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Original Article

Characteristics and serology of pregnant women with cytomegalovirus immunoglobulin G seroconversion during pregnancy in Japan

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ABSTRACT

Objective: Investigate the characteristics and serology of pregnant women with cytomegalovirus (CMV) immunoglobulin (Ig)G seroconversion during pregnancy to understand the risk factors associated with primary CMV infection and the occurrence of fetal congenital CMV infection.
Materials and methods: We retrospectively studied 3202 pregnant women who were CMV IgG-negative in early pregnancy and were retested for IgG in late pregnancy. Characteristics were compared between participants with and without IgG seroconversion, and serological parameters were compared between participants with and without fetal congenital CMV infection.
Results: Twenty-six participants showed CMV IgG seroconversion and fifteen showed fetal congenital CMV infection. Seroconversion rates were significantly higher in teens (5.0%) than in older women (20s: 0.8%; 30s and over: 0.6%) ($p < 0.001$). Titers of CMV IgM at IgG seroconversion were higher in women without (median 8.66) than with (median 6.54) congenital infection ($p = 0.045$). The congenital infection rate was high when IgM titers at IgG seroconversion were low (47.1% with 4.00–12.00 titers and 100% with 1.21–3.99 IgM titers) ($p = 0.048$).
Conclusions: Nulliparous pregnant teenagers have a high risk of CMV IgG seroconversion and the CMV IgM titer at IgG seroconversion may help predict the occurrence of fetal congenital CMV infection.

Introduction

Cytomegalovirus (CMV) is the most common pathogen causing intrauterine infection worldwide. In children, the magnitude of health problems related to congenital CMV infection is comparable to that caused by other childhood diseases, such as trisomy 21, fetal alcohol syndrome, and spina bifida. Mental retardation, motor disabilities, and sensorineural hearing loss are among the most severe neurological sequelae of fetal congenital CMV infection [1–4]. Two sources of CMV infection in pregnant women include direct contact with young children and sexual activity [5–8], with transmission occurring through direct contact with body fluids containing viable CMV [8].

In pregnant women with primary CMV infection during pregnancy, who are at higher risk of fetal congenital infection, serological tests such as CMV immunoglobulin (Ig) G, IgM antibody, and IgG avidity tests provide accurate results. The common pattern of CMV IgM antibody titer dynamics after primary infection involves an initial rise, followed by a rapid drop, whereas the persistence pattern involves an initial rise in IgM titers, followed by a gradual drop [9]. However, the influence of different patterns on fetal congenital CMV infection occurrence under primary infection is still unclear.

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In an effort to understand the risk of fetal congenital CMV infection, we investigated characteristics, such as age and parity, between pregnant women with and without IgG seroconversion. Additionally, CMV IgG, IgM antibody, and IgG avidity were serologically investigated between pregnant women with and without fetal congenital infection under IgG seroconversion during pregnancy.

Methods

In 2013, a maternal CMV antibody screening program using CMV IgG, IgM antibody, and IgG antibody avidity testing was initiated as a prospective cohort study in Mie, Japan; this study is ongoing [10]. Briefly, pregnant women with serologically confirmed primary CMV infection during pregnancy were selected, including women who were positive for both IgG and IgM, had low IgG avidity in early-stage pregnancy, and exhibited IgG seroconversion in late-stage pregnancy. For the current nested, case–control study, we obtained approval from the Clinical Research Ethics Review Committee of the Mie University Hospital (Approval No.: H2019-127) and adopted an opt-out approach instead of informed consent from participants.

Participants

In this study, we retrospectively included 3202 pregnant women who were negative for serum CMV IgG antibodies during early pregnancy (14 or fewer gestational weeks), received education on the risks of primary CMV infection during pregnancy via a note from their obstetrician, and were retested for IgG in late pregnancy (28 or more gestational weeks). All participants were selected from the antibody screening cohort between September 2013 and September 2016 at 17 centers in Mie, Japan (Fig. 1). Precautions taken to avoid primary infection during pregnancy were as follows: avoidance of direct contact with saliva and urine (from one's own children and other young children) and exposure to semen.

Reviewed data

Clinical characteristics of the participants, including age, parity, gestational weeks of CMV IgG antibody tests, both in early and late pregnancy, and the interval between IgG tests performed during early and late pregnancy, were reviewed. Fetal congenital CMV infection diagnosis was confirmed by the presence of CMV DNA in newborn urine within the first week of life. In some cases, a supporting diagnosis was made by positive viral isolation tests using the same urine samples. Participants were organized into age and parity groups (age groups: teens [16–19 years], 20s [20–29 years], and 30s and over [30–44 years]; parity groups: para 0, para 1, and para 2 and more). Characteristics were compared between pregnant women with and without IgG seroconversion during pregnancy.

Clinical characteristics, including birth weeks, birth weight, head circumference, Apgar score at 1 min, Apgar score at 5 min, umbilical cord artery pH, and placental weight in neonates born to women with IgG seroconversion during pregnancy, were reviewed. In neonates with congenital infection, results of neonatal hearing screening, brain magnetic resonance imaging, and auditory brain stem response were reviewed.

Test results of serum CMV IgG, IgM antibody, IgG avidity, and serum CMV DNA analysis at IgG seroconversion in late pregnancy from pregnant women with CMV IgG antibody seroconversion were reviewed. IgG titers (enzyme immunoassay [EIA] titer), IgG avidity index (%), IgM positivity number, IgM titers (index) in positive IgM cases only, and serum CMV DNA positivity number were compared between pregnant women with and without fetal congenital infection.

Serological and DNA tests

In serum CMV IgG or IgM antibody tests, Seiken assays for CMV IgG or IgM (Denka Seiken, Tokyo, Japan) were used [11,12]. For IgG antibody tests in early pregnancy, an EIA titer value of 0–1.9 was considered negative, while in late pregnancy, an EIA titer value of 4.0 or more was considered positive or indicative of seroconversion.

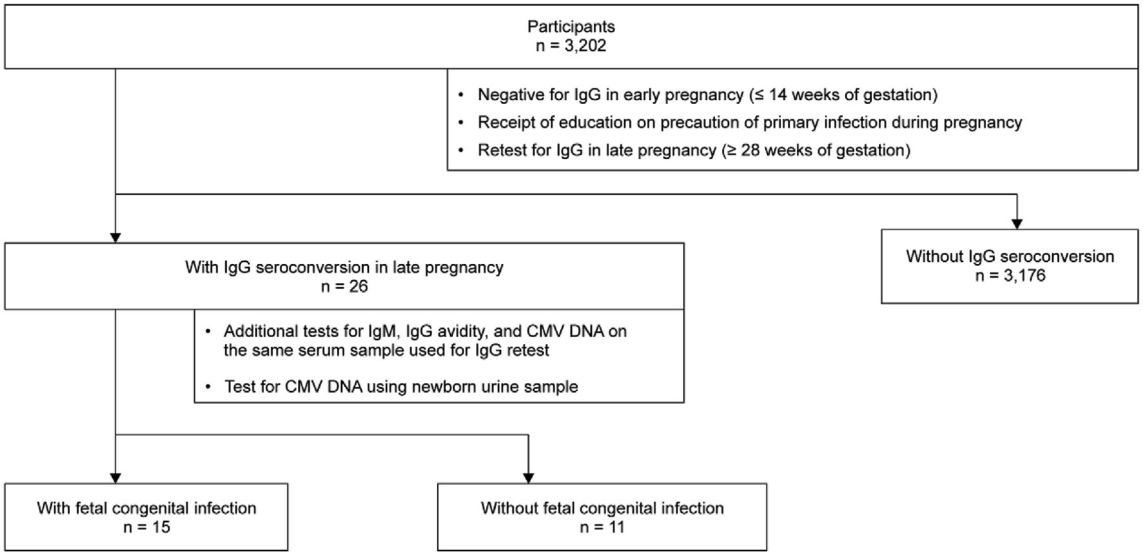

Fig. 1. Flowchart for inclusion in the nested, case control study. Diagnosis of fetal congenital cytomegalovirus (CMV) infection was made based on positive CMV DNA from urine samples of newborns.

In IgM antibody tests, an IgM titer value of 1.21 or more was considered positive, with 12.00 being the maximum titer in the study. The serum IgG avidity index (%) was measured at the Research Center for Disease Control, Aisenkai Nichinan Hospital [10]. Serum CMV DNA and neonatal urine CMV DNA were tested by real-time polymerase chain reaction (PCR) at the Central Laboratory, Mie University Hospital [10]. Viral isolation, using the newborn urine sample, was performed using human fetal fibroblasts to determine cytopathic effects at the Institute for Clinical Research, National Mie Hospital. Cytopathic effects were evaluated up to 6 weeks after culture onset.

Statistical analysis

To calculate statistical significance, we used Chi-squared tests, Mann–Whitney U tests, or Fisher's exact tests; $p < 0.05$ was considered statistically significant. Analyses were performed using SAS Enterprise Guide 6.1 (SAS Institute, Cary, NC, USA) software.

Results

CMV IgG seroconversion during pregnancy and fetal congenital CMV infection in each age and parity group

Of the 3202 participants, 26 (0.8%) had CMV IgG seroconversion during pregnancy with 15 (0.5%) of those resulting in fetal congenital CMV infection (Fig. 1, Table 1). The IgG seroconversion rate during pregnancy and the fetal congenital infection rate were significantly ($p < 0.001$) higher in primipara teenagers than in the other groups. In contrast, there were no significant differences in IgG seroconversion rate or fetal congenital infection rate between parity groups (Fig. 2).

Characteristics of pregnant women with and without CMV IgG seroconversion, and neonates born to pregnant women with IgG seroconversion during pregnancy

The median age (years) of pregnant women with CMV IgG seroconversion was 27 (ranging 16–37) and the median of women without IgG seroconversion age was 29 (ranging 16–44). The median parity (para) with and without IgG seroconversion was 1 (ranging 0–4) and 0 (0–5), respectively. The median number of gestational weeks of IgG testing in early pregnancy and IgG retesting in late pregnancy was 11 (ranging 8–14) and 34 (ranging 28–39), respectively, being 11 (ranging 5–14) and 34 (ranging 28–41) with and without IgG seroconversion, respectively. Median interval weeks between early and late pregnancy was 24 (ranging 18–28) and 23 (ranging 15–33) with and without IgG seroconversion, respectively. The only significant difference ($p = 0.037$) observed was the age between pregnant women with and without IgG seroconversion. The characteristics of neonates with and without congenital CMV infection are shown in Table 2. The only significant difference ($p = 0.033$) was found in placental weight between neonates with and without congenital infection. All 15 neonates with congenital infection underwent neonatal hearing screening within the first week of life and none showed "refer" results. Thirteen neonates underwent an auditory brain stem response test and none showed abnormalities. Nine neonates underwent a brain magnetic resonance imaging test; although 2 of those 9 neonates showed abnormalities (white matter lesions), none of them showed sequelae at 18 months of life.

Serological parameters at CMV IgG seroconversion in pregnant women with and without fetal CMV congenital infection

Serological parameters at IgG seroconversion are shown in Table 2 and Supplementary Table. The only significant difference ($p = 0.045$) observed was in the IgM titer at IgG seroconversion among IgM-positive women (Table 2). Plots of CMV IgM titers of pregnant women both with and without fetal congenital infection are shown in Fig. 3. In pregnant women with IgM positivity but low IgM titer levels, fetal congenital infection rate increased. Of the 17 pregnant women with IgM titer values between 4.00 and 12.00, eight (47.1%) had fetal congenital infection, whereas of the five pregnant women with titer values between 1.21 and 3.99, all five had fetal congenital infection (Fig. 4) ($p = 0.048$, calculated among positive IgM cases).

Discussion

In this study, we found that teenage pregnant women had a 6- to 8-fold higher risk of CMV IgG seroconversion during pregnancy than older pregnant women, with a rate of up to 5.5%. Teenage pregnant women in our study who showed IgG seroconversion during pregnancy were all nulliparous. In contrast, IgG seroconversion rates during pregnancy in nulliparous pregnant women in their 20s and in their 30s and older were both 0.5%. Therefore, an 11-fold higher risk of primary infection during pregnancy was found in nulliparous, teenage pregnant women, in comparison with that in nulliparous, older pregnant women.

Primary CMV infection occurs through direct contact with saliva, urine, or semen containing viable CMV [8]. Two sources of CMV infection in pregnant women include direct contact with their or others' young children and sexual activity [5–8]. In nulliparous pregnant women, the sources of primary CMV infection are limited to contact with saliva or urine of others' young children or with semen of their sexual partners with whom they have sexual intercourse during pregnancy. Fowler et al. [13] reported that teenage pregnant women possibly had more frequent sexual exposure to CMV. Indeed, the prevalence of CMV is increased during the teenage years, when individuals often become sexually active and may come in contact with semen from their sexual partners [14]. Moreover, Fowler and Pass [5] reported that pregnant women who became sexually active up to 2 years before delivery were at higher risk for fetal congenital infection. They suggested that maternal CMV infections resulted from sexual exposure to CMV and that

Table 1
Number of participants and rates of cytomegalovirus (CMV) IgG seroconversion and fetal congenital CMV infection according to age and parity.

Age and parity	Para 0	Para 1	Para ≥2	Total
	Number of participants, with CMV IgG seroconversion (%), and with fetal congenital CMV infection (%)			
Teens (16–19 years of age)	73, 4 (5.5), 2 (2.7)	7, 0 (0), 0 (0)	0, 0 (0), 0 (0)	80, 4 (5.0), 2 (2.5)
20s (20–29 years of age)	920, 5 (0.5), 3 (0.3)	529, 7 (1.3), 3 (0.6)	119, 1 (0.8), 1 (0.8)	1568, 13 (0.8), 7 (0.4)
30s and over (30–44 years of age)	626, 3 (0.5), 1 (0.2)	676, 2 (0.3), 2 (0.3)	252, 4 (1.6), 3 (1.2)	1554, 9 (0.6), 6 (0.4)
Total	1619, 12 (0.7), 6 (0.4)	1212, 9 (0.7), 5 (0.4)	371, 5 (1.3), 4 (1.1)	3202, 26 (0.8), 15 (0.5)

Abbreviations: CMV, cytomegalovirus; IgG, immunoglobulin G.

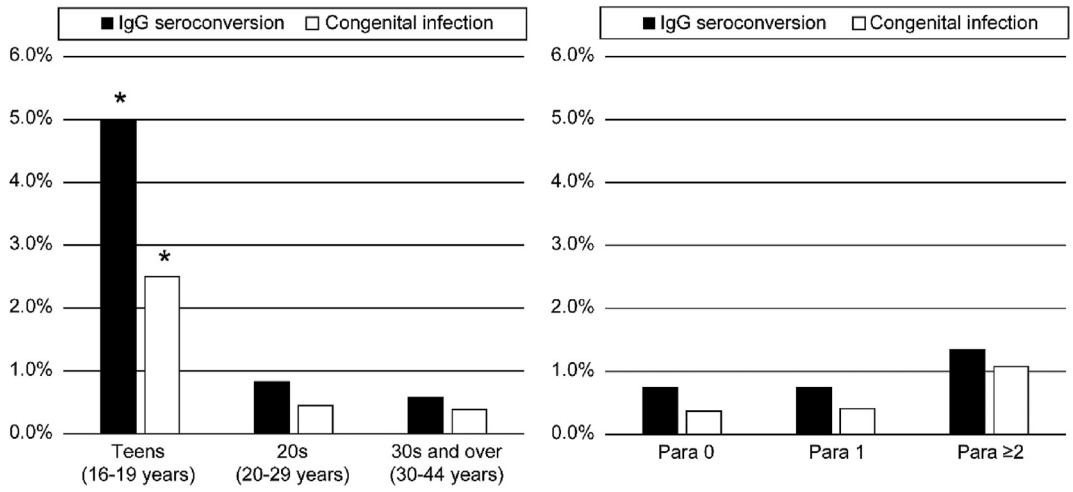

Fig. 2. Rates of cytomegalovirus (CMV) immunoglobulin G seroconversion and fetal congenital CMV infection according to age and parity. *Significant difference ($p < 0.001$). The IgG seroconversion rate and the fetal congenital infection rate were higher in teens than in the other groups. In contrast, there were no significant differences between parity groups.

Table 2
Serological parameters at cytomegalovirus (CMV) immunoglobulin (Ig) G seroconversion in pregnant women and characteristics of neonates, with and without congenital CMV infection.

	With fetal congenital CMV infection (n = 15)	Without fetal congenital CMV infection (n = 11)	*p* value
Serological parameters at CMV IgG seroconversion in pregnant women with IgG seroconversion			
CMV IgG titer at IgG seroconversion (EIA titer)	9.4 (6.3–19.8)	8.3 (4.8–18.5)	0.467
CMV IgG avidity index at IgG seroconversion (%)	23.1 (0.6–34.8)	17.5 (0.3–55.2)	0.876
CMV IgM positivity at IgG seroconversion (case)	13 (86.7%)	9 (81.8%)	0.555
CMV IgM titer at IgG seroconversion (index), n = 22	6.54 (1.49–12.00), n = 13	8.66 (4.21–12.00), n = 9	0.045
Serum CMV DNA positivity at IgG seroconversion (case)	4 (26.7%)	1 (9.1%)	0.356
Characteristics of neonates born to pregnant women with CMV IgG seroconversion			
Birth weeks (week)	38 (37–40)	38 (34–40)	0.789
Birth weight (g)	2896 (2070–3294)	3028 (1844–3838)	0.568
Head circumference (cm)	32.5 (30.0–34.0)	33.0 (30.1–35.6)	0.348
Apgar score at 1 min	9 (8–10)	9 (8–10)	0.313
Apgar score at 5 min	10 (9–10)	10 (8–10)	0.778
Umbilical cord artery pH	7.32 (7.16–7.48)	7.27 (7.18–7.35)	0.331
Placental weight (g)	517 (380–834)	574 (406–965)	0.033

Abbreviations: CMV, cytomegalovirus; IgG, immunoglobulin G.
Expressed as median (range) or number (%).

young maternal age might be a marker for recent exposure. As the age of pregnant women was reported to not be a predictor of CMV unawareness by Wizman et al. [7], in nulliparous teenage pregnant women, increased sexual activity itself was considered a risk factor for primary CMV infection, apart from CMV unawareness.

CMV antibody prevalence in women of childbearing age in Japan has declined to less than 75%, from greater than 90% in the 1970s [9,15–18], and thus, the number of pregnant women uninfected by CMV before pregnancy is expected to increase. In order to decrease congenital CMV infection, it is important for pregnant women to take preventative measures against primary CMV infection, including avoiding contact with saliva or urine from children, and the use of condoms during sexual intercourse [6]. In the current study, pregnant women who were CMV IgG negative in early pregnancy received education on precautionary measures to avoid primary CMV infection during pregnancy from their obstetrician. However, the information provided included mostly information on avoiding direct contact with saliva and urine. Thus, particularly in pregnant teenage women, education on avoidance of sexual contact without appropriate protection may be more important. In this study, environmental information was limited to parity, i.e., the existence of other children born to that participant. Information regarding whether the individual was in frequent contact with other children was not provided.

When analyzing pregnant women with primary CMV infection during pregnancy, serological tests, such as CMV IgG, IgM antibody, and IgG avidity tests, are used [10,19,20]. Seroconversion in IgG antibody, or positive IgM antibody plus low IgG avidity during pregnancy, indicates primary infection. Although CMV IgM antibody tests are highly sensitive in diagnosing an infection, the specificity for excluding primary infection is low. One reason for this poor specificity is that CMV IgM can be produced during re-infection or re-activation. Indeed, Koshizuka et al. [21–23] reported the first case of re-infection during pregnancy in a woman suspected of having original antigenic sin detected using both IgM assays and glycoprotein H-specific antibodies. Another reason for poor specificity is the persistence of IgM antibodies after primary infection. Indeed, CMV IgM antibodies are known to exhibit either a common or persistence pattern after primary infection, which may be related to the timing and type of infection [9].

The clinical influence of different dynamics of IgM antibody titers in pregnant women with fetal congenital CMV infection under primary infection has not yet been determined. In the current study, we found that the CMV IgG avidity index at IgG

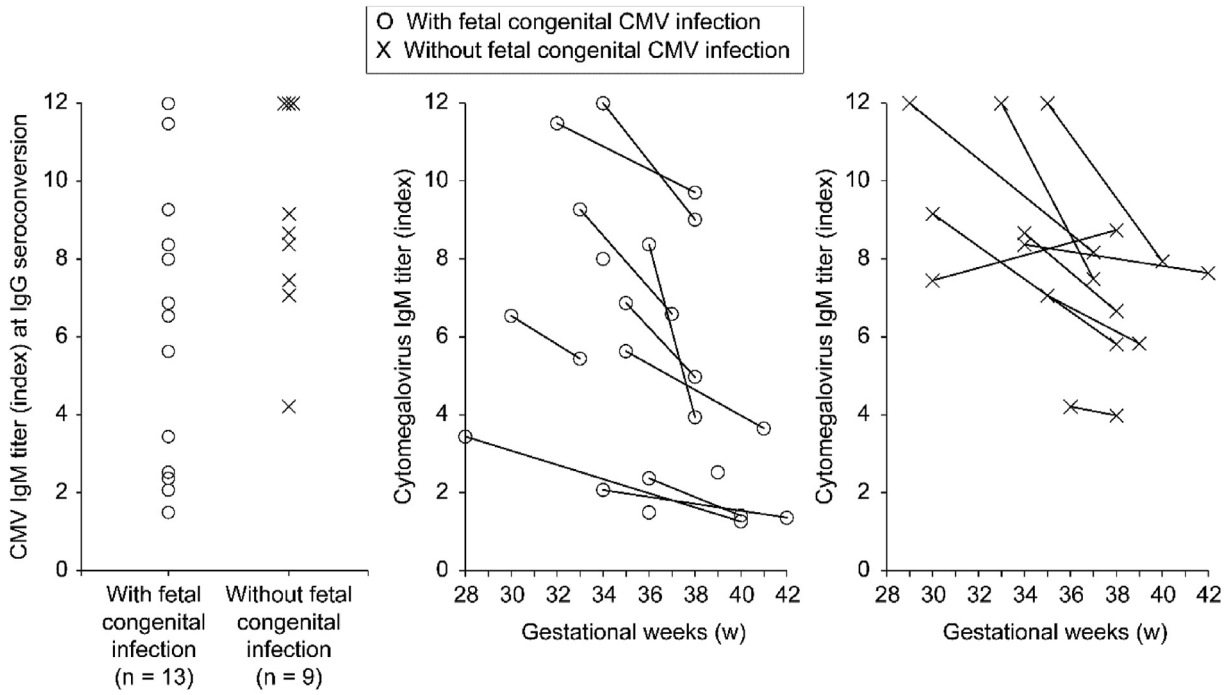

Fig. 3. Plots of cytomegalovirus (CMV) immunoglobulin (Ig) M titers (n = 22) at CMV IgG seroconversion and plots of CMV IgM titers from the time of IgG seroconversion until near the delivery date, in pregnant women both with and without fetal congenital CMV infection. The "O" symbols indicate cases with fetal congenital CMV infection, and "X" symbols indicate cases without fetal congenital CMV infection.

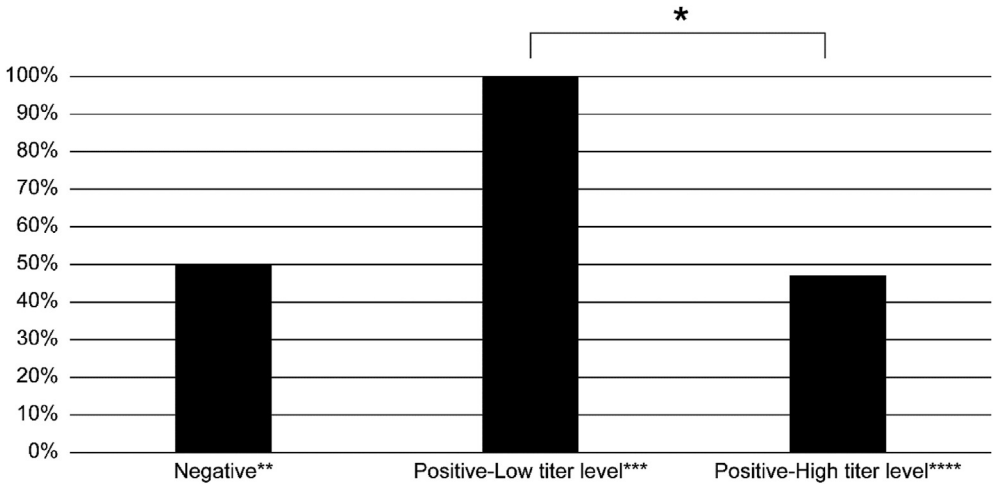

Fig. 4. Rates of fetal congenital cytomegalovirus (CMV) infection according to CMV immunoglobulin (Ig) M titer levels at IgG seroconversion. *Significant difference ($p = 0.048$). **0.00–1.20 index of CMV IgM titer. ***1.21–3.99 index of CMV IgM titer. ****4.00–12.00 index of CMV IgM titer.

seroconversion was not significantly different between pregnant women with and without fetal congenital infection. As CMV IgG avidity is currently the best way to estimate the timing of primary infection, the timing in this study may have been not so different between women with and without IgG seroconversion. The timing of primary infection may not have a major impact on occurrence of fetal congenital infection. Meanwhile, we found in this study that the CMV IgM antibody titer at IgG seroconversion in women with fetal congenital infection was significantly lower than that in women without fetal congenital infection. It was suggested that the CMV IgM titer at IgG seroconversion may be more associated with the occurrence of fetal congenital CMV infection than the IgG avidity index or the timing of primary infection during pregnancy.

Toriyabe et al. [10] demonstrated that high IgM titers in early pregnancy could predict the occurrence of fetal congenital infection under primary infection. Moreover, we observed a tendency for IgM titers in late pregnancy to decrease, in comparison with that in early pregnancy (Fig. 3). Accordingly, the differences in IgM titers at IgG seroconversion between pregnant women with and without fetal congenital infection may be related to differences in the dynamic patterns of IgM titers during pregnancy. The common pattern of CMV IgM titer dynamics after primary infection involves an initial rise, followed by a rapid drop, whereas the persistence pattern involves an initial rise in IgM titers, followed by a gradual drop [9]. In the current study, low IgM titers at IgG seroconversion might correspond to the common pattern, whereas high IgM titers

might correspond to the persistence pattern. Thus, the common pattern may be related to a higher frequency of fetal congenital infection than the persistence pattern in pregnant women with IgG seroconversion. Despite this observation, further studies are needed to better evaluate the correlation between the dynamics of IgM antibodies and the occurrence of fetal congenital infection under primary infection.

A limitation of this study is that the antibody status during the second trimester of pregnancy was unknown. The presence of IgG antibodies during the second trimester in pregnant women with IgG seroconversion in late pregnancy may indicate that seroconversion occurred during the second trimester. Thus, second-trimester antibody status would allow us to distinguish between IgG seroconversion in the second trimester from that in the third trimester. In addition, second-trimester titers of IgM in pregnant women with IgG seroconversion during the second trimester would provide additional information on the dynamics of IgM titer after primary infection.

In summary, we found a high risk of CMV IgG seroconversion during pregnancy in teenage pregnant women, and a high risk of fetal congenital CMV infection in pregnant women with low IgM titer at IgG seroconversion. Further studies are needed to investigate the source of primary CMV infection during pregnancy, and to develop appropriate approaches for patient education with regard to the risk of infection, particularly for teenage women. Additionally, in pregnant women with CMV IgG seroconversion during pregnancy, the CMV IgM titer at IgG seroconversion may help predict the occurrence of fetal congenital infection.

Ethics approval and consent to participate

This nested, case control study was approved by the Clinical Research Ethics Review Committee of the Mie University Hospital (Approval No. H2019-127). For the current study, we adopted an opt-out method instead of informed consent.

Consent for publication

Not applicable.

Authors' contributions

KS and KT were involved in writing, AK was involved in data collection, FM organized the antibody screening program in Mie, Japan, MI performed CMV DNA tests, TM performed CMV IgG avidity tests, and HN and SS performed viral isolation tests. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Appendix

Supplementary Table
Number of pregnant women with cytomegalovirus (CMV) IgG seroconversion and fetal congenital CMV infection stratified by CMV IgG, IgG avidity index, and IgM levels.

	Number of pregnant women	
	With CMV IgG seroconversion	With fetal congenital CMV infection
CMV IgG levels (EIA titer)		
4.0–7.9	9	4
8.0–15.9	14	9
16.0–31.9	3	2
CMV IgG avidity index levels (%)		
0.0–9.9	6	3
10.0–19.9	8	4
20.0–29.9	5	4
30.0–39.9	5	4
40.0–49.9	1	0
50.0–59.9	1	0
CMV IgM levels (index)		
0.00–1.20 (negative)	4	2
1.21–1.99	1	1
2.00–3.99	4	4
4.00–5.99	2	1
6.00–7.99	4	2
8.00–9.99	6	3
10.0–11.9	1	1
12.00	4	1
Total	26	15

Abbreviations: CMV, cytomegalovirus; IgG, immunoglobulin G; IgM, immunoglobulin M; EIA, enzyme immunoassay.

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