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Diagnostic Assays for Active Infection with Human Herpesvirus 6 (HHV-6)

Mary T. Caserta, M.D.¹, Caroline Breese Hall, M.D.^{1,2}, Kenneth Schnabel, M.B.A.¹, Geraldine Lofthus, Ph.D.², Andrea Marino, M.S.¹, Lynne Shelley, B.S.¹, Christina Yoo, B.S.¹, Jennifer Carnahan, B.S.¹, Linda Anderson, L.P.N.¹, and Hongyue Wang, Ph.D.³

¹Department of Pediatrics, at the University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

²Department of Medicine, at the University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

³Department of Biostatistics and Computational Biology at the University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

Abstract

Background—Human herpesvirus 6 (HHV-6) causes ubiquitous infection in early childhood with lifelong latency or persistence. Reactivation of HHV-6 has been associated with multiple diseases including encephalitis. Chromosomal integration of HHV-6 also occurs. Previous studies have suggested that the detection of HHV-6 DNA in plasma is an accurate marker of active viral replication.

Objective—We sought to determine whether PCR assays on plasma could correctly differentiate between primary HHV-6 infection, chromosomal integration of HHV-6 and latent HHV-6 infection.

Study Design—We performed qualitative PCR, real-time quantitative PCR (RQ-PCR), and reverse-transcriptase PCR (RT-PCR) assays on samples of peripheral and cord blood mononuclear cells, as well as plasma, from groups of subjects with well defined HHV-6 infection, including subjects with chromosomally integrated HHV-6.

Results and Conclusions—The detection of HHV-6 DNA in plasma was 92% sensitive compared to viral isolation for the identification of primary infection with HHV-6. All plasma samples from infants with chromosomally integrated HHV-6 had HHV-6 DNA detectable in plasma while only 5.6% were positive by RT-PCR. The specificity of plasma PCR for active replication of HHV-6 was 84% compared to viral culture while the specificity of RT-PCR was 98%. Our results demonstrate that qualitative or quantitative PCR of plasma is insufficient to distinguish between active viral replication and chromosomal integration with HHV-6. We found

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Corresponding Author: Mary T. Caserta, M.D., Associate Professor of Pediatrics, 601 Elmwood Ave., Box 690, Rochester, NY 14642, Tel: (585) 275-5944; Fax: (585) 273-1104, mary_caserta@urmc.rochester.edu.

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a higher specificity of RT-PCR performed on PBMC samples compared to PCR or RQ-PCR performed on plasma when evaluating samples for active HHV-6 replication.

Keywords

Human herpesvirus 6; chromosomally integrated HHV-6 (CI-HHV-6); polymerase chain reaction; real time quantitative polymerase chain reaction (RQ-PCR); reverse transcriptase polymerase chain reaction (RT-PCR)

HHV-6 infection causes an undifferentiated febrile illness, sometimes manifest as roseola (exanthem subitum).¹ HHV-6 is lymphotropic and unique among herpesviruses as it is chromosomally integrated in 0.2 to 0.8% of the population.^{2 3} Primary infection with HHV-6 is followed by latency or persistence at multiple sites including peripheral blood mononuclear cells (PBMCs) with reactivation associated with several diseases, especially among immunocompromised hosts.⁴

These characteristics of persistence and latency often confound the determination of HHV-6's role in disease, thus necessitating the development of assays which can distinguish active from latent infection. Detection of HHV-6 DNA in plasma has been proposed as a definitive marker of active viral replication similar to viral isolation in cell culture.^{5 6} However, since recognition of the chromosomal integration of HHV-6, the reliability of plasma assays to distinguish between active HHV-6 infection and integration or latency has not been re-evaluated. This has important clinical relevance when evaluating the results of diagnostic testing on patient samples.

OBJECTIVE

We have studied primary and reactivated HHV-6 infection in large groups of subjects using viral isolation and molecular assays.^{1 2 7-10} In this study, we examined specimens from groups of children with various types of well documented HHV-6 infections in order to evaluate the ability of different assays to identify actively replicating HHV-6 compared to viral isolation.

STUDY DESIGN

Blood samples were obtained from children with: 1) primary HHV-6 infection, defined by viral isolation from culture, HHV-6 DNA in PBMCs detected by PCR, and negative serology, 2) past HHV-6 infection, determined by a negative culture, positive HHV-6 DNA in PBMCs, and positive serology, 3) no prior HHV-6 infection, with negative serology, negative PCR, and negative culture, and 4) congenital HHV-6 infection, including those with chromosomal integration, (CI-HHV-6) defined by the presence of HHV-6 DNA in the cord blood. The University of Rochester RSRB approved all studies.

Cell pellets and plasma samples were used for qualitative nested DNA PCR⁹ and RQ-PCR of the U38 gene of HHV-6.⁷ DNA was purified from either 100µl or 200µl of plasma using the QIAamp® DNA Blood Mini Kit (Qiagen Inc., Valencia, CA). Two microliters were used for the qualitative nested HHV-6 DNA PCR, and 1ul was used for the RQ-PCR with results expressed as genome equivalent copies per microgram of DNA, (gec/µg DNA). RT-PCR assays were performed on cell pellets. HHV-6 antibody titers were determined by indirect fluorescent antibody assay.¹ Negative titers were defined as $\leq 3.32 \log_2$.

The RT-PCR assay amplifies the late structural gp82–105 mRNA of HHV-6 and detects <10 mRNA copies.^{2 11} Because this assay was introduced after our original studies of primary HHV-6 infection had begun not all samples were tested by RT-PCR. We defined active viral

replication as HHV-6 infection that resulted in the production of viral particles proven via viral isolation in cell culture.

Student's t-test was used to compare HHV-6 viral loads after log transformation and a p value of <0.05 was accepted as significant.

RESULTS

Of the 51 plasma and PBMC samples from children with primary HHV-6 infection examined, 92% had HHV-6 DNA detected in plasma (Table 1). Of the 20 PBMC samples appropriately stored for RT-PCR, 90% were positive, consistent with active replication of HHV-6.

HHV-6 DNA was not identified in any of the plasma samples from children with past HHV-6 infection, and all the RT-PCR assays were negative. Of 20 samples from children without evidence of HHV-6 infection, one plasma sample was positive for HHV-6 DNA by PCR while all of the RT-PCR assays were negative, (Table 1).

Plasma samples from newborns with CI-HHV-6 all contained HHV-6 DNA. Two of these samples were negative by both culture and RT-PCR. Only two of the 36 samples (5.6%) were positive by RT-PCR. These two were not tested in cell culture. Comparison of viral loads of HHV-6 in plasma samples showed that newborns with CI-HHV-6 had significantly greater viral loads than children with primary infection ($5.6 \pm 0.3 \log_{10}$ gec/ μ g DNA compared to $5.3 \pm 0.4 \log_{10}$ gec/ μ g DNA, respectively, $p < 0.01$), although the range of values between the two groups overlapped, (Figure 1). Sixteen blood samples from children with CI-HHV-6 were obtained at a mean of 12.3 months of age (range 1–30.3 months). Six were cultured and all were negative. All 16 samples had HHV-6 DNA detected in plasma with a geometric mean viral load of $4.5 \pm 0.3 \log_{10}$ gec/ μ g DNA. One culture negative sample was positive by RT-PCR. Ten children with CI-HHV-6 and peripheral blood samples also had cord blood samples available for comparison. In 70% the viral load in the plasma dropped at least 1 log between the cord blood specimen and the specimen obtained during the first two years of life.

Based upon the above results, the specificity of the plasma PCR compared to viral culture for the detection of active replication of HHV-6 was 84%. The RT-PCR assay was 98% specific for HHV-6 active infection compared to culture.

DISCUSSION

We performed molecular assays on well characterized samples from children with different types of infection with HHV-6 and found that a combination of assays was most instructive when attempting to identify actively replicating virus. Although prior studies have proposed that the presence of HHV-6 DNA in serum or plasma alone was a definitive marker of active viral replication, our results do not support that conclusion.^{5 6} Indeed, we found that all plasma samples from children with CI-HHV-6 had detectable DNA in high copy number. None of the 21% of cord blood samples cultured from children with CI-HHV-6 grew HHV-6, suggesting most infants with CI-HHV-6 do not have replicating virus detectable in culture. Although the production of late phase structural mRNA correlates well with isolation of HHV-6 in cell culture during primary infection, it is unclear if the presence of this same mRNA is a marker of infectious virion production in samples from individuals with CI-HHV-6. Our data support the findings of Achour and colleagues,¹² who reported that the HHV-6 DNA present in plasma was derived from the lysis of peripheral blood mononuclear cells and not necessarily reflective of active viral replication. Additionally, we detected HHV-6 DNA in the plasma of a child with no evidence of previous infection with

HHV-6 consistent with a false positive result, further supporting the assertion that a single assay is insufficient to characterize accurately the type of HHV-6 infection in individual patients.

The HHV-6 viral loads present in the plasma of our infants with CI-HHV-6 were significantly higher than the plasma viral loads of children with primary HHV-6 infection. However, substantial overlap existed between the two groups. Although sensitive and reliable quantitative molecular based assays have been developed for the determination of HHV-6 viral load,¹³ our data demonstrate that viral load determination alone may not differentiate the type HHV-6 infection present in an individual patient. Additionally, the drop of ≥ 1 log in viral load observed in 70% of plasma samples between the cord blood and subsequent plasma samples suggests that neither the height of the viral load nor changes in viral load between samples are necessarily reflective of viral activity. We have previously compared our RT-PCR assay for gp82–102 mRNA with culture and found it to be 95% sensitive and 98.8% specific, similar to the sensitivity and specificity determined in this study.¹¹ Based upon these results this test could potentially be very useful in distinguishing between chromosomal integration of HHV-6 and active viral replication.

In summary, our results demonstrate that qualitative or quantitative PCR of plasma is insufficient to distinguish between active viral replication and chromosomal integration with HHV-6.

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REFERENCES

1. Hall CB, Long CE, Schnabel KC, Caserta MT, McIntyre KM, Costanzo MA, et al. Human herpesvirus-6 infection in children. A prospective study of complications and reactivation. *N Engl J Med* 1994;331(7):432–438. [PubMed: 8035839]
2. Hall CB, Caserta MT, Schnabel K, Shelley LM, Marino AS, Carnahan JA, et al. Chromosomal integration of human herpesvirus 6 is the major mode of congenital human herpesvirus 6 infection. *Pediatrics* 2008;122(3):513–520. [PubMed: 18762520]
3. Leong HN, Tuke PW, Tedder RS, Khanom AB, Eglin RP, Atkinson CE, et al. The prevalence of chromosomally integrated human herpesvirus 6 genomes in the blood of UK blood donors. *J Med Virol* 2007;79(1):45–51. [PubMed: 17133548]
4. De Bolle L, Naesens L, De Clerc E. Update on human herpesvirus 6 biology, clinical features, and therapy. *Clin Microbiol Rev* 2005;18(1):217–245. [PubMed: 15653828]
5. Huang LM, Kuo PF, Lee CY, Chen JY, Liu MY, Yang CS. Detection of human herpesvirus-6 DNA by polymerase chain reaction in serum or plasma. *J Med Virol* 1992;38(1):7–10. [PubMed: 1328512]
6. Secchiero P, Carrigan DR, Asano Y, Benedetti L, Crowley RW, Komaroff AL, et al. Detection of human herpesvirus 6 in plasma of children with primary infection and immunosuppressed patients by polymerase chain reaction. *J Infect Dis* 1995;171(2):273–280. [PubMed: 7844362]
7. Caserta MT, Hall CB, Schnabel K, Lofthus G, McDermott MP. Human herpesvirus (HHV)-6 and HHV-7 infections in pregnant women. *J Infect Dis* 2007;196(9):1296–1303. [PubMed: 17922393]
8. Caserta MT, McDermott MP, Dewhurst S, Schnabel K, Carnahan JA, Gilbert L, et al. Human herpesvirus 6 (HHV6) DNA persistence and reactivation in healthy children. *J Pediatr* 2004;145(4):478–484. [PubMed: 15480370]

9. Hall CB, Caserta MT, Schnabel KC, Boettlich C, McDermott MP, Lofthus GK, et al. Congenital infections with human herpesvirus 6 (HHV6) and human herpesvirus 7 (HHV7). *J Pediatr* 2004;145(4):472–477. [PubMed: 15480369]
10. Hall CB, Caserta MT, Schnabel KC, Long C, Epstein LG, Insel RA, et al. Persistence of human herpesvirus 6 according to site and variant: possible greater neurotropism of variant A. *Clin Infect Dis* 1998;26(1):132–137. [PubMed: 9455521]
11. Norton RA, Caserta MT, Hall CB, Schnabel K, Hocknell P, Dewhurst S. Detection of human herpesvirus 6 by reverse transcription-PCR. *J Clin Microbiol* 1999;37(11):3672–3675. [PubMed: 10523572]
12. Achour A, Boutolleau D, Slim A, Agut H, Gautheret-Dejean A. Human herpesvirus-6 (HHV-6) DNA in plasma reflects the presence of infected blood cells rather than circulating viral particles. *J Clin Virol* 2007;38(4):280–285. [PubMed: 17339132]
13. Flamand L, Gravel A, Boutolleau D, Alvarez-Lafuente R, Jacobson S, Malnati MS, et al. Multicenter comparison of PCR assays for detection of human herpesvirus 6 DNA in serum. *J Clin Microbiol* 2008;46(8):2700–2706. [PubMed: 18550745]

Figure 1.
HHV-6 Viral Load (RQ-PCR) in Plasma from Children with Primary Infection and CI-HHV-6.

Results of HHV-6 Testing on Patient Samples

Table 1

N	Mean Age mos. (range)	PBMC Viral load (gec/μg DNA) [§]	HHV-6 titer (log ₂)	% PCR pos.	Plasma viral load (gec/μg DNA) [§]	% RT-PCR pos.
<i>Primary Infection (tissue culture positive, serology negative)</i>						
51	13.4 (2-51)	4.5±0.6 [¶]	2.34	92	5.3±0.4	90 [*]
<i>Past Infection (tissue culture negative, serology positive, HHV6 DNA present in PBMC)</i>						
30	38 (5-110)	2.9±0.4	9.19	0	---	0
<i>No Infection (tissue culture negative, serology negative, no HHV6 DNA in PBMC)</i>						
20	12.1 (4-25)	---	2.57	5	4.5	0
<i>Congenital Infection (HHV6 DNA present in cord blood mononuclear cells) CI-HHV-6</i>						
36	0	6.1±0.3 [^]	8.18	100	5.6±0.3	5.6
<i>CI-HHV-6, Samples obtained in infancy</i>						
6	5 (1-14)	6.5±0.2	5.99	100	4.5±0.4	16.7

[¶] 44 samples appropriately saved and tested

^{*} 20 samples appropriately saved and tested

[^] Cord blood mononuclear cells were tested

[§] mean±std deviation in genome equivalent copies/μg of DNA in log₁₀ scale