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# Rare detection of cytomegalovirus in severe fetal malformations in China

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ABSTRACT

*Background:* Cytomegalovirus (CMV) is a significant cause of fetal abnormalities in developed world. Whether this could be applied in developing world remains unknown.
*Objectives:* To investigate CMV infection in severe fetal malformations in China.
*Study design:* During 2007–2014, 436 fetuses (237 males) with severe malformations and terminated pregnancy at median gestational age of $26^{+1}$ weeks were enrolled. CMV DNA was detected in fetal kidneys and other tissues by real-time PCR, and CMV IgG and IgM were measured by ELISA.
*Results:* CMV DNA was positive in kidneys and other tissues of seven (1.60%) fetuses. Hematoxylin-eosin staining showed intranuclear and intracytoplasmic inclusion bodies in kidneys of three fetuses, which was also positive for CMV antigens in immunohistochemistry. CMV DNA was found in 5 (6.1%) of 82 fetuses with central nervous system anomalies, 1 (11.1%) of 9 fetuses with abdominal anomalies, 1 (0.59%) of 168 fetuses with multiple congenital malformations, and none of fetuses with other anomalies (177). Of 293 pregnant women with plasma available, 279 (95.2%) were CMV IgG positive only and 6 (2.1%) were CMV IgG and IgM positive. Of 5 mothers with infected fetuses 1 (20%) was CMV IgG and IgM positive, while 5 (1.7%) of 288 mothers with uninfected fetuses were positive respectively ($P = 0.099$).
*Conclusions:* Congenital CMV infection in fetuses with severe congenital malformations is rare, indicating no close association between CMV infection and severe fetal malformations in China. Maternal screening for CMV may have minimal value in identifying fetal malformations in developing world.


## 1. Background

Human cytomegalovirus (CMV) is a frequent cause of intrauterine infection [1–4], occurring in 0.2–2.5% of live births in developed countries [5–9] and 0.6–6.1% in developing countries [10]. Congenital CMV infection is the consequence of either maternal primary infection, resulting in 30–35% transmission, or non-primary active infection (reactivated infection or re-infection), leading to an estimated 2–3% infection in fetuses [3].

Congenital CMV infection is a significant cause of fetal abnormalities in industrialized countries [11]. Studies show that an estimated 9.9–15.4% of adverse pregnancy outcomes can be attributed to CMV infection [7,12,13]. Nearly 10% children with hearing loss are congenitally CMV infected [7]. The congenital infection in children with cerebral cortical malformations is higher (15.4%) than that (0.2–0.5%) in healthy children [12]. CMV DNA may be detected in 15% stillbirths [13], suggesting the association between CMV infection and stillbirth. The high congenital CMV infection rate in fetal abnormalities may be associated with the considerable proportion of pregnant women susceptible to CMV,

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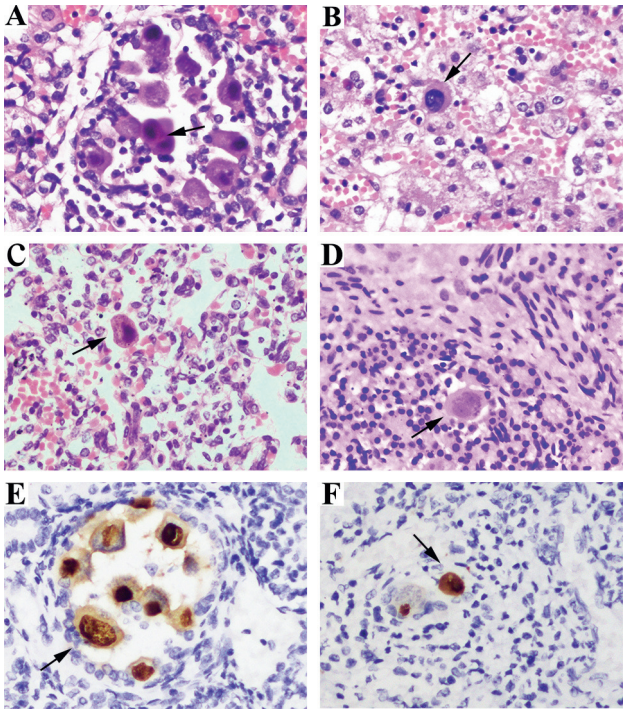


**Fig. 1.** Pathologic examinations in tissues with inclusion bodies. Intranuclear and intracytoplasmic inclusion bodies (*solid arrow*) were found by hematoxylin-eosin staining in the kidney (A, ×200) and liver (B, ×200) of Case 14009, lung of Case 12054 (C, ×200) and pancreas of Case 13055 (D, ×200). CMV antigens were stained brown with nuclear and cytoplasmic distribution by immunohistochemistry in the kidney (E, ×200) and liver (F, ×200) of Case 14009.

since only 36.9–68.3% women of reproductive age in industrialized countries are positive for CMV IgG [14–16]. The congenital infections caused by maternal primary infection have more severe consequences [17]. Additionally, non-primary active infection may also cause a certain proportion of fetal infection [2,18].

In developing countries, however, more than 95% child-bearing women are CMV IgG seropositive and have less chance of getting primary infection during pregnancy [19–22]. Thus, congenital infections in fetuses in developing countries could be associated with maternal non-primary, active infections. Since relevant data based on large sample size are lacking, the contribution of congenital CMV infection in severe fetal malformations remains elusive in developing countries.

## 2. Objectives

In this study, we aimed to investigate CMV infection in 436 fetuses with severe malformations that needed to terminate pregnancies in China, and to determine the role of congenital CMV infection in severe fetal malformations.

## 3. Study design

### 3.1. Subjects and specimens

During December 2007 to December 2014, 436 pregnant women at a median age of 28.0 years (20.0–47.0) opted to terminate pregnancy because of various severe fetal structural malformations confirmed by antenatal examinations, including ultrasound and/or magnetic resonance imaging in Nanjing Drum Tower Hospital, a genetic diagnosis center in China. The imaging findings showed a broad range of abnormalities (Table 1). There were 268 and 168 fetuses with single and multiple malformations, respectively. Of 436 fetuses (237 males) at gestational age of $26^{+1}$ weeks ($13^{+4}$–$37^{+5}$), 157 received chromosome analysis; 46 (29.3%) fetuses with chromosomal aberrations were identified, including triploid (1), aneuploid (34) and other chromosomal abnormalities (11) including deletion or rearrangement of chromosome segment. All the pregnant women were serologically tested for toxoplasmosis, rubella and syphilis, and none of them had current infection.

**Table 1**
Abnormalities by systems in the 436 fetuses and CMV infection in different abnormalities.

| | Number | Proportion of CMV-positive fetuses (%) |
|---|---|---|
| Anomalies in single system | 268 | 6 (2.24) |
| Central nervous | 82 | 5 (6.1) |
| Abdominal | 9 | 1 (11.1) |
| Circulatory | 65 | 0 |
| Face and neck | 40 | 0 |
| Urinary | 14 | 0 |
| Musculoskeletal | 21 | 0 |
| Respiratory | 1 | 0 |
| Hydrops fetalis | 36 | 0 |
| Multi-malformations[a] | 168 | 1[b] (0.59) |

[a] Multi-malformations refers to congenital malformations involving more than one system. 168 fetuses were multi-malformed, including anomalies of central nervous (85), abdominal (42), circulatory (84), face and neck (65), urinary (41), musculoskeletal (67), respiratory (41), and hydrops fetalis (26).
[b] Case 13055 had abnormalities in heart, abdomen, face and neck, and systems of respiratory and musculoskeletal (Table 2).

All fetuses underwent routine autopsy examinations. The diagnosis of malformations was based on the International Statistical Classification of Diseases and Related Health Problems 10th Revision [23]. Postmortem placenta, kidney, lung, liver, skin specimens, and amniotic fluid were all treated with two methods. For extraction of DNA, specimens were snap-frozen in liquid nitrogen and then kept at −70 °C. For histological examinations, specimens were fixed in formalin, dehydrated in alcohol and embedded in paraffin.

### 3.2. Detection of CMV DNA

Kidney tissue from each fetus (around 15–25 mg) was treated with 300 μl proteinase K (0.2 mg/ml) in proteinase K buffer in an incubator shaker at 55 °C for 3 h. DNA was subsequently extracted twice by phenol/chloroform, followed by dissolving in 50 μl Tris-EDTA buffer [24,25]. CMV DNA was detected by commercially available CMV fluorescence quantitative PCR diagnostic kit (DaAn Gene Co., Guangzhou, China), as described by Zhang et al. [24] on an ABI StepOne Plus Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The positive control, negative control, and four calibrations (4–7 log copies/ml) were included in each measurement. Positive results were confirmed by repeated tests in kidneys and other fetal tissues, including the lung, liver, skin, placenta, cord blood leukocytes and amniotic fluid.

### 3.3. Histological examinations

Postmortem kidneys, livers, lungs, pancreases, brains, and placentae were fixed in formalin, dehydrated and embedded in paraffin. Tissue sections of 4 μm were sliced transversally, followed by hematoxylin-eosin staining.

Immunohistochemistry was performed only in kidneys and livers in which inclusion bodies were found. The kidney and liver tissue sections were immunostained using monoclonal anti-CMV antibodies (Clone QB1/42, antibody against early antigen, and Clone QB1/06, antibody against late antigen respectively; OriGene Technologies, Rockville, MD, USA), which bind to CMV early antigen