ORIGINAL ARTICLE

Uvulo-Palatoglossal Junctional Ulcers - An Early Clinical Sign of Exanthem Subitum Due to Human Herpesvirus 6

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Summary

A provisional clinical diagnosis of exanthem subitum was made in six febrile infants seen in the Paediatric Unit of Assunta Hospital, Petaling Jaya, Malaysia with uvulo-palatoglossal junctional ulcers prior to the eruption of maculopapular rash. On follow-up, all six infants developed maculopapular rash with the subsidence of fever at the end of the fourth febrile day. Human herpesvirus 6 was isolated from the peripheral blood mononuclear cells during the acute phase of the illness and HHV 6 specific genome was also detected in these cells by nested polymerase chain reaction. All the six infants showed seroconversion for both specific IgG and IgM to the isolated virus. This study suggests that the presence of uvulo-palatoglossal junctional ulcers could be a useful early clinical sign of exanthem subitum due to human herpesvirus 6.

Key Words: Exanthem subitum, Uvulo-palatoglossal junctional ulcer, Human herpesvirus 6.

Introduction

Human herpesvirus 6 (HHV 6) was first isolated in 1986 by Salahuddin *et al*[1] from patients with various immunosuppressive and lymphoproliferative disorders. In 1988, a similar virus was isolated and confirmed as the causative agent of exanthem subitum (ES) by Yamanishi *et al*.[2] Presently, ES is seen mainly in infants under 2 years of age presenting with high fever and paucity of other clinical signs followed by the eruption of maculopapular rash with the subsidence of fever.

In Malaysia, a seroprevalence study of HHV 6 infection carried out in 1996 by Chua *et al*[3] showed 66% of the local population have seroconverted by the age of 2 years. This prevalence rate suggests that HHV 6 infection is common among local infants although clinical reporting of the incidence of ES is low. This could be due to clinicians overlooking clinical signs of ES or mistakenly diagnosed as measles, rubella or drug allergy. This study documents six infants who presented with uvulo-palatoglossal junctional ulcers at the early stage of ES and were confirmed virologically and serologically to be due to HHV 6.

Materials and Methods

During the period from early October 1996 to December 1996, six febrile infants with uvulo-palatoglossal junctional ulcers (UPJ) were identified. After obtaining parental consent, 1.5 ml of venous blood from each patient was collected into a 5-ml sterile tube with EDTA as anticoagulant. The infants were subsequently reviewed daily by the attending paediatrician either on an in-patient or out-patient basis until the subsidence of fever. Convalescent sample of venous blood from each infant was collected 3 to 5

weeks later. All the blood samples were transported in ice to the laboratory within 6 hours of collection. In the laboratory, the venous blood was separated into plasma (for serological testing) and packed cells by centrifugation at 800X g for 10 minutes. The packed cells were resuspended in 5 ml of phosphate buffer saline (PBS) and peripheral blood mononuclear cells (PBMC) were subsequently harvested by centrifuging in Ficoll-paque density gradient at 600X g for 15 minutes.

Virus isolation from the harvested PBMC with subsequent identification by indirect immunofluorescence was conducted as described by Chua *et al*[4]. Similarly, viral nucleic acids extraction from PBMC and amplification by nested polymerase chain reaction (PCR) was also performed following the methodology of Chua *et al*[4]. The sequences of the outer and inner oligonucleotide primers (Genosys USA) used were constructed according to Yamamoto *et al* [5].

Specific IgG and IgM antibodies to HHV 6 were detected from the patients sample by indirect immunofluorescence using the in-house activated human cord blood mononuclear cells (HCBMC) infected with the reference strain of HHV 6B (Hashimoto strain donated by K Yamanishi, Japan). The technique used was as described by Chua *et al*[4].

Electron microscopic examination of the isolated virus was also performed. HCBMC co-cultured with the patient's PBMC were pelleted down by centrifugation at 800X g for 10 minutes and subsequently fixed in 4% glutaraldehyde for 8 to 12 hours. The fixed cells were then transferred into cacodylate buffer with osmiun tetroxide for 2 hours and kept in cacodylate buffer overnight. The following day, the cells were washed with double distilled water and stained using uranyl acetate for 10 minutes. After an additional washing step with double distilled water, cells were dehydrated with graded concentration of alcohol from 35% to 100%, conditioned with propylene oxide followed by propylene oxide/epon mixture and finally embedded in epon at 60°C for 12 hours. The embedded cells were sectioned at 60-70 nm thickness, stained with uranyl acetate followed by lead acetate and viewed under a Philip CM-12 transmission electron microscope.

Results

Clinically, bilateral UPJ ulcers were noted in all six febrile infants (Table) before the eruption of maculopapular rash. All developed the rash at the end of the fourth febrile day with subsidence of fever (Table I). One of the patients (KWL) had a mild cough with running nose while another (LHW) had mild diarrhoea. None of the infants presented with febrile convulsions. No other abnormal clinical findings were noted in all the 6 infants.

Antibody responses to HHV 6 in paired sera of the 6

Table I
Clinical profile of infants with exanthem subitum

Patient	Sex	Age (months)	Duration of fever (days)	Rash eruption on day of fever
PJH	Male	6	4	4
LSY	Female	4.5	4	4
LWK	Male	6	4	4
TYS	Female	7	4	4
LHM	Female	8	4	4
KWL	Male	8	4	4

Table II
HHV 6 specific IgM and IgG titres in paired sera from infants with exanthem subitum

Patient	IgM		IgG	
	Acute	Convalescent	Acute	Convalescent
PJH	<1 : 10	1 : 80	<1 : 10	1 : 320
LSY	1 : 10	1 : 80	<1 : 10	1 : 640
LWK	1 : 10	1 : 80	<1 : 10	1 : 320
TYS	1 : 10	1 : 80	<1 : 10	1 : 320
LHM	<1 : 10	1 : 80	<1 : 10	1 : 160
KWL	<1 : 10	1 : 80	<1 : 10	1 : 320

infants with ES are shown in Table II. All six infants showed significant 4-fold rise in antibody to HHV 6. The highest HHV 6 specific IgM antibody titre recorded was 1 in 80 dilution for all infants while the HHV 6 specific IgG antibody titre ranges from 1 in 160 to 1 in 640 dilution. Measles and rubella specific IgM were not detected in both acute and convalescent sera using commercially available ELISA kits (Radim, Italy).

After 4 to 5 days of culture, ballooning cytopathic effect could be seen in a small population of the PBMC in all the six infants. These enlarged PBMC were immunofluorescence positive with monoclonal antibodies OHV-1 (specific for HHV 6A and 6B) and OHV-3 (specific for 6B) but not with monoclonal antibodies KR-4 (specific for HHV 7) and Gp110 (specific for HHV 6A), indicating that the PBMC were infected by HHV 6 variant B.

Electron microscopy of ultra-thin sections showed the presence of viral particles morphologically resembling members of the herpesvirus family[6]. These particles were found as naked particles (90 to 105 nm) in the nuclei and as enveloped particles (180 - 200 nm) in the cytoplasm, intracytoplasmic vesicles and extracellular spaces of the infected HCBMC co-cultured with the patients' acute PBMC (Figure 1). Prominent inclusion bodies could also be seen in the nuclei of the infected cells.

The presence of HHV 6 was detected by nested PCR. An amplified DNA fragment of about 400 base pairs was detected in all six samples (Figure 2, lane 1 to 6) and they corresponded in size to the amplified DNA fragment of the reference HHV 6B, Hashimoto strain (Figure 2, lane 7). Nucleic acid extracted from HSB-2 cells infected with HHV 6, GS strain was used as reference group A HHV 6 (Figure 2, lane 8). 100

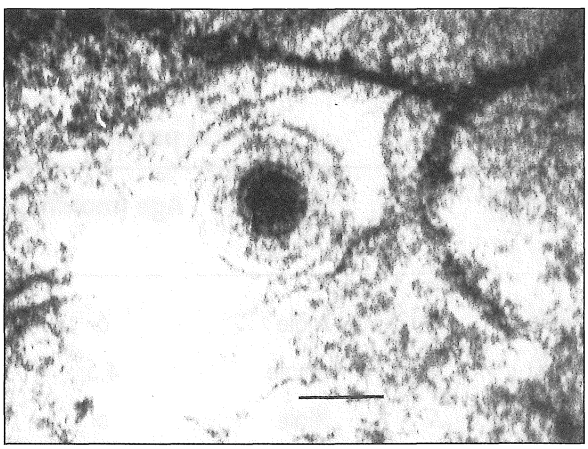

Fig.1: Electron micrograph of a herpesvirus particle in an infected cord blood lymphocyte. Bar scale denotes 100 nanometer.

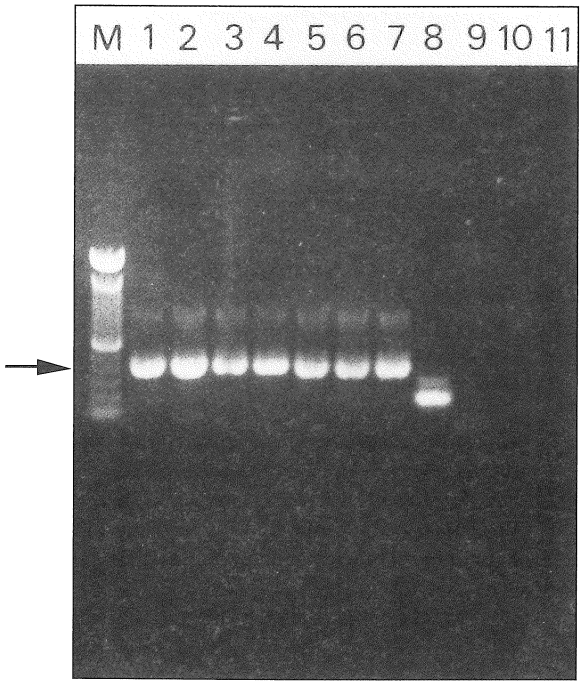

Fig. 2: Ethidium bromide stained agarose gel showing nested PCR amplification products of patients' PBMC.

Arrow indicates the amplified products of 6 patients' acute PBMC (lanes 1 to 6) and HCBMC infected with HHV 6, Hashimato strain (lane 7) as reference for group B. Nucleic acid extracted from HSB-2 cells infected with HHV 6, GS strain (lane 8) was used as the reference for group A. Activated HCBMC (lane 9) and milli-Q water (lanes 10, 11) were included as negative controls. 100 base-pair ladder markers (lane M) were used to indicate the molecular sizes of the amplified products.

base-pair molecular ladder markers were shown in lane M (Figure 2).

Discussion

The diagnosis of exanthem subitum (ES) is based on clinical features and can be confused with other childhood infections. Laboratory confirmation has not been possible until recently.

In this study, six infants with clinical ES confirmed virologically and serologically to be due to HHV 6 presented with typical ulcers at the junction of the base of the uvula with the palatoglossal (anterior pharyngo-tonsillar) folds prior to the appearance of typical maculopapular rash. All six patients showed significant seroconversion by detection of HHV 6 IgM and IgG. Virological evidence was obtained by isolation and confirmed by nested polymerase chain reation. All were found to belong to genotype HHV 6B.

The consistent finding of UPJ ulcers in these six patients with ES prior to the appearance of rash suggests that UPJ ulcers could be a useful early clinical sign of exanthem subitum caused by human herpesvirus 6. This finding may help paediatricians in better patient management to avoid unnecessary prescription of antibiotics and wrongful diagnosis of other viral exanthemata or drug allergy.

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