

Cytomegalovirus seroepidemiology in an urban community of São Paulo, Brazil

Soroepidemiologia da citomegalovirose em comunidade urbana de São Paulo, Brasil

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Keywords

Cytomegalovirus infections, transmission.[#] Seroepidemiologic studies.[#] Mathematical models.[#] Cytomegalovirus infections, epidemiology. Cytomegalovirus infections, congenital. Enzyme-linked immunosorbent assay, utilization. Disease transmission vertical. Disease transmission horizontal. Age factors.

Descritores

Infecções por cytomegalovirus, transmissão.[#] Estudos soroepidemiológicos.[#] Modelos matemáticos.[#] Infecções por cytomegalovirus, epidemiologia. Infecções por cytomegalovirus,

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Abstract

Introduction

After the era of rubella vaccine, cytomegalovirus (CMV) infection is one of the most frequently causes of mental retardation and congenital deafness. Seroepidemiological studies are necessary to understand the transmission dynamics of the disease. The purpose of the study was to quantify the transmission rate of CMV disease in a community in the state of São Paulo, Brazil.

Methods

Using ELISA test (IgG), a retrospective serological survey looking for CMV antibodies was performed in a non-immunized community. Frozen sera from 443 individuals, randomly selected by cluster sampling technique in the town of Caieiras, São Paulo, were collected from November 1990 to January 1991. Seroprevalence was stratified by age (0-40 years). Mathematical techniques were applied to determine the age-dependent decay function of maternal antibodies during the first year of life, the age-dependent seroprevalence function and the force of infection for CMV in this community.

Results

It was observed a descending phase of seropositivity in the first 9 months, but changes in antibody titration were observed between 8 months old and one year of age. The average age of the first infection was 5.02 months of age and 19.84 years, when the age-dependent seroprevalence and the force of infection were analyzed between 10 months of age and 10 years of age and from 10 to 40 years old, respectively.

Conclusion

CMV infection is highly prevalent among the population studied and infection occurs in the first year of life. This study shows that most women at reproductive age are vulnerable to the first infection, increasing the risk for congenital infection.

Resumo

Introdução

Após a era da vacinação contra a rubéola, a citomegalovirose tornou-se uma das causas mais frequentes de surdez congênita e retardamento mental. Estudos soroepidemiológicos são necessários para entender a dinâmica de transmissão da doença. O objetivo do estudo foi quantificar a transmissão dessa doença em

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Transmissão vertical de doença.
Transmissão horizontal de doença.
Fatores etários. – Citomegalovirose.

comunidade do Estado de São Paulo, Brasil.

Métodos

Foi realizado estudo sorológico retrospectivo utilizando anticorpos contra o citomegalovírus (CMV) de classe IgG, pelo método ELISA, em soros congelados de 443 indivíduos, randomizados e selecionados pela técnica de Cluster, provenientes da comunidade de Caieiras, uma pequena cidade localizada nos arredores de São Paulo. As amostras foram colhidas entre novembro de 1990 e janeiro de 1991. No estudo da soroprevalência estratificada por idade (0 a 40 anos), foram aplicadas técnicas matemáticas para a determinação da função de decaimento de anticorpos maternos durante o primeiro ano de vida, da função de soroprevalência por idade e da força de infecção para o CMV na comunidade.

Resultados

Foi observada uma fase descendente da soropositividade durante os primeiros nove meses, porém ocorreram mudanças nos títulos de anticorpos entre oito meses e um ano de vida. A idade média de aquisição da primeira infecção foi de 5,02 meses e 18,84 anos, quando se analisaram a soroprevalência e a força de infecção, idade dependentes, respectivamente entre 10 meses e 10 anos e 10 e 40 anos.

Conclusões

A citomegalovirose é altamente prevalente na população estudada e ocorre precocemente já no primeiro ano de vida. O estudo demonstrou que mulheres em idade reprodutiva se encontravam vulneráveis à primeira infecção, aumentando a chance de doença congênita.

INTRODUCTION

Cytomegalovirus is a common worldwide infection. Humans are believed to be the only reservoir for CMV, and natural transmission occurs by direct or indirect contact. Human CMV infection is endemic and occurs throughout the year, having no seasonal pattern. It occurs early in childhood and about 40-80% of individuals are seropositive at puberty.⁹

Primary CMV is followed by a persistent infection and under certain circumstances recurrences occur. After primary infection, specific antibody titers persist for life.⁴ Spread through susceptible population is enhanced by the prolonged excretion virus that continues for years after congenitally, neonatal and postnatal acquired infections.⁹ Prolonged replication following primary infection and recurrences are associated with intermittent shedding of CMV from many sites.¹⁴ The most common routes for neonatal and postnatal transmission of CMV to infants were the ingestion of breast milk and cervicovaginal secretions.⁶ It was observed that children in day-care centers from the age 5-36 months of age excreted CMV in their saliva or urine, which represents a horizontal spread of the virus and a risk to a pregnant woman.^{11,12}

Human CMV infection rates have been best defined in pregnant women because of interest in understanding the intrauterine transmission of the virus. Sexual transmission of CMV represents another

route of virus transmission in the adult population. Epidemiological findings suggest that CMV is transmitted in the sexual intercourse. Studies have shown an extremely high seroprevalence in sexually active male homosexuals.⁵

CMV disease is presently the leading infectious cause of mental retardation and congenital deafness.¹⁰ Despite that, it is not a noticeable disease in most countries. In fact, little has been done to determine the magnitude of the problem especially in developing countries. In Brazil the epidemiological data available were restricted to some urban areas, such as the state of São Paulo.^{2,3,12,13,15} Past serological studies using sera collected from healthy people of different age groups in São Paulo and tested for CMV antibodies showed a seroprevalence of 60% in children at the age 0-4 years old,¹⁵ with a slow rise after 15 years old² and 80% of positivity at age group 51-60 years old.³

The incidence of intrauterine CMV infection in developed and developing countries is about 1%.^{9,13} Among the infected neonates, at least 10% suffer some brain damage and a greater proportion remain with some degree of hearing loss.¹⁰

Data on congenital CMV infection in Brazil came from data collected by some institutions in different populations.¹³

The prevalence of CMV antibody varies according

to age, geographic location, and the population's socioeconomic conditions.⁹ Although the CMV vaccine was developed to prevent primary infection in women during pregnancy and to seroconvert non-infected patients before organ transplantation, mass vaccination against CMV is foreseeable, which makes population background information even more necessary for the design of control strategies.⁹

This study aims to quantify CMV transmission in a community in the state of São Paulo, Brazil.

METHODS

The community sampled

The community chosen was the population of Caieiras, a small town located in northern São Paulo. Its population (about 30,000 inhabitants in 1990) is distributed over an area of 104 km², most of them in the urban area (>90%). About 47% of the community were 20 years of age at the time of the study (November 1990–January 1991).

The economic activity of Caieiras is centralized in one big industry and 75 smaller factories. Its social structure is highly heterogeneous, a characteristic seen in many southern Brazilian cities.⁸

Population sampling

The community was stratified into 24 age groups to take into account the expected rates of seroprevalence changes with increasing age. Hence age groups were defined by monthly increases over the first year of life, yearly up to age 4, and five-yearly thereafter to age 40. Further details on population sampling were published elsewhere.¹

Blood collection

The blood sample was collected using a vacuum system or butterfly needle for children below 2 years of age. The sera obtained after centrifugation of clotted samples were stored at –20°C.

Laboratory tests

An enzyme immunoassay (ELISA) kit for determination of human IgG antibodies to human CMV was used (Enzygnost Anti-CMV/IgG, Behringwerke AG, Marburg, Germany). For each sample the value to be determined was the difference between the measured absorbances of the antigen and the control antigen. The cut-off value of 0.2, as recommended by the manufacturer, was used to

distinguish seropositive (immune) from seronegative (susceptible) individuals.

Data management

After establishing the proportion of seropositive for each age group, a continuous function was fitted to the data for each age-dependent phase, i.e., for maternal antibodies decay phase, $M(a)$, with the following general formula,

$$M(a) = \frac{1}{1 + \exp\left(\sum_{i=0}^n q_i a^i\right)} \quad (1)$$

where q_i are the fitting parameters, a is the age.

In a second phase, a modified Farrington⁷ was used to calculate the force of infection (per capita rate by which susceptible individuals acquire infection) between 7 months and 10 years old, and from 10 to 40 years old.

The age-dependent force of infection, $\lambda_1(a)$, and the seropositive proportion, $S_1^+(a)$, were estimated using a modified Farrington applied to the serological data of individuals between 7 months and 10 years old, where k_1, k_2 , are the fitting parameters, as follows:

$$\lambda_1(a) = k_1 a e^{-k_2 a} \quad (2)$$

$$S_1^+(a) = 1 - \exp\left\{\frac{k_1}{k_2^2} \left[(k_2 a + 1)e^{-k_2 a} - 1\right]\right\} \quad (3)$$

The age-dependent force of infection, $\lambda_2(a)$, and the seropositive proportion, $S_2^+(a)$, were estimated using a modified Farrington applied to the serological data of individuals between 10 to 40 years old, where c_1, c_2 , are the fitting parameters, as follows:

$$\lambda_2(a) = c_1 (a - L_c) e^{-c_2 (a - L_c)} \quad (4)$$

In this case, L_c is the age at which $\lambda_2(a=L_c) = 0$

$$S_2^+(a) = 1 - \exp\left\{\frac{c_1}{c_2^2} \left[(c_2 (a - L_c) + 1)e^{-c_2 (a - L_c)} - 1\right]\right\} \quad (5)$$

The average age of the first infection, A , was calculated as defined by Azevedo-Neto et al,¹ where $\lambda(a)$ is

the force of infection, $S^+(a)$ the seropositive proportion at age a .

$$A = \frac{\int_0^{\infty} a\lambda(a)[1-S^+(a)]da}{\int_0^{\infty} a\lambda(a)[1-S^+(a)]da} \quad (6)$$

RESULTS

Four hundred and forty-three sera samples were analyzed for CMV antibodies. In the first analysis it was observed a decline in the proportion of seropositive individuals, from newborns to 9 months of life. In a second phase, in individuals aged 10 months to 3 years, there was a first increase in the seropositivity that remains up to 14 years. A second increase in the seroprevalence occurred after 15 years up to 30 years of age when most individuals were seropositive (Table).

When the serological data were plotted to a continuous function for each age-dependent phase, for the maternal antibody decay, $M(a)$, using Equation (1), it showed that at birth more than 90% of the newborns had maternal antibodies against CMV. The decrease in maternal antibodies occurs during the first 9 months, when the proportion of seropositives decreases but never below 18%. In contrast, changes in antibody titration were observed between 8 months and the first year of life (Figure 1).

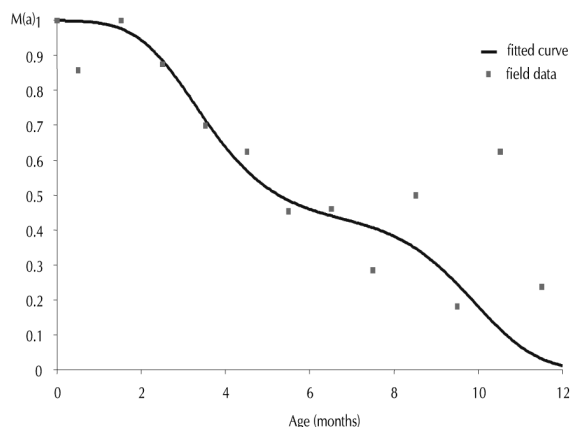


Figure 1 – Maternal antibody decay, $M(a)$, for CMV in Caieiras' individuals (dots) and the fitted function (continuous line).

The parameters for $M(a)$ were fitted ($q_0 = -7.711391$; $q_1 = +39.545561$; $q_2 = -67.717229$; $q_3 = +40.246744$), using the function showed in Equation 1, obtained by the least-square method (Table Curve, version 3.18, Jandel Scientific, USA).

Scatterplots illustrating individual antibody concentrations showing those individuals aged 0–12 months are presented in Figure 2. There was a clear decline in maternal antibodies from birth up to 9 months of age. Changes in antibody titration were observed between 8 months of age and the first year of life.

In another analysis step, the phenomenon was split in two age bands (10 months of age to 10 years and 10

Table - Seroprevalence for citomegaloviruses in Caieiras, São Paulo, Brazil.

Age group months (m) years (y)	Sero- negative	Sero- positive	Sample size	Proportion seropositives	Confidence Interval* (95%)
Newborn	0	36	36	1.000	0.903-1.000
<1 m	1	6	7	0.857	0.421-0.996
1 m	0	6	6	1.000	0.541-1.000
2 m	1	7	8	0.875	0.473-0.997
3 m	3	7	10	0.700	0.348-0.933
4 m	3	5	8	0.625	0.245-0.915
5 m	6	5	11	0.455	0.167-0.766
6 m	7	6	13	0.462	0.191-0.749
7 m	15	6	21	0.286	0.113-0.521
8 m	10	10	20	0.500	0.272-0.728
9 m	9	2	11	0.182	0.023-0.518
10 m	6	10	16	0.625	0.354-0.848
11 m	16	5	21	0.238	0.082-0.472
1 y	22	28	50	0.560	0.413-0.700
2 y	22	21	43	0.488	0.333-0.645
3 y	16	25	41	0.610	0.445-0.758
4 y	13	14	27	0.518	0.319-0.713
5 to 9 y	17	30	47	0.638	0.485-0.773
10 to 14 y	3	6	9	0.667	0.299-0.925
15 to 19 y	2	8	10	0.800	0.444-0.975
20 to 24 y	1	6	7	0.857	0.421-0.996
25 to 29 y	2	7	9	0.778	0.400-0.972
30 to 34 y	0	9	9	1.000	0.664-1.000
35 to 39 y	0	3	3	1.000	0.292-1.000
Total			443		

*Confidence intervals (95%) were calculated by the exact binomial method using Epi Info 6.0 (CDC, USA and WHO, Switzerland).

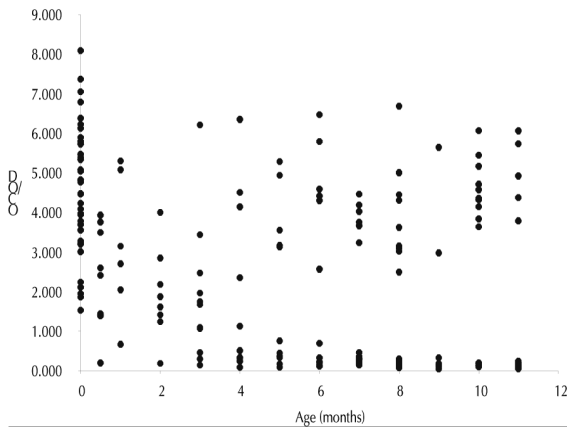


Figure 2 – Scatterplots of the decay of maternal CMV antibody enzyme immunoassay titers in the first year.

to 40 years old). A modified Farrington was applied for each phase to try to better explain the serological phenomenon, i.e. for the naturally acquired infection phase, $S^+(a)$ (Equations 3,5). The prevalence of CMV antibodies in the population of Caieiras increased in the first five years of life, showing about 60% of seropositivity, and there is no fluctuation until the age of 15 years. Between 15 and 40 years there is a second increase in the seropositivity. The results are shown in Figure 3.

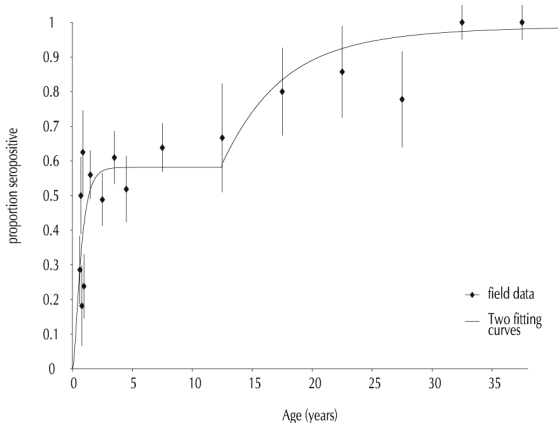


Figure 3 – Function $S^+(a)$ obtained through a modified Farrington (Equations 3, 5).

When a modified Farrington was used to fit the data, the average age of the first infection estimated from serological data, taking into account the two age bands, falls to 5.02 months and 19.84 years, respectively.

The parameters obtained were: $k_1=4.1\pm 1.4 \text{ year}^{-2}$; $k_2=2.17\pm 0.44 \text{ year}^{-1}$; $A=5.02 \text{ months (0.418 year)}$; $c_1=0.054\pm 0.032 \text{ year}^{-2}$; $c_2=0.109\pm 0.062 \text{ year}^{-1}$; $L_c=5 \text{ years}$; $A=19.84 \text{ years}$ (Equation 6).

The age-dependent force of infection, $\lambda_{(a)}$ (Equations 2,4), has its highest value early in childhood then falls at least up to 5 years of age. After that, it rises slowly again (5 to 15 years old) then decreases again at short marks (35 to 40 years old) as it shows in Figure 4.

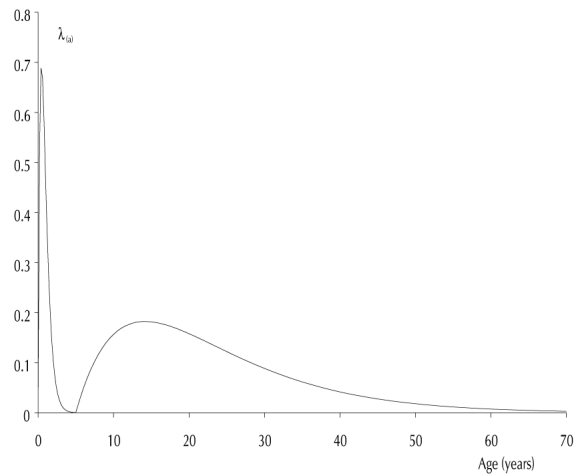


Figure 4 – Estimated force of infection, $\lambda_{(a)}$, using a modified Farrington (Equations 2, 4).

DISCUSSION

This retrospective CMV serological survey in the community of Caieiras was carried out to obtain baseline data from which epidemiological information about CMV occurrence might be analyzed.

The changes in antibody titration detected at 8 months of age and in the first year of life could be an evidence of primary infection.¹² However, identifying the differences between congenital and acquired infection needs further investigation.

The proportion of individuals with maternal antibodies is possibly summed up with the proportion of individuals who have antibodies from congenital or recent infections, making the interpretation of the events of this phase difficult. There's a need of further studies for this population in the first year of life to better understand the declining phase, including IgM analysis and virus detection by PCR technique.

The serological profile of this community indicates that CMV infection is high before the age of 5, showing the intense circulation of the virus in this community.¹⁵ This could be explained by the oral transmission, common to children at this age.^{11,12} At school ages, the proportion of individuals with detectable CMV antibodies remains around 60%. Between the ages 15 and 40 there is a second increase in the seropositivity, suggesting a sexual component that contributes spreading the infection.⁵ The fraction of susceptibles shows that an expressive number of individuals in reproductive age are vulnerable. It should be emphasized that the first infection occurs in 40% of the individuals in the reproductive phase, leading to a considerable risk for congenital CMV infection.

REFERENCES

1. Azevedo-Neto RS, Silveira AS, Nokes DJ, Yang HM, Passos SD, Cardoso MR et al. Rubella seroepidemiology in a non-immunized population of São Paulo, Brazil. *Epidemiol Infect* 1994;113:161-73.
2. Candeias JAN, Stewien KE, Barbosa V. Serological study of cytomegalovirus infections. *Rev Saúde Pública* 1974;8:257-63.
3. Carvalho RPS, Pannuti CS, Amato Neto V, Oselka GW, Ângelo MJO. Sero-epidemiological study of cytomegalovirus infection in São Paulo-Brasil. *Rev Inst Med Trop São Paulo* 1976;18:1-5.
4. Doerr HW, Braun R, Munk K. Human cytomegalovirus infection: Recent developments in diagnosis and epidemiology. *Klin Wochenschr* 1985;63:241-51.
5. Drew WL, Mintz L, Miner RC, Sands M, Ketterer B. Prevalence of cytomegalovirus infection in homosexual men. *J Infect Dis* 1981;143:188-92.
6. Dworsky M, Yow M, Stagno S, Pass RF, Alford C. Cytomegalovirus infection of breast milk and transmission in infancy. *Pediatrics* 1983;72:295-9.
7. Farrington CP. Modelling forces of infection for measles, mumps and rubella. *Stat Med* 1990;9:953-67.
8. Fundação IBGE. Annual report. Rio de Janeiro; 1992.
9. Gold E, Nankervis GA. Cytomegalovirus. In: Evans AS, editor. *Viral infections of humans: epidemiology and control*. New York: Plenum Medical Book; 1989 v. 8. p. 169-86.
10. Hanshaw JB. On deafness, cytomegalovirus, and neonatal screening. *Am J Dis Child* 1982;136:886-7.
11. Hutto SC, Ricks RE, Garvie M, Pass RF. Epidemiology of cytomegalovirus infection in young children: day care vs home care. *Pediatr Infect Dis* 1985;4:149-52.
12. Mello ALR, Ferreira EC, Vilas Boas LS, Pannuti CS. Cytomegalovirus infection in a day-care center in the municipality of São Paulo. *Rev Inst Med Trop São Paulo* 1996;38:165-9.
13. Pannuti CS, Vilas-Boas LS, Angelo MJO, Carvalho RPS, Segre CM. Congenital cytomegalovirus infection. Occurrence in two socioeconomically distinct populations of a developing country. *Rev Inst Med Trop São Paulo* 1985;27:105-7.
14. Reynolds DW, Stagno S, Hosty TS, Tiller M, Alford CA. Maternal cytomegalovirus excretion and perinatal infection. *N Engl J Med* 1973;289:1-5.
15. Veronesi R. Citomegalia: revelação da presença dos "vírus das glândulas salivares" em crianças de São Paulo, através de inquérito sorológico. *Rev Hosp Clin Fac Med Univ São Paulo* 1959;14:249-55.