonstrated in 13 of the 37 CMV IgM+ subjects at the first trimester (Table 2, Cases 1–13). CMV IgG seroprevalence in urban and downtown areas was 93.84% and 95.75%, respectively (p = 0.023). CMV IgM seroprevalence in urban and downtown areas was 1.32% and 1.24%, respectively (p = 0.843). Therefore, no significant difference in CMV infection rate was found by socioeconomic status or lifestyle in north China (Table 3). The highest seroprevalence of anti-CMV IgM occurred in autumn, with 2.45% (p = 0.021) (Table 4).

Table 2. Screening data from fourteen low IgG avidity women[a].

Case	Age	Period	CMV IgG	CMV IgM	CMV status at birth
1	26	2010-10	+	+	Uninfected
2	26	2011-03	+	+	Uninfected
3	25	2011-07	+	+	Asymptomatic
4	28	2011-11	+	+	Uninfected
5	29	2012-08	+	+	Asymptomatic
6	25	2012-11	+	+	Uninfected
7	34	2013-09	+	+	Uninfected
8	26	2013-12	+	+	Uninfected
9	34	2014-01	+	+	Uninfected
10	31	2014-07	+	+	Uninfected
11	27	2014-11	+	+	Symptomatic
12	35	2015-01	+	+	Uninfected
13	27	2015-05	-	+	Symptomatic
14	37	2013-07	-	+	Asymptomatic

[a]+, positive; -, negative.

Table 3. CMV seroprevalence and demographic characteristics among pregnant women in north China[a].

Subjects characteristic	CMV			
	IgG (%)		IgM (%)	
	+	-	+	-
Age				
22–29	1,262 (92.59)	101 (7.41)	23 (1.69)	1,340 (98.31)
30–39	998 (95.59)	46 (4.41)	10 (0.96)	1,034 (99.04)
40–44	474 (98.75)	6 (1.25)	4 (0.83)	476 (99.17)
Residence				
Urban	1,494 (93.84)	98 (6.16)	21 (1.32)	1,571 (98.68)
Downtown	1,240 (95.75)	55 (4.25)	16 (1.24)	1,279 (98.76)
Education				
Junior college	1,020 (95.68)	46 (4.32)	16 (1.50)	1,050 (98.50)
Graduate	904 (94.07)	57 (5.93)	11 (1.14)	950 (98.86)
Postgraduate	810 (94.19)	50 (5.81)	10 (1.16)	850 (98.84)
Gravidity				
Primigravid	1,859 (95.19)	94 (4.81)	22 (1.13)	1,931 (98.87)
Multigravid	875 (93.68)	59 (6.32)	15 (1.61)	919 (98.39)

[a]+, positive; -, negative.

Table 5. Rate of CMV seroconversion in 2,887 pregnant women[a].

First trimester	Third trimester	Subjects (%)
IgG-/IgM-	IgG-/IgM-	146 (5.06)
IgG-/IgM-	IgG-/IgM+	3 (0.10)
IgG+/IgM-	IgG+/IgM-	2,694 (93.31)
IgG+/IgM-	IgG+/IgM+	7 (0.24)
IgG+/IgM+	IgG+/IgM+	24 (0.83)
IgG+/IgM+	IgG+/IgM-	9 (0.31)
IgG-/IgM+	IgG-/IgM+	1 (0.03)
IgG-/IgM+	IgG+/IgM-	2 (0.07)
IgG-/IgM+	IgG+/IgM+	1 (0.03)

[a]+, positive; -, negative.

Age-Dependent Prevalence of CMV IgG and IgM among Pregnant Women in North China

All subjects enrolled in the study (aged 22–44 years old) were divided into three age groups and the CMV seroprevalence (IgG and IgM) was compared. As expected, an age-dependent change was evident in the CMV seroprevalence data. CMV IgG seroprevalence was significantly higher in the 40–44 age group than in the younger age groups ($p < 0.01$). In contrast, the 22–29 age group showed the highest CMV IgM seroprevalence ($p = 0.183$) (Table 3).

Identification of Pregnant Women with Seroconversion

A total of 22 subjects underwent seroconversion during the third trimester, and the rate of CMV seroconversion was 0.76% (22/2,887). Ten had evidence of CMV IgM seroconversion, whereas 12 demonstrated CMV IgG seroconversion (Table 5). Three subjects, who were initially CMV IgG-/IgM-, became IgG-/IgM+. Seven women, who were initially CMV IgG+/IgM-, became IgG+/IgM+; unfortunately, of these, one presented low IgG avidity, indicating a high risk of intrauterine transmission (Table 2, Case 14). Thus, the incidence of primary CMV infection during pregnancy was estimated to be 0.48% (14/2887). Two women, who were initially CMV IgG-/IgM+, became IgG+/IgM-, and one who was initially CMV IgG-/IgM+, became IgG+/IgM+, indicating a low risk of congenital infection. All 14 fetuses were born at full term.

Estimation of the Incidence of Congenital CMV Infections in North China

Fourteen singleton fetuses born to mothers with primary CMV infection (CMV IgM+ with low IgG avidity) were screened for CMV IgM antibody in the first 2 weeks of life. Five infants were CMV IgM positive. The congenital infection rate was estimated to be 0.17% (5/2,887) (Table 2). Furthermore, quantitative PCR was performed to detect CMV DNA in blood samples of the five infants with in utero CMV transmission and to assess the serological diagnosis. Of the five neonates, two showed CMV DNAemia, and symptomatic infection with sensorineural hearing deficits, whereas the other three infants were completely asymptomatic. The two neonates with symptomatic CMV infection received ganciclovir therapy. Fortunately, 2 weeks later, CMV viral loads decreased in the neonates and their clinical conditions improved partially.

Table 4. The prevalence of anti-CMV IgM in 2,887 pregnant women during different seasons[a].

	Spring (%)	Summer (%)	Autumn (%)	Winter (%)
Anti-CMV IgM	7 (0.87)	6 (0.77)	16 (2.45)	8 (1.24)

[a]Spring: March, April, and May; Summer: June, July, and August; Autumn: September, October, and November; Winter: December, January, and February.

Discussion

CMV infection is a well-acknowledged cause of birth defects and may account for a majority of perinatal deficits, especially in developing countries. In this study, we investigated the seroepidemiology of CMV infection among pregnant women and the rate of congenital infections in the Beijing area.

As shown in our study, the seroprevalence of anti-CMV IgG was 94.70%, which is consistent with previous studies [11–13], but higher than that of the United States (50.4%) [14]. 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 likely leads to an increased chance of reactivation within a host. CMV IgG seroprevalence increased strongly with age; in contrast, CMV IgM seroprevalence decreased across age groups. To our best knowledge, this is the first report of the age-related CMV serostatus in north China, and the results are in accordance with those from other regions [4]. In our neighborhood, Japan has found decreased CMV IgG seroprevalence with age [15], which possibly correlates with wealth, socioeconomic status, and hygiene. We found that our CMV IgM seroprevalence rate was lower (1.28%) than that reported in previous studies [7, 16, 17]. In Pakistan, IgM seroprevalence was as high as 12.71% and CMV DNA was detected in 20% of pregnant women [13]. The differences may be partly explained by variation in the types of populations sampled. Furthermore, IgM seroprevalence changed with the seasons, with the highest rates being in the autumn (16/37, 43.24%). Thus, pregnant women need to pay more attention to CMV infection in autumn.

The prevalence of anti-CMV IgG+/IgM+ was 1.14% (33/2,887), which is lower than reported previously [18]. Strengthening health awareness gradually can account for this condition. Our data differ from previous reports that revealed socioeconomic status, literacy, or exposure to young children were significantly associated with CMV IgM seroprevalence [3, 19]. In the present study, no significant differences in CMV IgM seroprevalence were shown for district of residence (urban or downtown), socioeconomic status, gravidity, or lifestyle. It is possible that the living environment, geographical variation, and ethnicity can account for this phenomenon. Women who were CMV IgG- during their entire pregnancy were at risk of severe congenital CMV infection. We performed preventive education, especially focusing on hygiene for these mothers, although it is still a debated issue.

A total of 22 subjects underwent CMV seroconversion, of whom one converted from CMV IgG-/IgM- to IgG-/IgM+ with low IgG avidity. This mother gave birth to a baby with asymptomatic CMV infection. Therefore, the overall primary infection rate was 0.48% (14/2,887), which was lower than previous reported rates of 0.75–1.50% [20]. Besides this, the other 21 subjects tested had high IgG avidity, suggesting none was recently infected. Among the other 13 subjects with primary CMV infection, two infants showed postnatal sequelae with hearing deficits, and two neonates were asymptomatic. Thus, the incidence of congenital CMV infection was estimated to be 0.17% (5/2,887), which was relatively low compared with previous reports (0.3–0.6%), probably due to the high IgG seroprevalence in our region. Thus, a high CMV IgG seroprevalence in our study possibly indicated a low incidence of intrauterine transmission, but reinfection with a different strain of the virus is possible [21]. We retrieved and reviewed the ultrasonographic examination results for gravida with primary infection and found no evidence of fetal abnormalities. Thus, ultrasonographic examination may not be appropriate in screening for congenital CMV infection, but further research is needed.

Forner *et al*. [22] reported that high levels of CMV DNAemia predicted CMV sequelae in asymptomatic congenitally infected newborns. Fortunately, the three asymptomatic infants had no CMV DNAemia. The two symptomatic neonates with CMV DNAemia received ganciclovir therapy. For reason of medical technology, the two neonates were transferred to the newborn center for further intensive treatment. We did not perform anti-CMV IgG serology in the neonates, and thus cannot establish the antibody levels of the neonates. We did not perform PCR testing to detect CMV DNA from the pregnant women with active infection, and therefore cannot deduce the relationship between the maternal CMV DNA levels and congenital infection rate.

In conclusion, in north China, many women of reproductive ages are still at risk of primary CMV infection during pregnancy. There is an urgent need for vaccine research and other strategies to prevent and treat congenital CMV infection. To reduce the burden of congenital CMV infection will require global awareness. Further studies are necessary for more precise surveys to gain accurate estimates of the risk of congenital CMV infection.

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