HHS Public Access

Author manuscript

J Pediatric Infect Dis Soc. Author manuscript; available in PMC 2018 August 24.

Published in final edited form as:

J Pediatric Infect Dis Soc. 2018 August 17; 7(3): e160–e162. doi:10.1093/jpids/piy048.

Viral Loads in Congenital Cytomegalovirus Infection from a Highly Immune Population

Aiqiang Xu[1,2], **Shiwen Wang**[3], **Wenqiang Zhang**[1,2], **Xiaofang Wang**[3], **Tongzhan Wang**[1,2], **Xialin Liu**[1,2], **Haiyan Wang**[1,2], **Wei Ma**[4], **Minal Amin**[5], **Sheila Dollard**[5], and **Chengbin Wang**[5]

[1]Shandong Provincial Key Laboratory for Infectious Disease Control and Prevention, Shandong Provincial Center for Disease Control and Prevention, Jinan, Shandong, China

[2]Academy of Preventive Medicine, Shandong University, Jinan, Shandong, China

[3]National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China

[4]School of Public Health, Shandong University, Jinan, Shandong, China

[5]U.S. Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Abstract

Among newborns with congenital cytomegalovirus infection from China, there was no difference in CMV viral load in saliva specimens dried and stored at room temperature compared to those kept wet and stored cold, even after longer storage time for the former than the later (74 vs. 58 days, P=0.02).

Keywords

Cytomegalovirus; Viral load; Congenital Infection

Congenital cytomegalovirus (CMV) infection is an important cause of sensorineural hearing loss and cognitive impairment worldwide. Following primary CMV infection or reinfection of seropositive individuals by another strain of CMV, the virus establishes latency and can then undergo intermittent reactivation[1]. During pregnancy, primary or non-primary maternal infection (reinfection and reactivation) can lead to vertical transmission to the fetus, resulting in congenital CMV infection.

Polymerase chain reaction (PCR) testing of infant saliva, urine or blood within 3 weeks of birth is the standard method for identifying congenital CMV. Saliva specimens have been shown to have higher sensitivity for detecting congenital CMV infection than dried blood

Correspondence: Chengbin Wang, MD, PhD, Centers for Disease Control and Prevention, 1600 Clifton Road MS A-34, Atlanta, GA 30333 (cwang1@cdc.gov).
Drs. Aiqiang Xu, Shiwen Wang, and Wenqiang Zhang contributed equally to this manuscript.

Financial Disclosure: The authors have no conflicts of interest to disclose

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

spot (DBS) specimens in developed and developing countries [2, 3]. In the current study, we compared CMV viral loads in two different types of saliva specimens (wet vs. dried), in saliva compared to blood specimens from infants of population with high maternal seroprevalence in China.

METHODS

With approval from China Center for Disease Control and Prevention Ethics Committee, newborn screening for congenital CMV infection was conducted in five birthing hospitals in Shandong Province, China, from March 2011 through September 2013 [2]. All parents were approached to participate and approximately 98% of them enrolled. Specimen collection, storage, and PCR testing has been previously described [2]. Wet saliva specimens (maintained as wet and cold-stored and transported) were collected for the full study period of 2.5 years, while DBS specimens (dried overnight at ambient temperature, cold-stored and -transported) were collected concurrently for the first 12 months of the study only. Concurrent dried saliva specimens (air-dried and stored and transported at ambient temperature) were added during the last 2 years of the study only. Different specimens were collected on the same day from an infant if enrolled. DNA was eluted from swabs with Extracta (Quanta, United States) and extracted from a 6 mm-diameter DBS punches using thermal shock [4, 5]. Detection of CMV DNA was performed with real-time PCR with TaqMan-based primers and probes targeting the glycoprotein B gene on Mx3000P qPCR Systems [4, 5]. The commercially standardized human CMV DNA quantitated with digital PCR by Advanced Biotechnologies, Inc. (Eldersburg, MD, USA) was included on every PCR plate to quantify the viral loads.

Viral load data were $\log_{10}$ transformed for statistical analyses. The viral loads among the three types of specimens (wet saliva, dried saliva, DBS) were compared with each other with unpaired method. The Pearson correlation coefficient (r) was used to examine the correlations of viral loads between wet and dried saliva specimens and between wet saliva and blood specimens from the same infants. Paired comparison of viral loads between dried saliva and DBS was not performed because there were too few infants with both specimens collected. Maternal and newborn factors assessed in a prior report on congenital CMV infection [2] were further evaluated on their association with viral loads in different specimens using Student's *t*-test or analysis of variance wherever appropriate. All analyses were performed with SAS V9.4 (SAS Institute, Cary, NC); statistical significance was defined as $P < 0.05$.

RESULTS

Among 75 newborns identified with congenital CMV infection from 10,933 newborns screened [2], mean viral loads in wet saliva (n = 66) and dried saliva (n = 52) specimens were 6.1 and 6.4 $\log_{10}$ copies/mL, respectively. There was no difference between wet and dried saliva specimens ($P = 0.39$) (Figure 1, Panel A), even the storage time before testing was longer for dried saliva specimens than for wet saliva specimens (74 vs. 58 days, P=0.02). Viral loads in wet and dried saliva specimens from the 46 newborns who were CMV positive in both types of saliva specimens were similar (mean: 6.5 $\log_{10}$ copies/mL for

both, P = 0.75) and highly correlated (r = 0.94, $P < 0.001$) (Figure 1, Panel B). The mean viral loads from DBS (n = 14) was 3.4 $\log_{10}$ copies/mL, significantly lower than that of wet and dried saliva specimens ($P < 0.001$ for both).

Among 3,953 newborns with both wet saliva and DBS available for CMV testing, 28 were CMV positive in wet saliva specimens, of which 11 were CMV positive in DBS. Viral loads in paired saliva and blood specimens from the same child did not correlate (r = −0.17, $P = 0.62$). In addition, we compared CMV viral loads in saliva specimens from children who were CMV-positive in blood (n=11) to children CMV-negative in blood (n=17), and found there were no significant difference (mean: 5.9 and 5.6 $\log_{10}$ copies/mL, respectively, $P = 0.64$). Average viral loads in saliva or DBS specimens were not associated with any maternal or newborn characteristics such as maternal age, birth to a mother who had a previous live birth, birth to a mother residing with children or not, sex, preterm birth, or type of delivery ($P > 0.05$ for all). As no symptomatic congenital CMV infection was identified in current study [2], the association of viral loads with symptomatic congenital CMV infection was not examined.

DISCUSSION

In a Chinese population with very high CMV seroprevalence (> 96%) [2], we found a high correlation of viral loads in wet and dried saliva specimens, indicating little if any DNA decay in the dried saliva specimens during the process of air drying, storage, and transportation of the saliva specimens at ambient temperature, even months after collection. As storage and transportation of dried saliva specimens is significantly easier than wet saliva specimens, along with reported high sensitivity [2, 6], dried saliva may be more convenient for potential newborn CMV screening. In addition, we verified substantially higher viral loads in saliva specimens than in DBS (approximately 3 logs), similar to other populations with moderate and high seroprevalence [1], which is likely the reason for the higher sensitivity of saliva specimens relative to blood specimens in detecting congenital CMV infection [2, 3].

There are a few possible explanations for the lack of correlation between viral loads in saliva and blood specimens in this study. First may be a true lack of correlation in CMV from saliva and blood, as indicated by the compartmentalized viral dynamics during and after treatment in infants with congenital CMV infection [7]. Second, the small number of infants with positive blood specimens and very low viral loads with little variations may make current study not powerful enough to detect an association if there is one. Future study with sufficient sample size in developed countries with lower seroprevalence may provide further information on whether there is a correlation between viral loads from blood and saliva, and the findings there will be of higher clinical significance for that population, just as the good performance of DBS in detecting congenital CMV infection with high risk of sequelae reported in France and Italy [8, 9]

In summary, we found similar viral loads in wet and dried saliva specimens, indicating little DNA decay in the preparation and transportation at ambient temperature for dried saliva specimens. We also verified that viral loads of congenital CMV infection were lower in

blood specimens than in saliva specimens in a highly immune Chinese population, similar to findings from lower seroprevalence populations. Our finding that dried saliva is a reliable type of specimen for identifying congenital CMV infection has important public health implications in that dried saliva is much easier and more economical to store and transport than wet saliva.

Acknowledgments

Funding: This work was supported by a cooperative agreement between the U.S. Centers for Disease Control and Prevention and the China Center for Disease Control and Prevention, and was part of the Taishan Scholar Program at the Shandong Provincial Center for Disease Control and Prevention.

We highly appreciate the help from the medical staff of the birthing hospitals in this study and the efforts of the local CDC staff in coordination, specimen storage and transportation, and performing data quality checks. We are also indebted to the parents for their involvement and willingness to have their children participate in this project, and to Ms. Mary Ann K. Hall for her editorial assistance.

References

1. Cannon MJ, Hyde TB, Schmid DS. Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. Rev Med Virol. 2011; 21(4):240–55. [PubMed: 21674676]
2. Wang S, Wang T, Zhang W, et al. Cohort study on maternal cytomegalovirus seroprevalence and prevalence and clinical manifestations of congenital infection in China. Medicine (Baltimore). 2017; 96(5):e6007. [PubMed: 28151899]
3. 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(14):1375–82. [PubMed: 20388893]
4. Koontz D, Baecher K, Amin M, Nikolova S, Gallagher M, Dollard S. Evaluation of DNA extraction methods for the detection of Cytomegalovirus in dried blood spots. J Clin Virol. 2015; 66:95–9. [PubMed: 25866346]
5. Kharrazi M, Hyde T, Young S, Amin MM, Cannon MJ, Dollard SC. Use of screening dried blood spots for estimation of prevalence, risk factors, and birth outcomes of congenital cytomegalovirus infection. J Pediatr. 2010; 157(2):191–7. [PubMed: 20400091]
6. Boppana SB, Ross SA, Shimamura M, et al. Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns. N Engl J Med. 2011; 364(22):2111–8. [PubMed: 21631323]
7. Luck SE, Emery VC, Atkinson C, Sharland M, Griffiths PD. Compartmentalized dynamics of cytomegalovirus replication in treated congenital infection. J Clin Virol. 2016; 82:152–8. [PubMed: 27500364]
8. Leruez-Ville M, Vauloup-Fellous C, Couderc S, et al. Prospective identification of congenital cytomegalovirus infection in newborns using real-time polymerase chain reaction assays in dried blood spots. Clin Infect Dis. 2011; 52(5):575–81. [PubMed: 21292661]
9. Forner G, Abate D, Mengoli C, Palu G, Gussetti N. High Cytomegalovirus (CMV) DNAemia Predicts CMV Sequelae in Asymptomatic Congenitally Infected Newborns Born to Women With Primary Infection During Pregnancy. J Infect Dis. 2015; 212(1):67–71. [PubMed: 25387583]

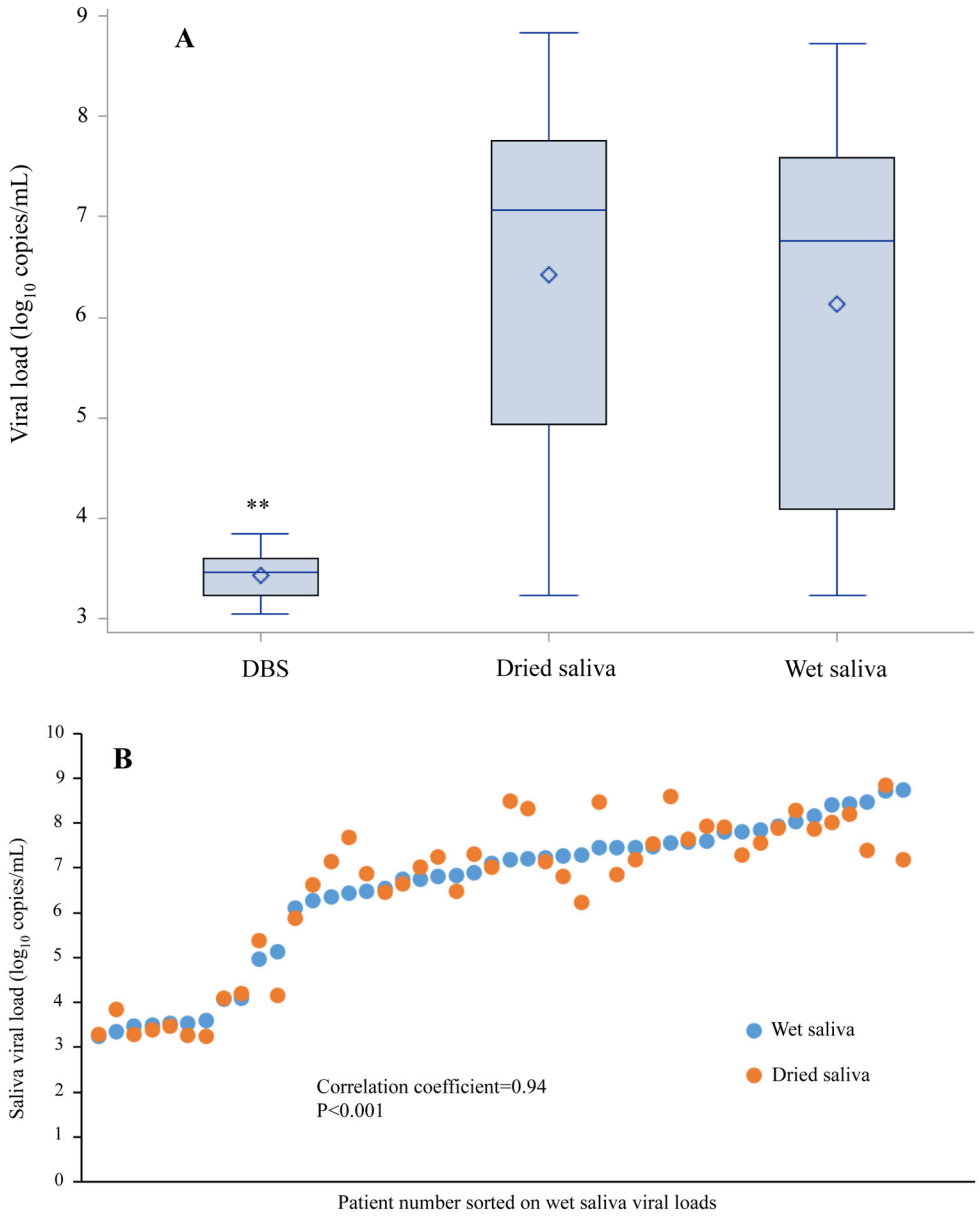

Figure 1.

Distribution and correlation of the CMV viral loads in wet and dried saliva and dried blood spot specimens (DBS) from newborns identified with congenital CMV infection in China.

Panel A. Viral loads in wet and dried saliva and dried DBS (** $P < 0.001$ compared with saliva specimens). The box indicates the interquartile range, the line inside box indicates the median, the diamond sign indicates mean, and whiskers above and below the box show maximum and minimum values.

Panel B. Correlation between viral loads in wet and dried saliva specimens among 46 newborns who were CMV positive in both specimens (each dot represents results for one sample).