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**Original Article**

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## **Cytomegalovirus, a Common Cause of Intrauterine Infection: A Case - Control Study in Tehran, Iran**

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### **ABSTRACT**

**Background and Aim:** Congenital cytomegalovirus (CMV) infection affects ~1% of live births in the United States. Ten percent of these infants have symptoms at birth and another 10 to 15% develop hearing loss or developmental problems. The aim of this study was to compare CMV infection (IgM and IgG) in infants suspected for intrauterine infection with the controls.

**Materials and Methods:** A case control study was performed in the Pediatrics department of Hazrat Rasool Akram Hospital in Tehran (2002-2003). We compared the serum CMV antibodies (IgM & IgG- ELISA) of 74 cases suspected of intrauterine infection (mean age =  $4.7 \pm 3.7$  months) with that of 65 controls (mean age =  $5.3 \pm 3.1$  months).

**Results:** Acute and previous immunity to CMV (IgM and IgG) was found in 41.9% (31/74) and 74% (54/74) of the cases, respectively. Meanwhile acute infection (IgM) was observed in 6.2% (4/65) and previous immunity (IgG) in 95.4% (62/65) of the controls. Acute infection (CMV-IgM) was higher in cases ( $p$ -value=0.000), but previous immunity (CMV-IgG) was higher in the controls ( $p$  value=0.001).

**Conclusion:** We conclude that like other countries, CMV is the most common cause of intrauterine infection in infants aged less than 6 months as compared to the healthy ones. We prefer, at least in our country, to consider seropositive (CMV-IgM) infants suspected of intrauterine infection (less than 6 months) as congenital form. To arrest the natural progression of congenital CMV, we recommend prolonged course of oral analogues of ganciclovir for children with symptomatic congenital CMV.

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**Keywords:** Congenital cytomegalovirus, Cytomegalovirus (CMV), Ganciclovir, Sensorineural Hearing Loss (SNHL)

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## **INTRODUCTION**

Congenital CMV is one of the most common causes of congenital infection in developed countries with reported incidences varying between 0.15 and 2.0%, with higher rates in populations having a lower standard of living (1,2).

The effects of congenital CMV infection may vary from a congenital syndrome to an asymptomatic course. Infants that are asymptomatic at birth, may still present handicaps at a later age (1,2). In approximately 5% of the infants, CMV becomes clinically manifested with damage to many organs including the liver, spleen, brain, eye, and inner ear. Most infants who are infected congenitally with CMV, are asymptomatic at birth (2-4). The percent of congenital CMV cases alone appears to account for all the cases previously attributed to all congenital infections (3,4).

Approximately 10% of congenitally infected infants have clinical evidence of disease at birth. The most severe form of congenital CMV infection is referred to as Cytomegalic Inclusion disease (CID). CID almost always occurs in women who have primary CMV infection during pregnancy, although rare cases are described in women with pre-existing immunity who presumably have reactivation of infection during pregnancy. CID is characterized by intrauterine growth retardation, hepatosplenomegaly, hematological abnormalities (particularly thrombocytopenia), and a variety of cutaneous manifestations including petechiae and purpura (i.e. Blueberry muffin baby). However, the most significant manifestations of CID are those involving the central nervous system.

Microcephaly, ventriculomegaly, cerebral atrophy, chorioretinitis, and Sensorineural Hearing Loss (SNHL) are the most common neurological consequences of CID (1-5).

Many studies suggest that congenital CMV infection has a more relevant role in the etiology of

SNHL than previously reported. More than 40% of the deafness cases with an unknown cause needing rehabilitation, are caused by congenital CMV (5,6). It accounts for approximately 4000 cases of deafness yearly in the US. This hearing loss is symmetric and may be progressive (7). Yet, 10 to 17% of these infants later may have unilateral or bilateral deafness (with often is progressive), differences in higher level auditory function and possibly other neuro-developmental sequel (6,7).

Diagnosis of congenital CMV is made by direct detection of CMV in urine (8), detection of IgM antibody in blood (9,10), or perilymphatic fluid (11). The diagnosis of perinatal infection with CMV is difficult, but is documented best by negative CMV viral culture and CMV-IgM antibody level at birth; positive viral culture and CMV-IgM antibody at 8 to 16 weeks of age; antibody and persistence of CMV-IgG antibody. Detection of antibody or virus in urine after the first year of life is of no use, as most children develop immunity to the virus (1,2,9,10). CMV-IgG seroconversion, presence of CMV-IgM antibody and viral shedding in saliva, urine and other body fluids all indicate postnatal CMV infection (1,2,10-12). Treatment of children with congenital cytomegalovirus infection with ganciclovir is recommended (13).

Previous studies in Tehran detected previous immunity (CMV-IgG) in 100% of the primigravid mothers living in Tehran (14,15). Congenital CMV infection (CMV-IgM in cord blood) diagnosed in 2.6% of their neonates (15,16). CMV was reported as the most common cause for Sensorineural Hearing Loss (SNHL) in children in Tehran (16). The goal of this study was to determine the role of CMV infection by serology (CMV-IgM & IgG ELISA) in cases suspected had intrauterine infection (<1 years old) in compare with normal infants.

## MATERIALS AND METHODS

This case-control study was carried out in the Pediatrics department of Hazrat Rasool Akram Hospital in Tehran (2002-2003). Our center is a tertiary care general hospital with 500 active beds. This study was approved by the Ethical Committee of the Iran University of Medical Sciences and Health Services. Our study group consisted of 74 infants suspected had intrauterine infection (case) and 65 healthy infants (control). All case and control groups were less than 1 years of age.

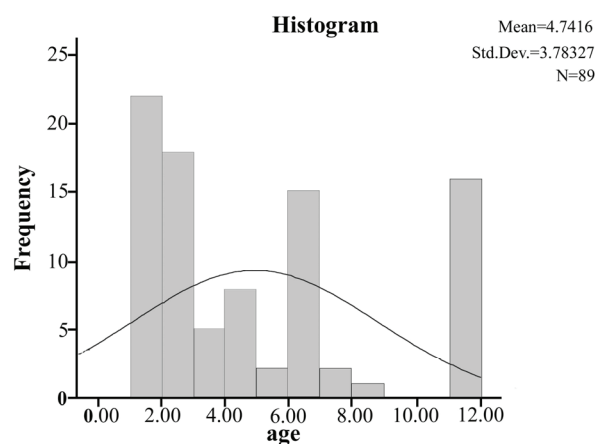
WHO criteria (2 major criteria/ or 1 major plus 2 minor) for intrauterine infection CRS (Congenital Rubella Syndrome) was used for selecting the cases.

Major criteria included: cataract/ glaucoma, congenital heart disease, SNHL, and pigmented retinopathy.

Minor criteria included: purpura, splenomegaly, microcephaly, mental retardation, meningoencephalitis, radiolucent osteitis, and icter on the first day of birth.

Our control group consisted of infants who were hospitalized for elective surgery (i.e. undescended testes, hernia, orthopedic procedures, etc). These infants were age matched with our intrauterine infection suspected group. They all underwent appropriate physical examination for health by expert pediatricians before surgery. We used their extra blood (which was taken for routine blood tests before their surgery) for the CMV serologic tests.

Initially a questionnaire was completed by an authorized physician for each case and control, followed by a complete clinical examination. Blood sample (2 ml) from each child was then centrifuged and transferred to our research laboratory. The serum was restored in -20°C freezer, until the serologic examination was performed. The centrifuged blood specimens were screened using an ELISA assay for CMV IgM and IgG antibodies.



Histogram 1. Age distribution (in months) in cases

**Serological test:** The evaluation of specific CMV IgM and IgG antibodies were carried out with commercial kits (Biochem, Germany). Both kits were used and the results were interpreted as suggested by the Manufacture. Results were calculated qualitatively.

**Statistical analysis:** The Student's t-test was used to determine the significant differences in means for all the continuous variables. Chi square values (CI 95%,  $p < 0.05$ ) were calculated for all categorical variables. All analyses were conducted using SPSS V.10 software.

## RESULTS

**Demographic pattern:** The age range of the cases (missing=4) was 1-12 months (mean=4.7±3.7 months) (Histogram 1). Also 47.2% of the patients were males, while 52.8% were females. The age range of the controls was 1-12 months (mean=5.3±3.1 months).

Serologic results in cases detected acute infection (CMV-IgM) in 41.9% (31/74), and previous immunity (IgG) in 74% (54/74) of them. Serologic results in controls detected acute infection

Table 1. Serologic tests between cases and controls

Serologic results	Case (74)	Control (65)	p-value
CMV-IgG	74%(54)	95.4%(62)	<0.001
CMV-IgM	41.9%(31)	6.2%(4)	<0.000

(IgM) in 6.2% (4/65), and previous immunity (IgG) in 95.4% (62/65) of them.

Acute infection was higher in the cases (p-value <0.000), but previous immunity (CMV-IgG) was higher in controls (p-value <0.001) (Table 1).

Mean age of TORCH infants with acute infection (CMV-IgM) was 6 months. Mean age was not different (p-value <0.3) between cases with and without acute infection (6 months vs. 4.2 months). Mean age of TORCH cases with previous immunity (CMV-IgG) was 4.8 months. Mean age was not different (p-value <0.6) between cases with and without previous immunity (4.8 months vs. 5.3 months) (Table 2).

## DISCUSSION

In the present study, acute CMV infection (IgM) was detected in 41.9% (31/74) of the suspected cases (with mean age of 6 months). Acute CMV infection was lower (6.2%) in controls. Mean age of suspected cases with acute and previous immunity was 6 months and 4.8 months, respectively. Mean age of the suspected cases with acute CMV infection (6 months) was close to cases without acute CMV infection (4.2 months).

We observed higher rate (p-value <0.001) of previous immunity (positive CMV-IgG) in normal infants than cases (95.4% vs 74%). Mean age of suspected cases with protective antibodies was near to cases without antibodies (4.8 vs 5.3 months). Indeed 95.4% of the controls had protective

antibodies (positive CMV-IgG). Acute CMV infection (positive CMV-IgM) was observed only in 6.2% of cases. Due to higher rate (p-value <0.000) of acute infection (positive CMV-IgM) and lower rate of previous antibodies (negative-CMV-IgG) in suspected cases, CMV infection may play a significant role especially in younger cases (age <6 months).

In present study, CMV infection probably happened in a seronegative susceptible fetus. Presence of previous antibodies (CMV-IgG) in intrauterine life was protective in controls. Absence of these protective antibodies (negative CMV-IgG) showed in suspected cases.

In the last decades, two studies in pregnant mothers in Tehran determined CMV-IgG positivity in 98% (<20 years old) and 100% (<20 years old), with 96% reduction in the risk of congenital CMV infection in their future pregnancies (14,15). So, most neonatal infections are due to recurrent or reactivated CMV infection in pregnant women; thus screening of CMV antibodies in Iranian pregnant women would not be helpful (15,16).

Previous immunity (CMV-IgG) was observed in 100% of the primigravid mothers living in Tehran (1999-2000). It provides lower risk of primary CMV infection in Iranian pregnant women as compared to women living in United States (1,2). Congenital CMV was reported in 2.6% of their neonates (positive CMV-IgM in cord blood); all being asymptomatic at birth (15). Probably the prevalence of congenital CMV infection in live births in Iran is ~2.5% which is higher than its prevalence (~1%) in United States (1,2).

Presence of symptoms and sequelae in congenitally CMV infected infants is related to the titer of CMV-IgM (ELISA) in cord blood (89% sensitivity, 100% specificity) (1-4,12,16).

We conclude that many cases of congenitally infected infants were asymptomatic (or had mild

**Table 2.** Comparison of mean age between cases with and without infection

Serology	Mean age	p-value
Positive-CMV-IgM	6 months	p< 0.3
Negative-CMV-IgM	4.2 months	
Positive-CMV-IgG	4.8 months	p<0.6
Negative-CMV-IgG	5.3 months	

symptoms) and were not evaluated and diagnosed in the period of present study. Only 10% of the infected infants with symptoms at birth needed follow up. Probably, 10 to 15% of all the infected cases will develop hearing loss or developmental problems in future. Thus, we recommend screening of neonatal cord blood at birth for specific CMV-IgM antibody (ELISA). When CMV infection is diagnosed in the first year of life, treatment is recommended and is associated with diminished occurrence of sequelae. Currently available oral analogues of ganciclovir may facilitate earlier and more prolonged therapy for children with congenital CMV (12,13).

## CONCLUSION

CMV infection is one of the most common causes of intrauterine infection in suspected infants admitted in our tertiary referral hospital. Congenital CMV affects ~2.5% of live births in our country. Most congenital CMV infections in our neonates are due to recurrent or reactivated CMV infection in their mothers. Most of these congenitally infected infants are asymptomatic at birth. Ten to 17% of these infants later may have unilateral or bilateral deafness (which is often progressive). Screening for CMV infection in the period of pregnancy is not helpful. CMV-IgM antibody (radioimmunoassay) screening of cord blood of all neonates is recommended.

We prefer at least in our country to consider seropositive (CMV-IgM) intrauterine infected suspected infants of less than 6 months of age as congenital form. We are not able to differentiate the congenital form from the acquired CMV infection only by seropositive (CMV-IgM) results in suspected infants after 6 months of age.

Prolonged course of ganciclovir will arrest the natural progression of congenital CMV infection. Search for CMV-DNA virus by the more sensitive

method (PCR) in the damaged end organs of the suspected infants, may increase the possible role of CMV infection in future.

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