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ORIGINAL ARTICLE

Cytomegalovirus a common cause of intrauterine infection: A case-control study

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ABSTRACT

Background: Congenital cytomegalovirus (CMV) infection affects nearly 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) rate in infants suspected for intrauterine infection with the control group.

Patients and methods: A case-control study was performed in the Pediatrics Department of Hazrat Rasool Akram Hospital in Tehran. The study population included 74 suspected cases of intrauterine infection (mean age, 4.7±3.7 months) and 65 normal healthy controls (mean age, 5.3±3.1 months). We compared serum CMV antibodies (IgM, IgG) with ELISA kits.

Results: Acute and previous immunity to CMV (IgM and IgG) was found in 41.9% (31/74) and 74% (54/74) of cases, respectively. These figures were 6.2% (4/65) and 95.4% (62/65) in controls, respectively. Acute infection (CMV-IgM) was more common among cases ($p<0.0001$), but previous immunity (CMV-IgG) was more prevalent among controls ($p<0.001$).

Conclusion: We concluded that 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.

Keywords: *Cytomegalovirus (CMV), Sensorineural hearing loss (SNHL), Congenital infections, Ganciclovir*.
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INTRODUCTION

Congenital cytomegalovirus (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

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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 neurodevelopmental 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, 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 salvia, urine and other body fluids all indicate postnatal CMV infection (1,2,10,11). Treatment of children with congenital cytomegalovirus infection with ganciclovir is recommended (12).

Prior studies in Tehran detected previous immunity (CMV-IgG) in 100% of primigravid mothers living in Tehran (13,14). However, congenital CMV infection (CMV-IgM in cord blood) was diagnosed in 2.6% of their neonates (14,15). CMV was reported as the most common cause for SNHL in children in Tehran (15). The goal of this study was to determine the role of CMV infection by serology (CMV-IgM & IgG ELISA) in <1 year old neonates suspected of having intrauterine infection in comparison with normal infants.

PATIENTS and METHODS

This case-control study was carried out in the Pediatrics Department of Hazrat Rasool Akram Hospital in Tehran. Our center is a tertiary care general hospital with 500 active beds. The study population included 74 infants suspected of intrauterine infection (case) and 65 healthy infants (control). All subjects aged less than 1 year.

WHO criteria (2 major criteria/ or 1 major plus 2 minor) for intrauterine infection CRS (Congenital Rubella Syndrome) was applied for case selection. Major criteria include: cataract/glaucoma, congenital heart disease, SNHL, and pigmented retinopathy. Minor criteria include: purpura, splenomegaly, microcephaly, mental retardation, meningoencephalitis, radiolucent osteitis, and icter on first day of birth.

The control group consisted of infants who were hospitalized for elective surgery (i.e. undescended

testes, hernia, orthopedic procedures, etc). They underwent appropriate physical examination by expert pediatricians before surgery. We used their extra blood (which was taken for routine blood tests before their surgery) for CMV serologic analysis.

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

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 guidelines.

Finally, t-test was used to determine the significant differences in means for all the continuous variables. Chi square values (95%confidence interval, $p<0.05$) were calculated for all categorical variables. All analyses were conducted using SPSS software (version 10.0, SPSS Inc., USA).

This study was approved by the Ethical Committee of the Iran University of Medical Sciences and Health Services.

RESULTS

The mean age of cases and controls was 4.7±3.7 and 5.3±3.1 months (a range, 1-12 month), respectively. Totally, 47.2% of subjects were boy and the remaining 52.8% were girls.

Serologic analysis revealed acute infection (CMV-IgM positivity) in 41.9% (31/74) and previous immunity (CMV-IgG positivity) in 74% (54/74) of cases. Among controls, acute infection was detected in 6.2% (4/65), while previous immunity was noted in 95.4% (62/65).

Thus, acute infection was more common among cases ($p<0.0001$), but previous immunity (CMV-IgG positivity) was more prevalent in controls ($p<0.001$).

Furthermore, among cases, the mean age of infants with acute infection (CMV-IgM positivity) was not significantly differed from cases without infection (CMV-IgM negative) (6 vs. 4.2 months, $p<0.3$). Similarly, the mean age of infants with previous immunity (CMV-IgG positivity) was slightly lower than cases with CMV-IgG negative status (4.8 vs. 5.3 months, $p<0.6$).

DISCUSSION

In the present study, acute CMV infection (IgM) was detected in 41.9% of 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 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 found higher rate of previous immunity (positive CMV-IgG) in normal infants than cases (95.4% vs 74%). Mean age of suspected cases with protective antibodies was close to cases without antibodies (4.8 vs 5.3 months). Indeed, 95.4% of controls had protective antibodies (positive CMV-IgG). Acute CMV infection (positive CMV-IgM) observed only in 6.2% of cases. With respect to higher rate of acute infection and lower rate of previous antibodies in suspected cases, CMV infection may play a significant role especially in younger cases (age <6months).

In our setting, 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) was demonstrated in suspected cases. In the last decade, two studies determined CMV-IgG

positivity in 98% and 100% of pregnant mothers, with 96% reduction in the risk of congenital CMV infection in their future pregnancies (13,14). Thus, most neonatal infections are due to recurrent or reactivated CMV infection in pregnant women; hence screening of CMV antibodies in Iranian pregnant women would not be helpful (14,15).

Previous immunity (CMV-IgG) observed in most primigravid mothers living in Tehran (13,14). It provides lower risk of primary CMV infection in Iranian pregnant women as compared to women living in United States (1,2). Congenital CMV reported in 2.6% of their neonates (positive CMV-IgM in cord blood), and all being asymptomatic at birth (14). The prevalence of congenital CMV infection in live births in Iran is nearly 2.5% which is higher than the figures (~1%) reported from United States (1,2).

Presence of symptoms and sequel in congenitally CMV infected infants is related to the titer of CMV-IgM in cord blood (89% sensitivity, and 100% specificity)(1-4,15,16).

We concluded that many cases of congenitally infected infants were asymptomatic (or had mild 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 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 during the early stage of life, treatment should be commenced. Currently available oral analogues of ganciclovir may facilitate earlier and more prolonged therapy for children with congenital CMV (12,16).

In conclusion, CMV infection is one of the most common causes of intrauterine infection in suspected infants. Most congenital CMV infections are due to recurrent or reactivated CMV infection in their mothers. Most of these congenitally infected infants are asymptomatic at birth, however 10 to 17% of these infants may have unilateral or bilateral deafness (which is often progressive). Screening for CMV infection in the period of pregnancy is not helpful, nevertheless, CMV–IgM antibody (radioimmunoassay) screening of cord blood of all neonates is recommended.

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