

## CYTOMEGALOVIRUS INFECTION IN A DAY-CARE CENTER IN THE MUNICIPALITY OF SÃO PAULO

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### SUMMARY

The prevalence of antibodies against cytomegalovirus (CMV) and the incidence of CMV infection were tested in 98 children aged 5 to 36 months who attended the day-care center of a University hospital in São Paulo. At the beginning of the study the overall prevalence of anti-CMV IgG antibodies was 44% (43/98). Saliva and/or urine samples were obtained from 38 of the 43 children that were seropositive at the beginning of the study for isolation of the virus, and 52.6% of these children were found to excrete CMV in one of the two materials. Among the 37 children that were initially seronegative from whom it was possible to obtain a new blood sample 6 to 12 months later, 22 (59.5%) presented seroconversion. The rate of viral excretion through urine or saliva from the children that seroconverted was 50%.

These results indicate that CMV infection is frequent and occurs early among the children who attend this day-care center. However, controlled studies using molecular epidemiology techniques are needed to define more precisely the role of day-care centers in CMV dissemination.

**KEYWORDS:** Cytomegalovirus; Day-Care center; Epidemiology.

### INTRODUCTION

Cytomegalovirus (CMV) is a frequent cause of congenital and perinatal infection in Brazil. Studies carried out in São Paulo have revealed 0.5 to 1.0 rates of congenital infections and a 30.9% risk to acquire perinatal infection by 4 months of age<sup>11, 15</sup>.

As has been shown in other countries, in Brazil the prevalence of CMV infection seems to be related to the socioeconomic status of the population studied. Thus, a sero-epidemiologic survey carried out in São Paulo has revealed 26% seropositivity among children aged 6 to 11 months<sup>3</sup>, whereas in children of lower socioeconomic status from Rio de Janeiro 47.3% were seropositive at 9 months of age<sup>14</sup>.

Several studies have also reported that CMV infection is more prevalent among children attending day-care centers than among children who stay at home<sup>9, 17, 23</sup>. In this setting, CMV dissemination is quite frequent, with rates of viral excretion

through urine and saliva ranging from 51% to 83%<sup>2, 10, 12, 13, 16, 18</sup>. The excretion rate is even higher among infants aged 12 to 24 months, reaching 71 to 83%<sup>2, 12, 17</sup>. Urine is considered to be the major source of CMV excretion, with the child often eliminating the virus for months or even years<sup>8</sup>. CMV has also been isolated from surfaces contaminated with the urine or saliva of infected children, indicating that indirect transmission of the virus through fomites may occur<sup>2, 7</sup>. There is also evidence that infants and children with subclinical infection are an important source of primary infection for mothers and employees of day-care centers in the childbearing age<sup>1, 6, 18, 19, 22</sup>.

The objective of the present study was to determine the prevalence of CMV antibodies and CMV excretion among children attending a city day-care center in São Paulo and the incidence of infection among susceptible children over a period of 6 to 12 months.

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## PATIENTS AND METHODS

The study was conducted at the day-care center of the University Hospital, Faculty of Medicine of São Paulo, which exclusively accepts children of hospital employees and cares for them during the parent's work day, with a mean yearly permanence of 300 children. All children aged 5 to 35 months who attended the day-care center from July 1991 to December 1992 were included in the study. Informed written consent to participate in the study was obtained from the parents. A first blood sample was collected at the beginning of the study from each child.

A form containing data about educational level, mother's obstetrical history and information about the current health and medical history of the children was filled out using the information in the day-care center files or, when possible, with the help of the mothers themselves.

Blood was then collected for the detection of anti-CMV IgG antibodies. Urine and/or saliva samples were obtained from seropositive children for viral isolation. Seronegative children were submitted to a new blood collection 6 to 12 months after the first for the evaluation of the incidence of infection among these susceptible children.

Blood was obtained by puncturing the fleshy tip of the index finger and placed on filter paper according to the technique of SOUZA & CAMARGO<sup>20</sup>. Saliva was collected by swabbing the oropharynx and placed in Hank's transport medium containing antibiotics. Urine was obtained at the day-care center itself using collection bags for the younger infants and sterile glass containers for older children, with care taken to maintain asepsis. The time between collection and preparation of the materials and inoculation into a cell system did not exceed 3 hours.

For viral isolation, 0.1 ml of saliva and urine samples were inoculated into tubes containing a monolayer of human foreskin fibroblast cells. After 30 min. 0.9 ml of culture medium (Eagle's Minimal Essential Medium, supplemented with 10% fetal bovine serum) was added. Cell cultures were maintained for 4 weeks and were examined twice a week. Cytomegalovirus was identified by the production of characteristic cytopathic effect.

An in-house enzyme-linked immunosorbent assay (ELISA) was used to detect specific IgG antibodies against CMV. Briefly, virus antigen was extracted from human foreskin fibroblasts infected with AD-169 strain and control antigen from uninfected cells by sonication and treatment with 0.2% sodium desoxycholate. Microtiter plates (Nunc-Polysorp) were coated with 50 µl of either CMV or control antigens by overnight incubation at 4°C. After

washing twice with PBS containing 0.1% Tween 80 (PBST), the plates were incubated for 1 hour at 37°C with blocking solution (5% skim milk, Molico-Nestlé, in PBST) and a 1:100 serum dilution were dispensed in duplicate into wells coated with either virus or control antigen. After incubation for 1 hour at 37°C, the plates were washed four times with PBST and horseradish peroxidase-conjugated anti-human IgG (Sigma, USA) was added to each well and incubated 40 minutes at 37°C. After washing plates 4 times with PBST, chromogenic substrate (o-phenylenediamine and H<sub>2</sub>O<sub>2</sub> in citrate buffer pH 5.0) were added and after 20 minutes incubation at room temperature, the enzymatic reaction was stopped with 2.5N H<sub>2</sub>SO<sub>4</sub>. The test was considered as positive when the absorbance of the antigen well, read at 492 nm in Titertek Multiskan Plus (Labsystems), minus the absorbance of the control well (differential optical density-DOD) was higher than 0.1.

## RESULTS

A total of 194 consent forms were distributed and 132 were returned, authorizing participation of the child in the study. Blood samples were collected from 98 of these (61 girls and 37 boys) for the determination of CMV antibodies and this group corresponded to our sample. The remaining 34 children could not be located at the time of blood collection and therefore were excluded from the study.

Data about educational level were obtained from 85 mothers, revealing that 20% of them had higher education and 80% were of technical level.

There were no reports of children born with congenital disorders and no mother had received a CMV diagnosis during pregnancy. The overall prevalence of antibodies in the population studied was 44% (43/98). No statistically significant differences were observed in the prevalence of CMV antibodies when the sample was stratified by age range in months (Table 1) or by the time (months) of child attendance at the day-care center (Table 2).

For 37 of the 55 children that were seronegative on the occasion of the first collection, it was possible to obtain a second blood sample 6 to 12 months after the first (mean: 6.7 months; median: 8 months). During this period, 22/37 (59.5%) of the children presented seroconversion, characterizing primary CMV infection. These children were not evaluated clinically.

For 38 of the 43 children that were seropositive at the beginning of the study it was possible to obtain saliva and urine samples for viral isolation. Saliva was collected from all 38 children and CMV was isolated in nine of them (23.7%). Eighteen of the 36 seropositive children from

**TABLE 1**

Prevalence of IgG-ELISA CMV antibodies in 98 children, attending a day-care center.

Age (months)	Number seropositive/total	%*
5-12	19/35	54.2
13-24	19/52	36.5
25-36	5/11	45.4
TOTAL	43/98	44.0

\* p = 0.26

**TABLE 2**

Prevalence of IgG CMV antibodies according to the time of attendance at a day care center.

Length of stay (months)	number seropositive/total	%*
2-8	21/41	51.2
9-15	11/31	35.5
16-22	7/16	43.7
23-36	4/10	40.0
TOTAL	43/98	44.0

\* p = 0.60

whom a urine sample was collected (50%) were positive. Overall, 20/38 (52.6%) were eliminating CMV in one of these materials and 7/38 (18.4%) presented concomitant elimination of the virus in saliva and urine.

Urine was collected from all of the 22 children who presented seroconversion during the study period and saliva was collected from 21/22. CMV was isolated in 11/22 (50%), from saliva in 3 cases (14.3%) and from urine in 10/22 (45.5%). Concomitant virus elimination through saliva and urine was observed in only two cases.

A new collection of material for viral isolation was possible 9 to 10 months after the first for 5 of the children that were eliminating CMV through saliva at the time of the first positive serology, and all 5 were negative. On the other hand, among the 8 children with positive urine at the time of first serologic determination, 3 (37.5%) continued to eliminate the virus through urine in samples collected 9 to 10 months after the first.

Furthermore, a child that was initially seropositive but whose urine and saliva were negative, showed the presence of CMV in urine collected 9 months after the first sample.

## DISCUSSION

The 44% rate of anti-CMV antibody prevalence detected here in children aged 5 to 36 months is comparable to the 41.6% rate reported in a previous study conducted on

children aged 5 months to 5 years in the city of São Paulo<sup>3</sup>. However, whereas only 26% of the children aged 6 to 11 months in that study had anti-CMV antibodies, in the present study 54.2% (19/35) of the children aged 5 to 12 months were seropositive, indicating that CMV infection occurs earlier among institutionalized children.

On the other hand, the fact that there was no difference in CMV seroprevalence in relation to the time (in months) that the children had been attending the day-care center leads us to assume that this may not be the factor determining earlier infection. Several studies have demonstrated that the major factor predisposing to infection at day-care centers may be age range, with significantly higher rates of infection being observed between 12 and 24 months of age<sup>2, 12, 17</sup>. Three possible factors facilitating infection may be present in this age range: one of them may be the loss of maternal antibodies which makes the children susceptible to infection when they are placed in contact with children eliminating the virus. The second may be contact of these susceptible children with children that were infected during the perinatal period, since it is known that under these circumstances the elimination of CMV through urine and saliva is frequent and persists for months or even years<sup>8, 17</sup>. In populations in which the incidence of perinatal infection is high, the probability that children attending day-care centers during this period were infected is relatively high. A previous study conducted in São Paulo demonstrated that approximately 34% of neonates born at a public hospital presented perinatal infection<sup>11</sup> and these children may be the major source of CMV introduction in day-care centers.

Finally, the habit that children of this age range have of bringing toys and objects to their mouths, together with the beginning of walking, which permits a more direct contact with other children, may be other factor facilitating viral dissemination.

The observation that approximately 50% of the children that were seropositive in the present study were eliminating CMV through urine or saliva indicates that there was a broad circulation of the virus during the study period. This probably explains the elevated rate of seroconversion (59.5%) observed among children that were previously seronegative for CMV within a mean period of only 6.7 months. Other studies have detected rates of CMV excretion through urine or saliva of the order of 51 to 83%<sup>12, 13, 18</sup>.

The present findings show that, in general, CMV excretion is frequent and prolonged among children attending the day-care center of this University hospital. The present study, however, was not controlled and therefore we cannot state that these findings were exclusively due to institutionalization of the children. In any case, the high rates

of excretion through urine and saliva emphasize the importance of hand washing and basic hygiene on the part of the employees of the day-care center when they handle these secretions in order to avoid spreading the virus among the children and the possibility of virus acquisition by susceptible employees. Comparative studies between children attending day-care centers and children who do not in terms of rates of seroprevalence, seroconversion and viral excretion through saliva and urine, as well as molecular epidemiology studies of isolated strains<sup>4,5,21</sup> would be necessary to confirm the role of day-care centers in the early acquisition of CMV.

## RESUMO

### Infecção por citomegalovírus em creche do município de São Paulo

A prevalência de anticorpos e a incidência de infecção por citomegalovírus (CMV) foram estudadas em 98 crianças de 5 a 36 meses de idade que freqüentavam a creche de um hospital universitário em São Paulo. No início do estudo, a prevalência geral de anticorpos para o CMV foi de 44% (43/98). Obteve-se para o isolamento viral, amostras de saliva e/ou urina de 36 das 43 crianças que eram soropositivas, constatando-se que 55,6% das mesmas estavam excretando CMV em um dos materiais. Das 37 crianças inicialmente soronegativas, das quais foi possível obter nova amostra de sangue 6 a 12 meses após, 22 (59,5%) apresentaram soroconversão. A taxa de excreção viral na urina ou saliva nas crianças que soroconverteram foi de 52,4%.

Estes resultados indicam que a infecção por CMV foi freqüente e ocorreu precocemente nas crianças que freqüentavam esta creche. Contudo, estudos controlados usando técnicas de epidemiologia molecular são necessários para definir mais precisamente o papel da creche na disseminação do CMV.

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