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BRIEF REPORT

Seroconversion for Cytomegalovirus Infection During Pregnancy and Fetal Infection in a Highly Seropositive Population: "The BraCHS Study"

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(See the Editorial Commentary Plotkin, on pages 1191–9.)

We determined the risk of seroconversion in seronegative pregnant women living in a high seroprevalence population. Cytomegalovirus (CMV)-immunoglobulin G reactivity was determined at the 1st trimester in all women and sequentially for seronegative women. A total of 1915 of 1952 (98.1%; 95% confidence interval [CI], 97.4%–98.7%) women were seropositive, and 36 (1.8%; 95% CI, 1.3%–2.6%) were seronegative. Five of the 36-seronegative women seroconverted for a cumulative rate of 13.9% (95% CI, 4.8%–30.6%). Congenital CMV infection was diagnosed in 1 of 36 infants (2.8%; 95% CI, 0.5%–63.9%) born to seronegative women compared with 8 of 1685 (0.5%; 95% CI, 0.2%–1.0%) infants born to seropositive mothers. Even with a high risk of primary infection in seronegative women, most CMV-infected infants were born to women with pre-existing seroimmunity.

Keywords. congenital infection; cytomegalovirus; pregnancy; seroconversion.

The prevalence of congenital cytomegalovirus (cCMV) infection has been reported to vary according to the epidemiological characteristics of women of childbearing age [1]. Both primary and nonprimary maternal CMV infection have been associated with intrauterine transmission of this virus, which can result in birth defects and long-term developmental disabilities. Most congenital infections in populations with high CMV seroprevalence are a consequence of nonprimary maternal infection among seropositive women [2]. However, opportunities for exposure to CMV in these settings could also pose a significant risk to pregnant seronegative women.

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We have previously demonstrated a high CMV seroprevalence in a Brazilian, urban, low-income, maternal population with a high prevalence of cCMV infection [3]. More importantly, these women were infected with CMV at a young age, and over 95% were seropositive before conception [3]. However, the risk of seroconversion during gestation and the frequency of mother-to-fetus CMV transmission in seronegative women in a highly seropositive population has not been reported. Defining the seroconversion rate of nonimmune women in this population could infer the relative risk of exposure to infectious CMV in this population, and it will aid in the design and implementation of public health interventions for preventing cCMV infections and its undesirable infant consequences.

In this study, we determined the incidence of seroconversion during gestation among seronegative women and compared the rate of fetal infection between women with primary infection during gestation and those who were CMV seropositive.

METHODS

Study Design

The overall objectives of the "Brazilian Cytomegalovirus Hearing and Maternal Secondary Infection Study" (BraCHS) are to understand the natural history of maternal and congenital CMV infections in a highly CMV-seropositive population. All study procedures were approved by the local and National Committee for Ethics in Research (16.928/2013), and written informed consent was obtained from the subjects.

In a cohort design, BraCHS recruited pregnant women who presented for prenatal care at <18 weeks' gestation in 6 public healthcare centers in Ribeirão Preto city, São Paulo state, Brazil. Most (99.2%) women belonged to the low-income group. They were enrolled at their first antenatal visit with follow-up evaluations in the 2nd (20–26 weeks) and 3rd (32–36 weeks) trimesters of gestation and at 1 month after delivery. Gestational age was determined based on the last menstrual period, and the ultrasound examination was performed in the 1st trimester of gestation when available. During the study visits, blood, saliva, vaginal swabs, and urine were obtained. A standardized questionnaire was administered at each study visit to collect information on demographics, household conditions, providing care for young children, and sexual history.

Infants born to study participants were screened for cCMV infection by testing the saliva obtained within 1 week of age with a CMV-deoxyribonucleic acid polymerase chain reaction (PCR) [4]. Congenital CMV was confirmed by testing urine samples, which were collected within the first 3 weeks of age, by PCR. All newborn infants with confirmed infection underwent complete physical examination, hearing screening, and

neurosonography. Other congenital infections such as syphilis and toxoplasmosis were excluded based on maternal and infants' serological testing.

Of the 2481 women enrolled into the BraCHS study (March 2014 to May 2015), 1952 (78.7%) women had at least 1 additional serum sample collected ≥14 weeks after the initial specimen was obtained in the 1st trimester of gestation and were included in this analysis, independently of the pregnancy outcome. Reasons for nonparticipation included lack of available samples due to miscarriage (239; 9.6%), ectopic pregnancy (4; 0.2%), fetal loss (12; 0.5%), consent withdrawal (141; 5.7%), migration to another town (90; 3.6%), or other reasons (43; 1.7%).

Serological Tests

Once collected, all blood samples had been processed, and sera were stored at −70°C for future batch testing. First-trimester serum samples of all women were tested for anti-CMV immunoglobulin (Ig)G antibodies using the commercial quantitative LIAISON CMV IgG II assay (DiaSorin S.p.A, Saluggia, Italy).

For women defined as seronegative in the 1st trimester, all subsequent samples obtained were tested for anti-CMV IgG antibodies. Upon detection of IgG seroconversion, the sample was tested for anti-CMV IgM using the LIAISON CMV IgM II assay (DiaSorin S.p.A).

Data Analysis

Cytomegalovirus seroprevalence was determined by the number of CMV IgG-positive participants in all valid test results, and 95% confidence intervals (CIs) were calculated. Seroconversion was defined as IgG positivity in a subsequent sample in women who were seronegative in the 1st trimester of gestation. The gestational age of CMV seroconversion was estimated using the mean time between the last negative and the first positive test. Seroconversion rate was calculated as an incidence rate using, as the denominator, the number of person-days at risk. The annual seroconversion rate was estimated based on the median follow-up time.

The distribution of quantitative variables among groups was compared using Wilcoxon non-parametric test, and the association with categorical variables was evaluated by Fisher's exact test. We used SAS 9.4 and a significance level of 5% for all analysis.

RESULTS

Cytomegalovirus Seroprevalence and Seroconversion

Maternal 1st-trimester samples were obtained at a median gestational age of 8 weeks (range, 1–18). Among 1952 women tested in the 1st trimester, 1915 were positive for CMV-IgG antibodies (98.1%; 95% CI, 97.4%–98.7%), 36 were negative (1.8%; 95% CI, 1.3%–2.6%), and 1 (0.1%) had equivocal results.

As shown in Table 1, CMV-seronegative women had more years of schooling, were slightly older at sexual debut, and were less frequently exposed to children before and during gestation than CMV-seropositive women. However, other baseline characteristics, including those related to sexual activity, were not different between the 2 groups.

Of the 36 seronegative women, 33 (91.7%) were tested as planned (1st, 2nd, 3rd trimesters, and 1 month postpartum), whereas 3 of them were tested 3 times during gestation but not after delivery. The median interval between the first and last sample was 252 days (range, 152–312), and 141 sera samples were tested. The overall observation period at risk encompassed 8877 person-days.

Five of the 36-seronegative women became CMV-IgG positive. The timing of seroconversion was estimated to be at 10, 12, 14, 19, and 28 weeks of gestation, respectively. All 5 women had consistently positive CMV-IgG results in 1 to 3 subsequent serum samples. Cytomegalovirus-IgM was positive in 4 of the 5 subjects in the first IgG-positive specimen. The overall cumulative incidence of seroconversions was 13.9% (95% CI, 4.8%–30.6%) during a median follow-up period of 36 weeks (seroconversion rate of 5 of 8877 person-days or 5.6 per 10 000 person-days at risk). Based on these findings, the annualized rate of seroconversion was estimated to be 19.5%.

Congenitally Cytomegalovirus-Infected Infants

All 36 pregnant women with primary CMV infection had a single live newborn infant. Among the 1915 seropositive pregnant women, 1685 newborns were screened for CMV. Congenital CMV infection was diagnosed in 1 of the 36 infants (2.8%; 95% CI, 0.5%–14.2%) born to initially seronegative mothers and in 8 of 1685 (0.5%; 95% CI, 0.2%–1.0%) infants born to seropositive mothers.

None of the newborns with cCMV had clinically detectable findings of congenital infection. The infant born to the mother with primary CMV infection at approximately 28 weeks of gestation passed the hearing screening and cranial sonography showed bilateral subependymal cysts. Six of the 8 infants born to seropositive mothers passed hearing screening, and the remaining 2 infants did not undergo hearing screening. Cranial imaging exams were done in 7 of these 8 infants. One of them had a concomitant hypoxic ischemic encephalopathy. Minor findings (bilateral subependymal cysts [1]; lenticulostriate vasculopathy [1]; lower volume of white matter [1]) were found in 3 infants, whereas the remaining babies had normal cranial sonogram.

DISCUSSION

In this study, only a small proportion (1.9%) of low-income, urban, Brazilian women were seronegative for CMV during the 1st trimester of pregnancy, a result that was consistent with our previous findings that most women in this maternal population have acquired CMV at a young age [3]. The finding that seronegative women in this population have a high risk of becoming

Table 1. Characteristics of CMV-Seropositive and CMV-Seronegative Women

Characteristics	Seropositive (n = 1915)	Seronegative (n = 36)	*P* Value
Maternal age in years[a,b]	24 [13–45]	25 [16–36]	.50
Schooling (years)[a,b]	10 [0–15]	11 [7–13]	.03
Number of previous pregnancies[a,b]	2 [2–10]	1 [1–3]	.15
Race[c]			
White	843 (45.7%)	21 (58.3%)	.17
Non-White	1002 (54.3%)	15 (41.7%)	
Ilicit Drugs Use (During Pregnancy)[c]			
No	1255 (83.9%)	27 (84.4%)	1.00
Yes	241 (16.1%)	5 (15.6%)	
Age at sexual debut[a,b]	16 [4–34]	16 [13–25]	.03
No. of Partners/Years of Sexual Activity[c]			
≤0.5	1411 (82.4%)	29 (82.9%)	
0.51–0.99	163 (9.5%)	4 (11.4%)	.88
≥1	139 (8.1%)	2 (5.7%)	
Number of sexual partners 1 year before pregnancy[a,b]	1 [0–40]	1 [1–2]	.81
History of Partners With Sexually Transmitted Infections[c]			
No	1701 (95.6%)	34 (94.4%)	.67
Yes	79 (4.4%)	2 (5.6%)	
Use of Condom[c]			
Never/Rarely	1565 (87.9%)	32 (88.9%)	1.00
Frequently	215 (12.1%)	4 (11.1%)	
Number of members ≥6 years in the household[a,b]	3 [1–19]	3 [1–12]	.61
Number of children <6 years in the household[a,b]	0 [0–13]	0 [0–2]	.07
Before Pregnancy			
Children <6 years in the household[c]			
No	995 (59.9%)	25 (69.4%)	.09
Yes	850 (46.1%)	11 (30.6%)	
Child Close Contact[c,d]			
No	1224 (66.4%)	30 (83.3%)	.03
Yes	619 (33.6%)	6 (16.7%)	
Children <6 Years Attending Day Care[c]			
No	1226 (66.4%)	27 (75.0%)	.37
Yes	619 (33.6%)	9 (25.0%)	
During Pregnancy			
Children <6 Years in the Household[c]			
No	1012 (60.4%)	25 (75.8%)	.10
Yes	664 (39.6%)	8 (24.2%)	
Child Close Contact[c,d]			
No	1307 (77.8%)	32 (97.0%)	< .01
Yes	374 (22.2%)	1 (3.0%)	
Children <6 Years Attending Day Care[c]			
No	1150 (68.7%)	26 (78.8%)	.26
Yes	524 (31.3%)	7 (21.2%)	

Abbreviations: CMV, cytomegalovirus.

[a]Wilcoxon nonparametric test.

[b]Median [range].

[c]Fisher's exact test.

[d]To provide direct child care such as changing diapers, bathing, and feeding a child in either the woman's family and/or a friend's.

[e]To live with or take care of children attending day care.

infected during gestation with a cumulative incidence rate of 13.9% and an annualized seroconversion rate of 19.5% indicates that CMV exposure in this maternal population is significant. The risk of fetal infection after primary CMV infection during pregnancy remains high in this population, similar to that described in infected women from low seroprevalence settings.

The rate of annual seroconversion among seronegative pregnant women observed in our study is higher than most reports

from several countries and populations. In a review by Hyde et al [5], the annual seroconversion rates in pregnant women, mostly from populations with low to intermediate CMV seroprevalence (< 77%), ranged from 1% to 7% (summary annual rate = 2.3%; 95% CI, 2.1%–2.4%). Other large studies have also documented lower seroconversion risks including a 3.1%–3.5% rate in pregnant women from United Kingdom [6] and 1.8%–2.1% in North American women [7, 8]. Although we have monitored a large cohort of pregnant women, relatively few women were seronegative, limiting the precision of the seroconversion estimates. However, even with wide CIs, the lower limits of the 95% CIs are higher than the upper limits reported in previous studies, suggesting that the higher seroconversion rate in our study population is likely reliable.

Similar to our findings, seroconversion rates as high as 22.5% and 14.8% have been reported in Japanese maternal populations with very high (94% and 84%, respectively) CMV seroprevalence rates [9, 10]. From these data and our findings, it could be argued that CMV seroconversion rates in women is a function of the exposure to CMV in the population that is reflected by the seroprevalence in the population. In particular, close contacts of pregnant women (such as sexual partners and young children) in high seroprevalence settings are more likely to be infected and shedding CMV, a known risk factor for CMV seroconversion [5]. This is especially relevant in the low-income, urban population where crowded living households with extended families and providing care to young children is common, leading to increased opportunities for exposure and transmission of CMV. We have recently shown that virus shedding in seropositive pregnant women from the same population was associated with caring for young children as well as crowded living conditions [11]. Furthermore, seroprevalence rates as high as those observed by us have been also detected among Brazilian children in day care [12] and in mothers with children in day care who shed CMV [13].

Because only a small number of seronegative women were available for monitoring in our study, it was not possible to carefully examine the risk factors associated with seroconversion and, perhaps of greater interest, why these women remained seronegative before pregnancy in a setting with significant CMV exposures. We did observe that the seronegative women had higher education level, were older at sexual debut, and significantly fewer women had contact with children either before or during pregnancy. However, population-based seroconversion studies are needed to elucidate the relative influence of different types of exposure to CMV.

The strengths of this study were the ability to monitor a large cohort of women until delivery and the ascertainment of the vertical transmission of CMV. Birth prevalence estimates of congenital CMV infection among seropositive women (0.5%; 95% CI, 0.2%–1.0%) are comparable to our previous studies in a similar population and in studies of other highly immune populations [14]. We observed a high vertical transmission rate (20%; 95% CI, 0.5%–63.9%) in women who acquired CMV during pregnancy, which is consistent with studies in other populations that have documented higher transmission rates in women with primary CMV infection [1, 15]. Despite this high transmission rate, approximately 90% (8 of 9) infants with cCMV were born to women with pre-existing seroimmunity, emphasizing the contribution of nonprimary maternal infections to the overall burden of cCMV in maternal populations with high seroprevalence.

CONCLUSIONS

This report adds to the natural history of CMV infection in this highly seropositive maternal population, and it highlights the relevance of developing intervention strategies for the prevention of maternal and congenital CMV infections that are culturally appropriate.

Notes

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