

Thymidine Kinase Sequence Analysis of Herpes Simplex Virus Type 1 Strains Present in Different Compartments in an Atypical Impetiginous Rash on the Lesional Skin of a Burn Patient[▽]

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We report the case of a 23-year-old male burn patient with an unusual herpes simplex virus (HSV) skin manifestation. The clinical symptoms and results of HSV type 1 (HSV-1) UL23 polymorphism analysis from saliva and lesional skin underscores the need for performing molecular analysis of HSV-1 infections in burned patients presenting unusual skin lesions.

CASE REPORT

An otherwise healthy, 23-year-old male patient with 32% total body surface area burns was transferred to our burn center. The patient sustained superficial second-degree burns on the face and neck, on both arms, extending from the hand to the upper arms, and on front and back areas of both lower legs and knees. The injury was caused by a workplace-related gas burner explosion.

Initially the patient was intubated and a bronchoscopy was performed. There were no signs of an inhalation injury. The wounds were surgically debrided, and a Vaseline gauze dressing was applied. Daily dressing changes were performed.

Due to gram-positive bacteria in tracheal secretions, antibiotic treatment with a cephalosporin was administered for 7 days. The patient was extubated on day 7.

On postburn day 10, a sudden increase in the patient's temperature to near 39°C was noticed, and a deterioration of his general condition was evident. Pneumonia was excluded by X rays of the chest. At this point we also removed the central line catheter.

Twenty-four hours after the onset of fever, the patient developed yellowish seromucus-covered erosions in the burned skin areas. The focal point of the rash was on the arms, the neck, and the underpart of the face (Fig. 1 and 2). The patient reported merely some light tension on the affected areas but severe pain. Based on the symptoms and the shape of the blisters, we suspected both bacterial and herpes simplex virus type 1 (HSV-1) infection. Swabs of the blister revealed *Staphylococcus epidermidis*, and molecular analysis for HSV-1, cyto-

megalovirus (CMV), and varicella-zoster virus (VZV) revealed HSV-1 infection.

On admission the patient was immunoglobulin G (IgG) positive for HSV, CMV, and VZV (20 July 2006). No enhanced CMV IgG levels were observed, and no antiherpesvirus IgM was detectable by enzyme-linked immunosorbent assay. A lesional wound swab from skin of the upper arm on postburn day 11 (1 August 2006) revealed the presence of HSV-1 DNA (H724) by a real-time PCR protocol with melting-point analysis for discrimination between HSV-1 and -2 (5). From the same swab, PCR for VZV and CMV DNA was negative. Serology for HSV IgM was negative, and complement fixation revealed an enhanced titer of 160 at 11 days postburn (1 August 2006). On day 12 after the thermal insult (2 August 2006), HSV-1 was isolated from a throat swab (H723) and a throat wash by microculture using monolayers of human foreskin fibroblasts and Vero cells. The HSV-specific cytopathic effects in both cell cultures were confirmed by immunoperoxidase staining of HSV glycoprotein D by an in situ enzyme-linked immunosorbent assay. At the same time, PCR results for CMV from leukocytes, plasma, and throat wash were negative. After confirmation of HSV infection, local antiseptic treatment was continued and an intravenous dose of 500 mg acyclovir three times per day was administered over a period of 7 days. Subsequently the efflorescences healed uneventfully (Fig. 2). On postburn day 17, the patient was discharged from the intensive care unit.

In order to analyze a potential HSV genotype variation between lesional virus from wound swabs and nonlesional HSV isolates from throat swabs, we sequenced the viral UL23 gene as described by Saijo et al. (21) using primers outside the thymidine kinase (TK) open reading frame S6f/S1r and four overlapping sense internal primers, pf, f(0), f(392), and f(782). HSV-1 TK is a 376-amino-acid (aa) protein encoded by a gene of 1,128 bp. It contains an ATP binding site (aa 51 to 63) and a nucleoside binding site (aa 168 to 176) (11). Conserved

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FIG. 1. Postburn day 11. HSV infection of a 23-year-old male burn patient, presenting as an impetiginous rash on the neck.

regions are located at aa 50 to 66, 79 to 91, 162 to 178, 212 to 226, and 281 to 292 (2). The polymorphism of the HSV thymidine kinase gene is larger than that for other HSV genes, and the HSV-1 genome is four times more variable than that of HSV-2. Mutations in the viral TK gene are associated with either polymorphism or drug resistance. The vast majority of mutations unrelated to acyclovir resistance are located outside TK conserved sites (10, 19). Table 1 summarizes the HSV-1 UL23 sequencing data for the viral isolates from throat (H723) and skin (H724) lesions that showed differences from published sequences from both human herpesvirus 1 (HHV-1) strain 17 (17) and the HHV-1 strain KOS mutant KG111 (14).

In 14 UL23 codons, both HSV isolates derived from our burn patient showed point mutations in reference to sequence of either strain 17 or KOS. The UL23 coding nucleotide sequences of both viral isolates H723 and H724 are absolutely identical. Compared to HHV-1 strains 17 and KOS, only codon 34 and/or codons 23, 36, and 232 do not differ in nucleotide sequence, respectively. In 8 out of 14 UL23 nucleotide substitutions (codons 23, 36, 204, 251, 267, 268, 286, and 376), our viral strains showed an exchange of amino acids compared to reference strain 17.

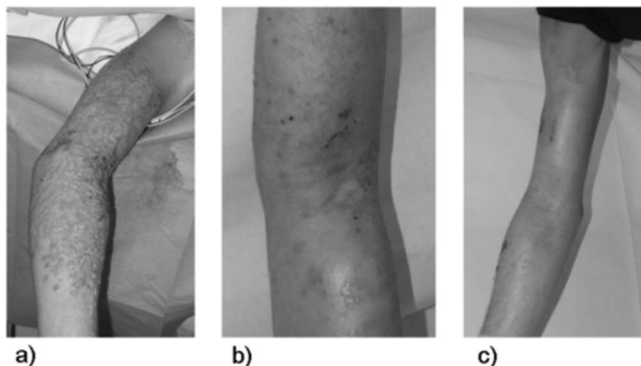


FIG. 2. HSV skin rash on the arm of a 23-year-old burned patient at postburn day 11 (a), 18 (b), or 28 (c).

TABLE 1. HSV-1 UL23 coding sequence data^a

Codon	nt sequence (aa residue or mutation) ^b			
	Strain 17	KOS	H723	H724
23	AAC (N)	AGC (N23S)	AGC (N23S)	AGC (N23S)
34	CAA (Q)	CAG (Q)	CAA (Q)	CAA (Q)
36	AAA (K)	GAA (K36E)	GAA (K36E)	GAA (K36E)
171	TGC (C)	TGC (C)	TGT (C)	TGT (C)
232	CTG (L)	TTG (L)	TTG (L)	TTG (L)
240	GGG (G)	GGG (G)	GAG (G204E)	GAG (G240E)
241	CTG (L)	CTG (L)	CTA (L)	CTA (L)
251	GGC (G)	GGC (G)	TGC (G251C)	TGC (G251C)
267	GTG (V)	GTG (V)	TTG (V267L)	TTG (V267L)
268	CCG (P)	CCG (P)	ACG (P268T)	ACG (P268T)
286	GAC (D)	GAC (D)	GAA (D286E)	GAA (D286E)
305	TAT (Y)	TAT (Y)	TAC (Y)	TAC (Y)
351	CAC (H)	CAC (H)	CAT (H)	CAT (H)
376	AAC (N)	AAC (N)	CAC (N376H)	CAC (N376H)

^a Data for published strain 17 (GeneID: 2703374, NC 001806) (17), strain KOS, mutant KG111 (NCBI J04327) (14), strain H723 from throat swab (postburn day 12), and strain H724 from lesional skin wound swab (postburn day 11) are given. Nucleotide exchange is shown in bold letters and underlined, and amino acid exchange is also given in bold letters.

^b nt, nucleotide; aa, amino acid.

The following codons are already known to be associated with UL23 polymorphism: 23, 36, 240, 251, 267, 268, and 286.

Invasive infection represents one of the major threats to the burned patient. While bacterial and fungal infections are commonly recognized, the same does not hold true for viral infections. Little information is available regarding the incidence, epidemiology, and diagnosis of HSV infections and the risk of serious HSV diseases in burn patients (4, 22). Kagan and colleagues described a prospective study of 52 patients with severe burn injuries in order to determine the seroprevalence of HSV and HCMV infections. Twenty-one (40%) of these patients developed HSV infections (15).

Our results underline the notion that HSV infection in burn patients occurs more often than is generally anticipated. This report may contribute to preventing potential misdiagnosis of HSV infections in burned patients presenting unusual skin rash morphology. As described in the case report, a skin rash could truly be misdiagnosed as an impetiginous rash caused by bacterial infection.

Clinical manifestations range from asymptomatic viral shedding to prolonged fever with eruption of vesicles to rare cases of systemic visceral dissemination with a lethal outcome (3, 4, 7, 9, 13, 18). Additionally, Byers et al. have demonstrated a strong association between HSV and adult respiratory distress syndrome (ARDS) in burn patients (6). In their study, 13 out of 16 ARDS cases were positive for HSV. The relative risk of HSV positivity for patients with ARDS compared with those without ARDS was 2.21 (95% confidence limit).

Given that the atypical clinical manifestation of skin rashes in burned patients is due to HSV infection, early molecular diagnosis of HSV and treatment with acyclovir seems obligatory (9).

In the past, the routine laboratory procedure for burned patients was restricted to serological analysis (3, 13, 15).

In contrast, our clinical case clearly shows that HSV should be verified by either virus culture, nucleic acid amplification, or both to detect productive virus replication and to document HSV reactivation in burned patients using skin lesion swabs and throat washes. Molecular analysis of the viral UL23 gene region revealed that viral isolates from the two specimens have identical sequences. The patient's initial HSV IgG-seropositive result confirms latent HSV infection, followed by reactivation of HSV by the burns, resulting in productive virus replication in different compartments. Nosocomial infection is ruled out, since endogenous virus reactivation was confirmed, including the presence of HSV in the respiratory tract.

Since HSV burn wound infections are being recognized, it is likely that nosocomial outbreaks of HSV infections will occur in burn units, as happens in newborn nurseries (16) and pediatric intensive care units (1, 12). In burn units, the introduction of such viral diagnostic procedures as virus culture and PCR will enable the recovery of HSV isolates from skin lesions and throat washes for subsequent molecular analysis. This will allow the verification or exclusion of nosocomial HSV transmission.

Sequencing of the HHV-1 UL23 gene from both skin lesions and throat swabs after a period of 12 days postburn revealed the molecular identities of both viral strains. Based on the published sequence of HHV-1 strain 17 and KOS, the eight UL23 point mutations of our proven acyclovir-sensitive clinical strains are known to be associated with UL23 polymorphism: 23, 36, 240, 251, 267, 268, 286, and 376 (8, 10, 20). Furthermore, we were able to describe an additional six UL23 point mutations (34, 171, 232, 241, 305, and 351), which are not known to be associated with acyclovir resistance. Except for codon 171, all of these mutations are localized outside the conserved HHV-1 TK gene regions. We attribute these mutations to nondescribed TK polymorphism, since the patient was initially acyclovir naive and the clinical HSV isolate H723 was susceptible to acyclovir with a 50% inhibitory dose of $<5 \mu\text{M}$ using a modified screening plaque reduction assay. Interestingly, the UL23 mutation in codon 171 (TGT instead of TGC) of our clinical isolates is localized in the nucleoside binding site (168 to 176) (19).

This report, therefore, underscores the need for molecular analysis of HSV infections in burned patients in cases where unusual skin lesions are observed. Shedding of HHV-1 in saliva can be used to document virus reactivation 10 days after the burn and to exclude nosocomial virus transmission in burn units by comparing the UL23 sequence variations of viral strains derived from throat and skin swabs. Furthermore, early initiation of antiviral therapy with acyclovir may have prevented pneumonia and reduced morbidity.

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