HHS Public Access

Author manuscript

J Neonatal Perinatal Med. Author manuscript; available in PMC 2016 July 31.

Published in final edited form as:

J Neonatal Perinatal Med. 2015 July 31; 8(2): 137–141. doi:10.3233/NPM-15814057.

Determining the Prevalence of Cytomegalovirus Infection in a Cohort of Preterm Infants

Mitchell M Pitlick[a], **Kristin Orr**[b], **Allison M Momany**[a], **Erin L McDonald**[a], **Jeffrey C. Murray**[a], and **Kelli K. Ryckman**[c,*]

[a]Department of Pediatrics, University of Iowa, Iowa City, IA

[b]Department of Obstetrics and Gynecology, University of Iowa, Iowa City, IA

[c]Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA

Abstract

Background—Preterm birth is a global public health problem that is a significant cause of infant morbidity and mortality. Congenital cytomegalovirus (CMV) infection has been proposed as a risk factor for preterm birth, but the rate of CMV in infants born preterm is unclear. CMV is the leading infectious cause of sensorineural hearing loss, which will affect 15%–20% of congenitally infected infants later in their childhood. 90% of infected infants are asymptomatic at birth and are not recognized as at risk for CMV-associated deficits.

Objective—To determine the prevalence of CMV infection in a large cohort of preterm infants.

Methods—DNA was extracted from cord blood, peripheral blood, saliva, and buccal swab samples collected from preterm infants. A total of 1200 unique DNA samples were tested for CMV using a nested PCR protocol. The proportions of preterm infants with CMV was compared by sample collection type, race, gender, and gestational age.

Results—A total of 37 infants tested positive for CMV (3.08%). After excluding twins, siblings, and infants older than two weeks at the time of sample collection, two out of 589 infants were CMV positive (0.3%), which was lower than the proportion of CMV observed in the general population. All positive samples came from buccal swabs.

Conclusions—Our work suggests that while CMV infection may not be greater in preterm infants than in the general population, given the neurologic consequences of CMV in preterm infants, screening of this population may still be warranted. If so, our results suggest buccal swabs, collected at pregnancy or at birth, may be an ideal method for such a program.

Keywords

Congenital cytomegalovirus; preterm birth; newborn screening; polymerase chain reaction

[*]Corresponding Author: Kelli K Ryckman, Department of Epidemiology, College of Public Health, University of Iowa, 145 North Riverside Drive, 1130 S400 CPHB, Iowa City, IA 52242, Telephone: 319-384-1562, Fax: 319-384-4155, kelli-ryckman@uiowa.edu.

Disclosure Statements

The authors have no financial, human research, animal research, or other disclosures.

I. Background

Preterm birth, defined as a birth before 37 weeks gestation, is a worldwide public health problem. Apart from a significant association with mortality, preterm birth carries an increased risk for numerous morbidities including intraventricular hemorrhage, retinopathy of prematurity, and respiratory distress syndrome. While a portion of preterm deliveries are medically indicated for conditions such as preeclampsia, the majority of preterm births are spontaneous [1]. There are both genetic and environmental risk factors that contribute to spontaneous preterm birth. One such environmental risk factor is infection. While there is a large body of evidence linking bacterial infection to preterm birth, evidence regarding the role of viral infection is limited [1]. Bacterial infection is thought to lead to preterm birth through an increase in uterine contractions due to increased prostaglandin production induced by the innate immune response [2, 3]. Viral infections can be trans-placental and cause the same placental-uterine inflammation as bacterial infections. Thus, it is logical to reason that viral infections could also play a role in preterm birth [4, 5].

Congenital cytomegalovirus (CMV) is the most common cause of congenital-acquired infection in the United States [6]. Vertical transmission of CMV can occur through the following routes: transplacental, breast milk, or via vaginal or cervical secretions during the intrapartum period [6]. Congenital infection via transplacental transmission is the greatest public health burden associated with CMV, occurring in approximately 0.7–1.0% of live births worldwide, although this figure can fluctuate based on racial, ethnic, and socioeconomic background [6–8]. Despite the fact that approximately 90% of congenitally infected infants are asymptomatic at birth, 15–20% of all infected infants experience permanent deficits later in life [6]. These deficits include sensorineural hearing loss, cognitive deficits, and other permanent neurologic sequelae, and CMV is the leading infectious cause of these deficits [2, 6, 7]. Sensorineural hearing loss, the most common deficit associated with congenital CMV infection, does not typically manifest until childhood, and thus is rarely detected during the course of routine newborn screening. In addition, few pregnant women in the United States are routinely screened for CMV, making it difficult to recognize when an infant is at risk for congenitally acquiring CMV. It is well established that CMV infects placental tissue and has recently been shown to be an etiologic factor in stillbirth [9]. Whether or not this placental infection is a trigger for preterm birth is a subject of much debate. There have been relatively few large population-based studies investigating the role congenital CMV infection has in preterm delivery, and the results of these studies have been mixed [10–14]. The primary purpose of this study was to determine the prevalence of congenital CMV infection in a population of preterm infants. If congenital CMV occurs at a significantly higher percentage in preterm infants, screening of all preterm infants may be warranted to identify those at risk for the neurologic sequelae associated with the virus. This is an important question to address, as it has recently been demonstrated that preterm infants infected with CMV have worse neurologic outcomes than preterm infants without CMV infection [10].

II. Methods

1. Study Population

Samples were collected from preterm infants admitted to the Neonatal Intensive Care Unit at the University of Iowa Hospitals and Clinics, University of Pittsburgh, University of Rochester, and Wake Forest University between the years 1995 and 2011. Cord or peripheral blood was obtained when possible, and saliva or buccal swabs were used if blood samples were not available. Samples were banked for use in studies investigating diseases of the newborn (IRB 199911068). Preterm birth was defined as delivery occurring prior to 37 weeks gestation, based on best obstetrical estimate (last menstrual period or ultrasound). DNA was extracted from these samples using standard DNA extraction protocols with Qiagen products. A total of 1200 unique DNA samples were tested for CMV nucleic acids.

2. Detection of CMV infection

Nested polymerase chain reactions (PCRs) were performed on an ABI GeneAmp9700 thermocycler using primers and conditions optimized from previous studies performing detection of CMV nucleic acids from human DNA [15]. Products were run on 2% agarose gels to determine which samples contained the viral CMV product indicating infection. A sample was required to test positive two out of three times to be considered CMV positive. A DNA sample from a symptomatic CMV positive infant, confirmed by urine analysis, was used as a positive control.

3. Statistical Analysis

Differences in CMV by sample type, age at sample collection, gender, and severity of prematurity (early vs. late preterm) were compared using Fisher's exact tests.

III. Results

Demographics of our cohort are presented in Table 1. Of the 1,200 premature infants tested, 37 tested positive for CMV (3.08%). Data regarding initial hearing screening was available for 30 of 37 infected infants, and of these three failed the initial hearing screening. There were no significant differences in CMV by gestational age or gender. Infants reported by their mothers as African-American or those infants missing ethnicity information were more likely to have a positive CMV test than Caucasians ($p<0.001$). Additionally, there was a significant ($p<0.001$) difference in CMV by sample type with all of the CMV positive samples coming from buccal swabs (Table 1).

Of the 1,200 premature infants 346 (28.8%) infants had samples collected two weeks or later after birth or had missing information on when the sample was collected. CMV was present in 2 of the 854 (0.2%) infants whose sample was collected within 2 weeks after birth. Excluding siblings (twins or other siblings), CMV was present in 2 of 589 (0.3%) infants whose sample was collected within 2 weeks after birth.

IV. Discussion

This is one of the largest studies to date investigating the prevalence of CMV infection in preterm infants in a primarily Caucasian population. Previous studies have established an association between infection and preterm birth, but the majority of evidence linking these two conditions exists for bacterial infections rather than viral [15]. The mechanism by which infection leads to preterm birth is through an inflammatory response at the placental-uterine interface resulting from the innate immune response [2, 3]. Since the pathogenesis of congenital CMV infection results in inflammation at this interface, it is biologically feasible to hypothesize that congenital CMV infection will be present more often in preterm infants [4, 5, 9]. However, attempts to answer this question have yielded mixed results, with a recent study finding that congenital CMV occurs in 0.39% of very low birth weight preterm infants, a lower proportion than what is observed in term infants [10].

We observed a complementary proportion of preterm infants, screened within two weeks after birth, with CMV (0.3%) as the previous study [10]. Our proportion of preterm infants with CMV was also lower than what has been reported for infants born in Iowa at term (35/7229=0.48%) [16]. While our study was not designed to directly compare CMV prevalence in preterm infants to those in term infants, our study does suggest a lower prevalence, similar to what has been shown previously in a different population [10]. One explanation for this unexpected finding is that the placental inflammation caused by congenital CMV infection may not trigger preterm labor, or it could be possible that early preterm labor might result in birth before transplacental transmission of CMV can occur. Additional large population based cohort studies are needed to further determine the incidence of congenital CMV in preterm infants compared to term infants.

The vast majority of infants infected with congenital CMV are asymptomatic at birth, yet as many as 20% will experience permanent neurologic deficits later in life [2, 6, 7]. Testing for CMV is not part of normal newborn screening, so those who are asymptomatic at birth are not recognized as being at-risk for future complications. In addition, it was recently shown that preterm infants with congenital CMV infection have far worse neurological outcomes than preterm infants without congenital CMV infection [10]. Recent studies have also shown that infants with CMV infection have improved hearing and neurologic outcomes when treated with anti-viral medication [17, 18]. Thus, one could argue that screening all infants that are born preterm for CMV infection would be appropriate since treatment exists that could alleviate some of the deleterious effects the virus has on neurologic function.

For screening to be effective, a CMV test would have to exist that is rapid, highly sensitive and specific, and cost-efficient. A recent study demonstrated that such a test exists for detecting CMV [19]. Ideally, congenital CMV should also be frequent in the preterm population for a screening program to be effective. While this is not supported by our study or others [10], further investigation into the efficacy of antiviral treatment in improving the neurologic outcomes of preterm infants born with CMV is needed.

Another finding from this study that deserves attention is the stark difference in CMV among buccal swab and blood samples. Buccal swabs accounted for 31 of 37 CMV positive

samples despite making up only 14.3% of all samples tested, while only three cord or peripheral blood samples tested positive despite making up 81.0% of all samples tested. A probable explanation for this finding is that buccal swabs may be a more sensitive sample to use for CMV detection than blood. Recent diagnostic studies found that CMV detection by PCR of dried blood spots had sensitivities between 28% and 34% compared to the gold standard of urine and saliva culture, whereas CMV detection by PCR of buccal swab samples had sensitivities between 97% and 100% [19, 22]. Taking into account the results of these diagnostic studies, our results could be indicative that blood is a less sensitive sample to use for detecting CMV.

While our study was not designed to determine the efficacy of a diagnostic test, the similarities are intriguing. One interesting avenue of future investigation would be to obtain both a blood sample and a buccal swab sample from both term and preterm infants and then test the DNA extracted from the samples for CMV. Not only would this investigation provide more information on the prevalence of congenital CMV in term and preterm infants as well as the sensitivity of each of these sample types for CMV detection, but it could also provide more information as to the pathogenesis of congenital CMV infection. If many individuals test positive for CMV from a buccal swab sample but not a blood sample, it could be indicative not only of sensitivity issues, but potentially that not all congenitally infected infants are viremic at birth. This would be consistent with reports that viral load in infected newborns is higher in saliva than blood [23].

V. Conclusion

We demonstrated that congenital CMV infection occurs in a population of preterm infants at a lower proportion than what has been observed among term infants from Iowa. Despite the fact that the number of CMV positive infants failing initial hearing screens was higher than the national average failure rate in all newborns [24], the majority of CMV positive infants passed initial hearing screens. When this finding is taken into account along with the fact that the majority of congenitally infected infants are asymptomatic at birth, it is clear that the risks posed by congenital CMV infection are often unknown to parents and clinicians. Our study suggests that further population-based prospective research of pregnant women and preterm infants is needed to determine the incidence of CMV in preterm infants to determine if screening this population for CMV would be worthwhile. Despite the low prevalence reported in this study, discussion of screening is still warranted since neurologic outcomes in CMV positive preterm infants are far worse than in CMV negative preterm infants, and anti-viral therapy may improve these outcomes. Buccal swabs may prove to be the ideal sample type for this type of program given our results and recent diagnostic studies that found this sample type to be highly sensitive to CMV detection.

Acknowledgments

The authors would like to acknowledge the research staff at the participating universities for consenting patients, data collection, and coordination efforts, the research staff in Dr. Murray’s laboratory for help with experiments, and all the patients and families that participated in the study.

This research was supported, in part, by funding from the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health (HD057192, HD052953, HD065786), a CTSA

award (UL1 TR000442) and the March of Dimes (FY11-261 and FY13-19). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008; 371:75–84. [PubMed: 18177778]
2. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassam S. The role of inflammation and infection in preterm birth. Semin Reprod Med. 2007; 25(1):21–39. [PubMed: 17205421]
3. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med. 2000; 342:1500–1507. [PubMed: 10816189]
4. Pereira L, Maidji E, McDonagh S, Tabata T. Insights into viral transmission at the uterine-placental interface. Trends Microbiol. 2005; 13(4):164–174. [PubMed: 15817386]
5. McDonagh S, Maidji E, Chang H, Pereira L. Patterns of human cytomegalovirus Infection in term placentas: a preliminary analysis. J Clin Virol. 2006; 35:210–215. [PubMed: 16386950]
6. Ross S, Boppana S. Congenital cytomegalovirus infection: outcome and diagnosis. Semin Pediatr Infect Dis. 2005; 16:44–49. [PubMed: 15685149]
7. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol. 2007; 17:355–363. [PubMed: 17542052]
8. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol. 2007; 17:253–276. [PubMed: 17579921]
9. Iwasenko J, Howard J, Arbuckle S, et al. Human cytomegalovirus infection is detected frequently in stillbirths and is associated with fetal thrombotic vasculopathy. J Infect Dis. 2011; 203:1526–1533.10. [PubMed: 21592980]
10. Turner KM, Lee HC, Boppana SB, Carlo WA, Randolph DA. Incidence and impact of CMV infection in very low birth weight infants. Pediatrics. 2014; 133:609–615.
11. Lorenzoni F, Lunardi S, Liumbruno A, et al. Neonatal screening for congenital cytomegalovirus infection in preterm and small for gestational age infants. J Matern Fetal Neonatal Med. Epub 2014.
12. DePasquale J, Freeman K, Amin MM, et al. Efficient linking of birth certificate and newborn screening databases for laboratory investigation of congenital cytomegalovirus infection and preterm birth: Florida, 2008. Matern Child Health J. 2008; 16:486–494. [PubMed: 21203810]
13. Gibson C, Goldwater P, MacLennan A, Haan E, Priest K, Dekker G. for the South Australian Cerebral Palsy Research Group. Fetal exposure to herpesviruses may be associated with pregnancy-induced hypertensive disorders and preterm birth in a Caucasian population. BJOG. 2008; 115:492–500. [PubMed: 18271886]
14. Yamamoto AY, Mussi-Pinhata MM, Cristina P, Pinto G, Moraes Figueiredo LT, Jorge SM. Congenital cytomegalovirus infection in preterm and full-term newborn infants from a population with a high seroprevalence rate. Pediatr Infect Dis J. 2001; 20:188–192. [PubMed: 11224840]
15. Hamprecht K, Steinmabl M, Einsele H, Jahn G. Discordant detection of human cytomegalovirus DNA from peripheral blood mononuclear cells, granulocytes and plasma: correlations to viremia and HCMV infection. J Clin Virol. 1998; 11:125–136. [PubMed: 9785214]
16. Murph JR, Souza IE, Dawson JD, et al. Epidemiology of congenital cytomegalovirus infection: maternal risk factors and molecular analysis of cytomegalovirus strains. Am J Epidemiol. 1998; 147:940–947. [PubMed: 9596472]
17. Kimberlin DW, Lin CY, Sanchez PJ, et al. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: a randomized, controlled trial. J Pediatr. 2003; 143:16–25. [PubMed: 12915819]
18. Oliver SE, Cloud GA, Sanchez PJ, et al. Neurodevelopmental outcomes following ganciclovir therapy in symptomatic congenital cytomegalovirus infections involving the central nervous system. J Clin Virol. 2009; 46:22–26.

19. Boppana SB, Ross SA, Shimamura M, et al. Saliva polymerase chain reaction assay for cytomegalovirus screening in newborns. N Engl J Med. 2011; 364:2111–2118. [PubMed: 21631323]

20. Yilmaz CD, Vardar F. Effect on hearing of oral valganciclovir for asymptomatic congenital cytomegalovirus infection. J Trop Pediatr. 2011; 57:132–134. [PubMed: 20576693]

21. Lackner A, Acham A, Alborno T, et al. Effect on hearing of ganciclovir therapy for asymptomatic congential cytomegalovirus infection: four to 10 year follow up. J Laryngol Otol. 2009; 123:391–396. [PubMed: 18588736]

22. Boppana SB, Ross SA, Novak Z, et al. Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection. JAMA. 2010; 303:1375–1382. [PubMed: 20388893]

23. Boppana SB, Fowler KB, Pass RF, et al. Congenital cytomegalovirus infection: association between virus burden in infancy and hearing loss. J Pediatr. 2005; 146:817–823. [PubMed: 15973325]

24. U.S. Centers for Disease Control, E.H.D.I. Summary of 2009 National CDC EDHI Data. 2011. Retrieved from http://www.cdc.gov/ncbddd/hearingloss/2009-Data/2009_EHDI_HSFS_Summary_508_OK.pdf

Table 1

Demographic Characteristics of the Full Study Cohort, N=1200 infants

Characteristic	CMV+ N=37	CMV− N=1163	P-value
Family Relationship			<0.001
Multiple Birth	0 (0.0%)	311 (26.7%)	
Siblings	9 (24.3%)	119 (10.2%)	
Singleton	28 (75.7%)	733 (63.0%)	
Age at Sample Collection			<0.001
≤2 weeks	2 (5.4%)	852 (73.3%)	
2–4 weeks	1 (2.7%)	10 (0.9%)	
>4 weeks	29 (78.4%)	138 (11.9%)	
Unknown	5 (13.5%)	163 (14.0%)	
Sample Type			<0.001
Blood (Cord or Peripheral)	3 (8.1%)	969 (83.3%)	
Buccal swab	31 (83.8%)	141 (12.1%)	
Saliva	3 (8.1%)	50 (4.3%)	
Unknown	0 (0.0%)	3 (0.3%)	
Gender (Male)	21 (56.8%)	648 (55.7%)	1.00
Race			<0.001
Caucasian	26 (70.3%)	1,055 (90.7%)	
African American	4 (10.8%)	65 (5.6%)	
Other/Unknown	7 (18.9%)	43 (3.7%)	
Gestational age (weeks)			0.84
22–31 (Very preterm)	17 (46.0%)	573 (49.3%)	
32–34 (Moderate preterm)	13 (35.1%)	353 (30.4%)	
35–36 (Late preterm)	7 (18.9%)	237 (20.4%)	