

Human Herpesvirus Infection in Children with Fever and Maculopapular Rash

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Fever with maculopapular rash without a localized sign is a common problem in children. It may be due to viral infection, bacterial infection, or drug allergy.^{1,2} Viral infections commonly causing maculopapular rash are attributed to cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus (HHV)-6, HHV-7, enterovirus and parvovirus B-19.¹⁻⁶

Human herpesviruses (HHVs) can cause primary infection, which may proceed towards latency.^{7,8} They are responsible for acute febrile illnesses in children. Viral reactivation in immunocompromised patients has been linked to a number of diseases and causes significant morbidity and mortality, especially in transplant recipients and HIV patients.^{9,11}

Serologic evidence, such as a four-fold or greater increase of the IgG titer, does not differentiate between primary or reactivated latent infection.¹²⁻¹⁴ The detection of HHV6 and HHV7 in peripheral blood leucocytes (PBL) only is of

SUMMARY Fever with maculopapular rash is a common problem in children. Infection with human herpesviruses is one of the common etiologies in fever with rash. The aim of this study has been to examine patients presenting with fever and maculopapular rash without respiratory symptoms for human herpesviruses infection by using multiplex nested-polymerase chain reaction. A descriptive and prospective study was conducted at King Chulalongkorn Memorial Hospital, Bangkok, Thailand from June 2000 to December 2001. One hundred patients, 43 boys and 57 girls, aged between 2 months and 14 years were recruited. Human herpesvirus 6 (HHV6) was the most common (24%) whereas HHV7, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) were present in 9%, 3% and 2% of the patients, respectively. Four percent of the patients simultaneously harbored HHV6 and HHV7. Only one patient had CMV, HHV6 and HHV7. Patients with HHV7 had a mean age of 4.5 years, whereas those with HHV6 had a mean age of 1.6 years. HHV6 and HHV7 were commonly found as causes of fever and maculopapular rash without respiratory symptoms. Co-infection with different herpesviruses can be found in the same patient.

limited significance since viral DNA persists in the PBL of healthy persons. Viral DNA detection in a cell free body fluid such as plasma has been shown to correlate with active viral replication.^{15,16} Plasma polymerase chain reaction has demonstrated diagnostic accuracy in detecting primary HHV6 infection in immunocompetent children with a sensitivity and specificity amounting to 90% and 100%, respectively.^{12,17} Polymerase chain reaction (PCR) has become one of the most widely used techniques in human virology diagnostic. Multiplex nested-PCR

represents a modification of the original PCR protocol applied to simultaneously amplify DNA originating from different pathogens using several primer pairs in the same reaction.¹⁸ Based on a technique described previously,¹⁸ we applied a sensitive multiplex nested-PCR to determine the prevalence of human lymphotropic herpesviruses in children with fever and maculopapular rash.

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MATERIALS AND METHODS

The study protocol was approved by the ethics committee, Faculty of Medicine, Chulalongkorn University. Prior to enrollment, we informed the subjects' parents about the purpose of the study and obtained their written consents.

Study population

We recruited 100 patients presenting with fever and maculopapular rash without respiratory tract infection, who attended the Out-Patient Clinic, Department of Pediatrics, King Chulalongkorn Memorial Hospital, from June 2000 to December 2001. Blood samples were taken for complete blood count; sera were separated and kept at -70°C until subjected to multiplex nested-PCR for HHV.

Laboratory diagnosis

HHV DNA was extracted from 50- μl serum specimens applying the proteinase K-phenol-chloroform extraction procedure.¹⁹ After removing the denatured protein, the DNA was precipitated in 0.3 M sodium acetate, 60 μg glycogen and absolute ethanol at -20°C for an hour. DNA was precipitated by centrifugation at $12,650 \times g$ for 15 minutes. The pellet was then washed twice with 70% ethanol and air-dried. The DNA pellet was re-suspended in 10 μl sterile water.

Sera were investigated for HHV by multiplex nested-PCR modifying the protocol previously described by Pozo and Tenorio.¹⁸ The primary PCR (1 $^{\circ}$ PCR) was modified to use newly designed degenerated primers instead of specific primers, and DNA was amplified at low an-

nealing temperatures based on the T_m of the degenerated primers. Specifically, the 1 $^{\circ}$ PCR was performed in a total volume of 50 μl containing 5 μl viral DNA, 20 pmol degenerated primers (HHV/1+ 5'-GTCATTTATG-GBGAYACKGA-3', HHV/1- 5'-ATC-CCCAATGTATCKYTTYTT-3', where B = C or G or T, Y = C or T, K = G or T) (Gibco, Life Technologies, USA), 200 μM each of dNTPs (Perkin-Elmer, USA), 1.25 U *Taq* DNA polymerase (Qiagen, Germany), 4 mM MgCl_2 and 1x PCR buffer. Amplification was carried out in a Thermal Cycler 2400 (Perkin-Elmer, USA). An initial denaturation step at 94°C for 4 minutes was followed by 30 cycles consisting of 30 seconds at 94°C , 1 minute at 45°C and 30 seconds at 72°C . A final extension step at 72°C was carried out for 10 minutes. For the secondary PCR (2 $^{\circ}$ PCR), 3 μl of the 1 $^{\circ}$ PCR product

were added to 47 μl of a reaction mixture consisting of 10 pmol each of the specific primers, 300 μM each of dNTPs, 2 U *Taq* DNA polymerase, 2 mM MgCl_2 and 1x PCR buffer. Amplification was performed applying the same conditions as in the 1 $^{\circ}$ PCR, except that the annealing temperature was 47°C . As an internal control of the PCR hepatitis B virus (HBV) DNA was amplified. Finally, 10 μl each of the 2 $^{\circ}$ PCR products were subjected to electrophoresis on a 3.5% agarose gel (Invitrogen, USA) stained with ethidium bromide on preparation in 0.5x TBE buffer at 90 Volts. The PCR products could thus be visualized on a UV transilluminator (Gel Doc 1000, Bio-Rad). The differential sizes were 54 bp for EBV, 68 bp for HHV6, 78 bp for CMV, 122 bp for HHV7 and 278 bp for HBV as the internal control (Fig. 1). The detail

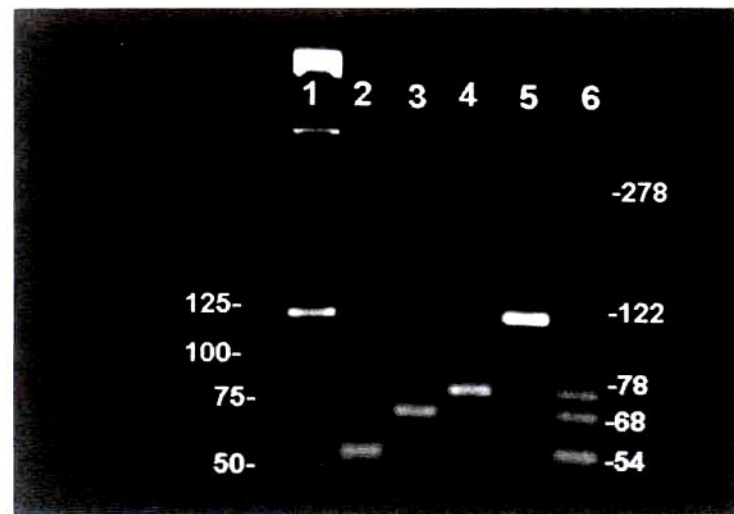


Fig. 1 Agarose gel showing the specific bands obtained for EBV, CMV, HHV6, HHV7 and multiplex nested-PCR products after two rounds of amplification. Lane 1, 25 bp DNA ladder; Lane 2, 54 bp (specific PCR products of EBV); Lane 3, 68 bp (specific PCR products of HHV6); Lane 4, 78 bp (specific PCR products of CMV); Lane 5, 122 bp (specific PCR products of HHV7); Lane 6, multiplex nested-PCR products. Internal control was 278 bp (HBV DNA products)

and sensitivity of the test have been reported elsewhere.²⁰

Statistic analysis

The results obtained were analysed by using mean, standard deviation and unpaired T-test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Among the 100 patients presenting with fever and maculopapular rash, 43 were boys and 57 were girls. Their age ranged from 2 months to 14 years. Forty-three patients were positive for herpesviruses by multiplex nested-PCR. HHV6 was the most common (24%) infection. HHV7, EBV and CMV were found in 9%, 3% and 2%, respectively. Four percent of the patients simultaneously harbored both HHV6 and HHV7. One patient had CMV, HHV6 and HHV7. The specific bands obtained for each herpesvirus after two amplification rounds represented DNA fragments of 54 bp for EBV, 68 bp for HHV6, 78 bp for CMV, and 122 bp for HHV7, as shown in Fig. 2. The result of multiplex nested-PCR and clinical mani-

festations of 100 patients are summarized in Table 1. Among twenty-five patients with a positive PCR for HHV6 DNA, only two patients were older than 2 years (age 4 and 12 years). The mean age of the patients with HHV6 was significantly lower than that of patients displaying negative results ($p < 0.001$).

DISCUSSION

This study has shown that

HHV6, HHV7, CMV, and EBV can cause fever with maculopapular rash in children. Among these viruses, HHV6 was the most common cause of infection. Patients with HHV6 infection had a lower mean age than those infected with HHV7, a similar result as reported in previous study.²¹ HHV6 usually infects young children; the peak age of acquiring HHV6 was 6-9 months.²² Only about 20% of the patients with primary HHV6 infection had maculo-

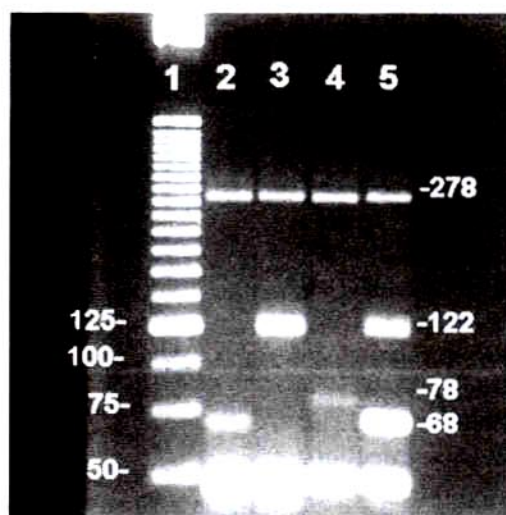


Fig. 2 Results of multiplex nested-PCR products on clinical specimens. Lane 1, 25 bp DNA ladder; Lane 2, positive for HHV6; Lane 3, positive for HHV7; Lane 4, positive for CMV; Lane 5, positive for HHV6 and 7 in the same patient.

Table 1 Result of multiplex nested-PCR and clinical manifestations in 100 patients that presented with fever and maculopapular rash

HHV-DNA (multiplex nested- PCR)	No. of patients (n = 100)	Mean age range (years)	Mean duration of fever range (days)	Mean duration of rash range (days)	Mean onset of rash range (days)
Negative	57	3.6 ± 3.9 (0.3-14)	4.0 ± 1.7 (1-9)	2.6 ± 1.7 (2-8)	2.5 ± 1.4 (1-7)
HHV6	24	1.7 ± 2.6 (0.4-12)	4.1 ± 1.2 (1-7)	3.7 ± 1.3 (2-6)	3.7 ± 1.3 (2-5)
HHV7	9	4.5 ± 4.2 (0.9-6.5)	3.9 ± 1.5 (1-5)	3.7 ± 1.8 (3.6)	2.3 ± 1.4 (2-5)
HHV6, HHV7	4	0.6 (0.3-0.9)	3.0 (3-4)	4.8 (2.4)	3.0 (1-5)
EBV	3	2.5 (1-5)	3.3 (2-4)	3.7 (3-5)	2 (1-3)
CMV	2	0.4 (0.3-0.5)	3.5 (3-4)	3 (3)	3 (1-3)
CMV, HHV6, HHV7	1	1.7	5	6	2 (3)

papular rash of the roseola type.²³ The infection is most probably transmitted through saliva.⁸ The virus can also be recovered from cervixes and vaginal secretions.⁸ HHV6 persisted in peripheral blood mononuclear cells in 66% of the patients after primary infection. Reactivation with febrile illness was suggested by both a subsequent rise in antibody titers and PCR.²²

Most studies included patients of younger age groups only. In this study we included patients of up to 14 years and found only one patient above the age of 5 years with HHV6 DNA in the plasma. Although rare, primary infection with HHV6 in otherwise healthy adults has been reported.²⁴ Differentiating active infection from reactivation may be difficult, and HHV6 serology does not constitute a reliable method to do so. Immunoglobulin M (IgM) antibodies to HHV6 are commonly detected within the first 5-7 days of primary infection. However, anti-HHV6 IgM can be found in 5% of healthy adults and some culture-positive children do not develop any measurable IgM response.^{25,26} A four-fold or greater rise of the IgG titer also may occur as a consequence of reactivation as well as in association with other infections.²⁷ Detection of HHV6 DNA in blood or saliva does not differentiate between persistent and primary infection because of persistence of HHV6 in the peripheral blood mononuclear cells and their secretion into the saliva is common in normal children after primary infection.^{22,26,28} HHV6 viremia rarely occurs in normal children outside the primary infection.^{17,28} Detection of free viral DNA in the plasma by PCR is a marker of active infection.^{12,15,17} Plasma PCR has shown

a sensitivity and specificity of 90% and 100% respectively, and therefore seems to be a good diagnostic tool.^{12,29}

HHV7 is also common in children. Probably more than 85% of the U.S. population are infected with HHV7.³⁰ Primary HHV7 infection seems to occur slightly later than that with HHV6, at a mean age of 3 years in the U.S.³¹ compared to 4.5 years in this study. HHV7 can reactivate HHV6 from latency in peripheral blood mononuclear cells.³² HHV6 could also cause fatal encephalitis in bone marrow¹⁰ and other organ transplant recipients.³³ There has been a report on HHV6 and HHV7 DNA detection in cerebrospinal fluid and PMBC by nested PCR in children with neurological signs and symptoms.⁹ We suggest that the 4 patients had HHV6 infection prior to HHV7 infection and that HHV7 reactivated HHV6 from latency. In those patients positive for CMV, HHV6 and HHV7 DNA in serum might be similarly explained. There has been a report on HHV6 reactivation associated with severe CMV-induced disease in orthotopic liver transplant recipients¹¹ and another report on CMV and HHV7 in renal allograft recipients.³⁴

In conclusion, HHV infection is common among children. Almost all children acquired HHV6 and HHV7 infections at a very young age. HHV6, HHV7, EBV and CMV can cause fever with maculopapular rash. Simultaneous infection with different herpesviruses could be found in some patients.

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