Seroprevalence and Risk Factors for Cytomegalovirus Infections in Adolescent Females

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Background. Congenital cytomegalovirus (CMV) is a leading cause of disability, including sensorineural hearing loss, developmental delay, and mental retardation. Understanding risk factors for acquisition of CMV infection in adolescent females will help determine vaccine strategies.

Methods. Females (12–17 years) were recruited from primary care settings in Cincinnati, Galveston, Houston, and Nashville from June 2006 to July 2010 for a seroepidemiologic study, from which seronegative participants were recruited for a CMV vaccine trial. Participants (n = 1585) responded to questions regarding potential exposures. For those with young children in the home (n = 859), additional questions were asked about feeding and changing diapers, and for those > 14 years of age (n = 1162), questions regarding sexual activity were asked. Serum was evaluated for CMV antibody using a commercial immunoglobulin G assay.

Results. Cytomegalovirus antibody was detected in 49% of participants. In the univariate analyses, CMV seroprevalence was significantly higher among African Americans, those with children < 3 years of age in the home, and those with a history of oral, anal, or vaginal intercourse. Among those with young children in the home, feeding children and changing diapers further increased the association with CMV infection. However, in the final multivariate analysis, only African Americans and household contact with young children were associated with CMV infection.

Conclusions. By age 12, evidence of CMV infection was common. Multiple factors regarding race and personal behaviors likely contribute to seroconversion earlier in life.

Key words. Adolescents; Computer-Assisted Screening Interview; CASI; Cytomegalovirus; CMV; Epidemiology
Cytomegalovirus (CMV) is a common infection with an estimate of seroprevalence in adolescents of 47%–89% [1–6]. Congenital CMV infection is the leading cause of birth defects and disability in the United States [7], affecting an estimated 8000 infants per year with sensorineural deafness, mental retardation, and/or seizure disorders [8–10], and an annual estimated financial burden of $1–2 billion [11]. The Institute of Medicine indicated a vaccine targeting 12 year olds would be highly cost-effective and designated it as a level I priority [12].

Because encouraging results from recent trials have renewed interest in CMVs vaccines [13–16], it is important to learn more about the risk factors associated with acquisition of CMV, particularly in young women 12–17 years of age who may have increased rates of infection [6]. Previous studies indicate that CMV may be transmitted via saliva, urine (or handling of diapers), blood, cervical secretions, and semen [2, 17–23]. Because young women frequently engage in behaviors with children at the peak age of CMV shedding (1–2 years old), they are at risk for coming into contact with body fluids containing CMV. This suggests that exposure to young children could be a major risk factor for transmission [24–25]. Therefore, we aimed to evaluate specific behaviors that may place adolescent females at increased risk of infection.

MATERIALS AND METHODS

Study Population and Design

To determine the seroprevalence of CMV and factors associated with CMV infection, females (12–17 years) were recruited from established patient populations of adolescent outpatient clinics affiliated with Baylor College of Medicine ([BCM] Houston, TX), Cincinnati Children’s Hospital Medical Center ([CCHMC] Cincinnati, OH), The University of Texas Medical Branch at Galveston, TX ([UTMB] Galveston, TX), and Vanderbilt University Medical Center ([VUMC] Nashville, TN) from June 2006 to July 2009 to learn more about the seroepidemiology of CMV. Cytomegalovirus serum immunoglobulin G antibody was evaluated using a commercial enzyme-linked immunosorbent assay (Wampole, Inverness Medical Professional Diagnostics) in accordance with the manufacturer’s instructions at CCHMC laboratories. During recruitment, potential participants were told that if eligible (CMV seronegative), they may be invited to participate in a CMV vaccine trial, but that their subsequent participation was voluntary.

To learn more about the epidemiology of CMV, participants completed a questionnaire that was developed from previous studies [24–25] and the experience of the investigators. The questionnaire included approximately 6 categories of risk: race/ethnicity, age, exposure to children 3 years of age in the household, group living situations, saliva-sharing behaviors (including kissing), and sexual activity. The questionnaire was administered using a computer-assisted screening interview (CASI). Questions regarding sexual activity were restricted to participants aged 14 years (the legal age of consent). For this study, race was defined by participants listing all applicable terms from the following list: African American/black, American Indian/Alaskan Native, Asian, Hawaiian/Pacific Islander, and Caucasian/white. Each participant was asked to describe herself as “Hispanic” or “non Hispanic,” regardless of race. Before study initiation, the institutional review boards of each site approved this study, and a certificate of confidentiality was obtained. A parallel seroepidemiology study with the same questionnaire was performed in adolescent males at CCHMC, UTMB, and VUMC from June 2006 to July 2007 as has been previously reported [26].

Statistical Analysis

Data analyses were performed with SAS version 9.2 (SAS Institute, Cary, NC). Point prevalence calculations determined the overall seroprevalence of CMV. To determine factors associated with CMV seroprevalence, we first performed univariate χ² analyses for categorical variables and Wilcoxon tests for continuous variables. Logistic regressions were then performed for multivariate analyses and additive models. Questions qualifying various exposures as “sometimes,” “often,” or “never or rarely” were collapsed into “sometimes or often” versus “never or rarely” due to small sample size. Individuals were then grouped according to Hispanic, African American (Non-Hispanic), White (Non-Hispanic), and Other (Non-Hispanic) categories for the purposes of the analyses. After the univariate analysis of 6 variables (discussed above) was performed, risk factors were entered into a multivariate logistic regression model with a cutoff value of P < .10.

RESULTS

Demographics

Of the 1627 adolescent females enrolled in the study, 26 subjects did not complete the questionnaire and
another 16 subjects did not have serology results. Therefore, the number of subjects who completed the questionnaire (ie, had laboratory results available) and were included in the statistical analysis was 1585. There were no significant differences between those included in the analysis and those with incomplete data.

Table 1 displays the CMV serostatus of participants by site. Overall, 779 of the 1585 (49%) participants were seropositive for CMV. The CMV serostatus varied significantly across sites ($P = 0.025$); however, race/ethnicity also varied significantly across sites ($P < 0.0001$). When stratifying the CMV serostatus comparison by race/ethnicity, the CMV serostatus was not significantly different across sites ($P = .23$), and including site in the multivariate models did not significantly improve the model fit. Because our goal was to evaluate personal behaviors and racial and ethnic characteristics, the participant population as a whole was evaluated.

Over half of participants (810) indicated they were of African American (Non-Hispanic) (51%). Of the remainder (775), 431 reported being White (Non-Hispanic) (27%), 214 White (Hispanic) (14%), and 130 reported Other, Non-Hispanic race/ethnic group (8%). The mean age of the sample was 15.2 years with a standard deviation of 1.6. Overall, 779 of the 1585 (49%) participants were seropositive for CMV. CMV antibody was detected in 49% of 12-year-old participants and ranged from 46% to 51% in those 13–17 years. When a category of age <4 years of age and 14 years of age was studied, there was no statistically significant increase in CMV seropositivity with age (odds ratio [OR], 1.04; confidence interval [CI], 0.83 and 1.30).

**Risk Factors**

Table 2 displays the univariate analysis of the 7 major risk factors evaluated with regard to CMV serostatus. Analysis by race/ethnicity revealed that using the reference group of White (Non-Hispanic), African American (Non-Hispanic) descent had over a 2-fold increased odds ratio (OR, 2.49) of being CMV seropositive (CI, 1.95 and 3.17).

**Exposure to Young Children in the Home**

Of the 1585 participants, 859 (54%) reported children <3 years of age in the home. Of these 859 participants, 614 reported 1–2 children in the home, whereas 245 had >3 children in the home. Exposure to a young child was associated with an almost 2-fold increased odds (OR, 1.88) of CMV infection (CI, 1.54 and 2.30). When specific activities were evaluated among those with young children in the home, changing diapers and feeding children were both associated with increased odds of infection ([OR, 1.54; CI, 1.14 and 2.09] and [OR, 2.02; CI, 1.37 and 2.98], respectively).

**Group Living Situations**

Half of the adolescents studied (793) had previously participated in a group living situation, defined as having been an overnight camp counselor for one week or more ($n = 65$), attended overnight camp ($n = 712$), stayed overnight in juvenile detention or jail ($n = 96$), or stayed overnight in a treatment program for drug, alcohol, or mental problems ($n = 50$). Overall, adolescent females who had been in a group living situation did not have an increased odds of CMV infection compared with those who had no reported history of group living (OR, 0.84; CI, 0.69 and 1.02).

**Saliva-Sharing Behaviors**

Approximately three fourths of adolescents reported sharing at least 1 item that likely contained saliva; 171 shared toothbrushes, 930 shared lip balm or lipstick, and 958 shared drinks. Of females who shared items, 598 (49%) were CMV positive; there was no difference in serostatus based on sharing these items (OR, 1.05; CI, 0.83 and 1.32). Of 1585 adolescents studied, 1134 (72%) reported kissing another adolescent (male or female). Of those participants, 51%
were CMV seropositive. When kissing alone was evaluated, there were no statistically significant differences with regard to CMV infection between those who reported a history of kissing and those who did not (OR, 1.22; CI, 0.98 and 1.52). When evaluating the saliva-sharing category (including sharing items and kissing), 1412 (89%) of adolescent females engaged in this behavior and it was not predictive of CMV infection (OR, 1.25; CI, 0.90, 1.73).

### Sexual Activity

**Of the 1162 adolescent girls > 14 years of age eligible to answer questions regarding sexual activity, 1150 responded. Of these, 554 (48%) indicated they had a history of intimate sexual contact (defined as oral, anal, and/or vaginal intercourse). Of those who were sexually experienced, 72 (13%) had a history of anal intercourse, 336 (61%) had a history of oral intercourse, and 456 (82%) had a history of vaginal intercourse. Vaginal intercourse was associated with CMV infection in the univariate analysis (OR, 1.30; CI, 1.02 and 1.65). Of those with a history of any type of sexual contact, 292 (53%) were CMV positive (OR, 1.31; CI, 1.04 and 1.65) compared with those participants without a history of these sexual activities.**

Further evaluation of factors within the category of sexual activity revealed that the age of first sexual contact was not associated with CMV antibody, regardless of whether it was evaluated as an ordinal value ($P = .67$ using 2-sided Wilcoxon test) or categorically using an age <14 years vs 14 years variable (OR, 1.14; CI, 0.76 and 1.72).

Neither the time from first intimate sexual contact to participation in the study (1.54 years +/- 1.3 years,
Infection with CMV is very common in adolescent females, our study showed that 49% of teenage girls was seropositive. This result is comparable to previously published US estimates in this age group (41%–89%) \([5, 6, 23, 25-26]\). The fact that roughly half of this population is already infected with CMV should be taken into account when determining the optimal target age for a CMV vaccine. Therefore, the age of optimal vaccination may be much earlier in life.

African Americans and Hispanics have been previously reported to have increased rates of positive CMV antibody (in both males and females) compared with Caucasians \([2, 3, 5, 27-29]\). Our study confirmed higher rates in African American (Non-Hispanics) females compared with other groups, which is similar to Staras et al’s \([5]\) National Health and Nutrition Examination Survey sample from 1988 to 1994 and Bate et al’s \([6]\) 1988–2004 population. It is of interest that we were not able to confirm the results of a smaller study conducted by Wilms et al \([30]\), who detected a much lower seroprevalence (ranging from 22% to 33% in 13–20 year olds) in African Americans in 2005–2006 in Virginia. It is unclear whether factors such as sample size, gender, geographic location, and sexual activity may be related to the lower seroprevalence found in that study.

It is likely that exposures to and behaviors associated with body fluids known to transmit CMV contribute to the risk of infection. CMV transmission has traditionally thought to be bimodal with acquisition occurring either in infancy (secondary to breast feeding) and/or early childhood (as documented by day care studies) \([24, 31-34]\) or later, in young adulthood (likely due to intimate exposures because CMV has been detected in saliva \([22, 24]\), cervical specimens \([18, 24]\), and semen \([17, 24]\)). Many studies in young women have shown a relationship between sexual activity and CMV infection with regard to early sexual debut \([2, 32]\), heterosexual contact \([19, 24]\), increased number of sexual partners \([3]\), and a history of sexually transmitted infections \([18]\). We found a relationship (OR, 1.31; CI, 1.04 and 1.65) between sexual exposure and CMV infection in the univariate, but not in the multivariate analysis (OR, 1.13; CI, 0.864 and 1.47). Further evaluation of the type(s) of intercourse, number of sexual partners, and sexually transmitted infections did not reveal any additional associations with CMV. It appears that exposure to young children is a more important source of CMV infection than sexual activities in adolescents.

When evaluating intimate behaviors in adolescents, sharing of items that likely contain saliva was commonly reported (77%) as was kissing (72%). It is interesting that the use of saliva-sharing items, kissing and saliva-sharing behaviors, were not linked to CMV infection because CMV is commonly detected in this

**DISCUSSION**

**Table 3. Multivariate Analysis**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Odds Ratio</th>
<th>95% CI for Odds Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (Non-Hispanic)</td>
<td>2.49</td>
<td>(1.83, 3.38)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1.45</td>
<td>(0.96, 2.21)</td>
<td></td>
</tr>
<tr>
<td>Other (Non-Hispanic)</td>
<td>2.02</td>
<td>(1.26, 3.23)</td>
<td></td>
</tr>
<tr>
<td>Diapering/feeding children</td>
<td>1.24</td>
<td>(1.08, 1.41)</td>
<td>.0016</td>
</tr>
<tr>
<td>Group living</td>
<td>0.99</td>
<td>(0.77, 1.28)</td>
<td>.964</td>
</tr>
<tr>
<td>Kissing</td>
<td>1.24</td>
<td>(0.88, 1.76)</td>
<td>.222</td>
</tr>
<tr>
<td>Sexual activity</td>
<td>1.13</td>
<td>(0.86, 1.47)</td>
<td>.388</td>
</tr>
</tbody>
</table>

Boldfaced values indicate P < .05.

Abbreviations: AA, African American; CI, confidence interval.
body fluid. However, it is problematic to know how to ask about the frequency and/or intensity of kissing to explore this possible risk factor. In addition, the existing literature has documented that living in crowded situations (in a communal Kibbutz living situation) and group living has been associated with CMV infection [5, 35–38]; however, after studying specific group living situations individually and collectively as a variable, we found that it was not associated with CMV infection. While some studies have evaluated the number of individuals living in a home as a measure of “crowding” or “group living,” we were concerned that adolescents answering a CASI may not know of the number of individuals living in the home earlier in life (from birth to the time of the questionnaire). It is possible that these variables were not of any significance in that we did not sufficiently capture the personal behaviors or exposure that is linked before CMV infection. A limitation of a cross-sectional seroprevalence study is that we may not be able to detect the specific behavior or exposure that a child may have had much earlier in life. A risk factor study at a younger age (before CMV infection) or a prospective design study may be more helpful in determining the specific risks linked with CMV antibody.

In the final multivariate analyses, only African Americans feeding/diapering young children in the home were significantly associated with CMV infection in adolescent females. We previously reported on a parallel study of adolescent males from the same clinic populations from Cincinnati, Galveston, and Nashville [26]. The findings were remarkably similar; overall, the CMV seroprevalence of adolescent males was 47% and that of adolescent females 49%, which is also similar to the most recent US estimates [5, 6]. In addition, the results of both studies indicated that African Americans were at increased risk for CMV infection. It is not clear why race was associated with CMV infection in these clinic populations, and it may be a marker for some other behavioral or cultural factor. In adolescent males, age was associated with CMV infection, although it was not in adolescent females. For both males and females, exposure to children 3 years in the home was associated with infection in the univariate models; however, it did not remain in the multivariate model for the adolescent males. Perhaps adolescent women participate in the care of the children (by feeding and changing diapers and thus possibly being exposed to saliva and urine, respectively) more than adolescent males.

There are a number of limitations to this study, including a sample size that may have been too small to detect subtle differences between groups or in the in the number or amount of specific behaviors and exposures. Participants were also recruited in conjunction with a CMV vaccine trial, and although adolescent females were not required to have an interest in this study, it may have skewed participation towards those individuals who were interested in receiving the CMV vaccine. There may be differences in adolescent females willing to complete a questionnaire versus those who are not willing to do so. Therefore, these participants may have different characteristics than those attending the adolescent clinics. In addition, there may have been a cohort effect as well as geographic differences that may have affected our results, and thus our findings may not be generalizable of those living in other geographic locations. It is also possible that adolescents had errors in self-reporting behaviors and exposures. In the study, only 1162 of 1583 participants were 14 years of age (and thus allowed to answer questions pertaining to sexual activity), reducing the ability to assess sexual activity as a risk factor and eliminating the possibility to evaluate those individuals under the age of consent. In general, it is difficult to assess possible lifetime exposures using a cross-sectional study design in adolescence. Specifically, factors relating to the participant’s gestational period, neonatal period, and early childhood (including origin of children, breastfeeding, and daycare) were not evaluated in an effort to focus on adolescent exposures and personal behaviors. In addition, we were concerned that participants’ answers regarding this early information may be unknown, unavailable, or unreliable. Regardless of the question, adolescents may error (whether intentionally or unintentionally) in the self-report process. Although the study adds to the current understanding of CMV infections of adolescent females in the United States, its cross-sectional design limits the ability to determine the annual incidence in the population in a prospective fashion. Given that half of adolescent females have already been infected with CMV, future studies should prospectively evaluate exposures and personal behaviors in younger children in preparation for a vaccine aimed at preventing congenital CMV infection.

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