Clinical markers of herpes simplex virus infection in patients with pemphigus vulgaris

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Background: Herpes simplex virus (HSV) is known to influence the course of pemphigus vulgaris. Relapse, exacerbation, and treatment resistance in patients with pemphigus vulgaris can be due to HSV infection.

Objectives: To characterize the clinical markers of HSV infection among patients with pemphigus.

Methods: This was a hospital-based, descriptive study performed with 60 consecutive patients with pemphigus vulgaris. The clinical and laboratory features of patients with documented HSV infection were then compared with those of patients without infection.

Results: HSV infection was confirmed in 23 (38.33%) patients. On univariate analysis, it was noted that male sex (P = .03); presence of fissures (P = .001), hemorrhagic crusts (P = .003), erosions with angulated margins (P = .024), and linear erosions (P = .001); and raised erythrocyte sedimentation rate (P = .015) were found to be significantly associated with HSV infection. In a multivariate analysis, hemorrhagic crusts (P = .015) and linear erosions (P = .008) were found to be independent predictors of HSV infection.

Limitations: We did not use polymerase chain reaction to detect HSV infection, which could have yielded more cases of HSV infection.

Conclusion: In the clinical setting of pemphigus vulgaris, the presence of fissures, hemorrhagic crusts, linear erosions, erosions with angulated margins and raised erythrocyte sedimentation rate must alert the clinician to the possibility of HSV superinfection. (J Am Acad Dermatol 2023;88:587-92.)

Key words: herpes simplex virus; pemphigus.

Pemphigus is a group of autoimmune blistering skin diseases caused by autoantibodies directed against desmogleins.[1] Various exogenous/environmental factors are known to influence the course of pemphigus vulgaris, of which the most widely studied is the herpes simplex virus (HSV).[2] The sources of HSV in pemphigus lesions can be from an infected site or the environment (exogenous), the latent form already present in the skin (occult colonization), or the mucosae and intraepidermal release from free nerve endings (endogenous).

HSV is known to be associated with severe, persistent, recalcitrant pemphigus lesions and also with exacerbations and relapse of pemphigus.[3] Impaired skin barrier and attenuated immune response are the underlying factors that favor the occurrence of HSV superinfection, which can be either localized to the erosions or disseminated with systemic involvement (eczema herpeticum [EH]).[4-6]

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Funding sources: None.
Conflicts of interest: None disclosed.
Accepted for publication June 4, 2019.

Reprints not available from the authors.
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Published online June 10, 2019.
0190-9622/$36.00

https://doi.org/10.1016/j.jaad.2019.06.002

It is challenging to diagnose HSV superinfection in cases of pemphigus because both conditions present with flaccid blisters that rupture to form painful erosions on the skin and mucosae. The timely recognition of herpetic superinfection helps avoid unnecessary changes of immunosuppressive treatment and start an effective antiviral treatment without delay.

HSV can be detected by direct fluorescent antibody test, viral culture, polymerase chain reaction, and immunohistochemistry. All positive results must be confirmed by immunohistochemistry to rule out asymptomatic colonization.[7] Although there are many reports[6-9] that document the association of HSV infection with pemphigus, the clinical markers of HSV infection among patients with pemphigus vulgaris has not been well studied. Hence, we undertook this study to determine the frequency of HSV infection and to identify the clinical markers of HSV infection in patients with pemphigus.

CAPSULE SUMMARY

- Herpes simplex virus is known to be associated with severe, persistent, recalcitrant pemphigus lesions and also with exacerbations and relapse of pemphigus vulgaris.
- Presence of linear erosions and hemorrhagic crusts are significant clinical markers of herpes simplex virus superinfection among patients with pemphigus, which may allow for empirical treatment for herpes simplex virus infection.

MATERIALS AND METHODS

We carried out a hospital-based descriptive study involving 60 consecutive patients with pemphigus vulgaris with active skin or mucosal lesions who were admitted as inpatients of the dermatology department of the Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India. The Institute's Ethics Committee (Human Studies), (SEC/2011/4/54) approved the project. We obtained written informed consent from all participants before the study began. This study was conducted by following the guidelines of the Helsinki Declaration of 1975, as revised in 1983.

Inclusion and exclusion criteria

All patients with pemphigus vulgaris identified through biopsy and direct immunofluorescence, with active skin or mucosal blisters/erosions, irrespective of treatment status, were included in the study. Patients with pemphigus vulgaris in remission and those with other autoimmune bullous disorders were excluded from the study.

Clinical examination and assessment of severity of pemphigus. Clinical examination of the skin and mucosae was performed to look for the presence or absence of the following features based on prior clinical experience: grouped vesicles, polycyclic margins of the erosions, linear erosions, erosions with angulated margins, periorbital edema, fissures, pustules, and hypopyon. Severity and activity of pemphigus was measured with the Pemphigus Disease Activity Index (PDAI).[10] Laboratory investigations such as complete hemogram with differential counts and erythrocyte sedimentation rate (ESR) were performed in all cases.

Sample collection and investigations for the detection of HSV. Swabs were taken from blisters and erosions on skin, scalp, and oral mucosa for viral culture and indirect immunofluorescent (IIF) antibody test (Merck Millipore, Burlington, MA). The viral transport medium tubes with the swabs were then placed on ice packs and transported to the virology laboratory, where they were vortexed for 30 seconds. Swabs were then discarded after expressing the medium from the swab. The fluid was then centrifuged at 3500 revolutions/min for 30 minutes at 4°C, and the supernatant fluid was collected. Medium was aspirated from the cell monolayers, and 0.2 mL of the supernatant was inoculated onto each Vero cell culture monolayer for viral culture and smeared onto the glass slide for indirect immunofluorescence.

Presence of cytopathic effects (enlargement and rounding of Vero cells in viral culture) and apple-green fluorescence under the fluorescent microscope in IIF were indicative of HSV infection. Biopsy of the lesional site including the blister, if present, or the erosion was performed under proper sterile conditions, and the specimen was transported in a bottle of formalin to the pathology laboratory for immunohistochemistry (IHC). IHC was performed in accordance with the manufacturer's standard protocols (BioGenix, Houston, TX). Specimens showing 10% or more nuclear or 50% or more cytoplasmic staining were considered to be positive for HSV infection. Those specimens with staining confined to the superficial layers were considered to be HSV colonizers.[7] All the procedures were performed with standard protocols and standard biosafety precautions.

Patients with evidence of herpes virus infection by any of the 3 methods discussed were treated with oral acyclovir 400 mg 3 times daily for 10 days, and

Abbreviations used:

EH: eczema herpeticum
ESR: erythrocyte sedimentation rate
HSV: herpes simplex virus
IHC: immunohistochemistry
IIF: indirect immunofluorescence
PDAI: Pemphigus Disease Activity Index

the response of the lesions to the therapy was assessed by repeating the severity and activity scoring. There was no change in the treatment of pemphigus vulgaris except for the addition of acyclovir. The response to acyclovir therapy was assessed by clinical improvement and decreased PDAI scores.

Statistical analysis. The data collected were tabulated in a Microsoft (Redmond, WA) Excel spreadsheet. Statistical analysis was performed using Deducer R software (R Core Team, Austria, Vienna). We used descriptive statistics for categorical variables. Mean with standard deviation and median with interquartile range were estimated for all continuous variables. Chi-squared test or Fisher exact test was used to identify the association of categorical variables. Independent *t* test and Wilcoxon rank sum test were used to assess the difference in means/medians. Paired data were analyzed using the paired *t* test and Wilcoxon signed rank test. Variables in the univariate analysis with *P* less than .05 were taken up for multivariate analysis to identify the independent clinical markers of HSV infection.

RESULTS

Sociodemographic characteristics

The mean age of the 60 recruited patients was 44.37 ± 13.67 years (median, 45 years). Of the 60 patients, 26 (43.33%) were men and 34 were women (56.67%), with a male-to-female ratio of 0.76:1. Among the 60 patients, 16 (26.67%) had diabetes mellitus, 16 (26.67%) had hypertension, and 10 (16.67%) had osteoporosis. The mean duration of pemphigus vulgaris in this study was 17.85 ± 21.04 months (median, 11 months).

Of the 60 recruited patients, 6 were had a new diagnosis of pemphigus vulgaris and were not receiving any immunosuppressive therapy. The mean duration of therapy (corticosteroids and other immunosuppressants) among the remaining 54 patients was 22.22 ± 29.18 weeks (median, 8 weeks). None of the patients had a history suggestive of herpetic infection.

Clinical presentation

Of the 60 recruited patients with pemphigus vulgaris, 48 patients presented with both skin and oral mucosal lesions, 8 with only skin lesions, and 4 with only oral mucosal lesions. We recruited patients at the following observation points: baseline (n = 6), control of disease activity (n = 32), end of the consolidation phase (persistent lesions) (n = 12), and relapse (n = 10 cases). Pustules, fissures, periorbital edema, and grouped vesicles were observed on the skin. Hemorrhagic crusts were seen on the skin and lips. Erosions with polycyclic margins, erosions with angulated margins (Fig 1, *A*), and linear erosions were seen on the skin and the oral mucosa.

Diagnosis of HSV infection

HSV infection was confirmed in 23 of 60 (38.33%) patients. Fourteen cases were confirmed with IHC (Fig 2), 11 by viral culture, and 6 with IIF. One patient had colonization of erosions by HSV, as confirmed by IHC. HSV was isolated from the skin lesions in 15 (65.22%) (trunk, n = 6; extremities, n = 5; face, n = 4), oral mucosal lesions in 6 (26.09%), and skin and mucosal lesions in 2 (8.70%) patients. All cases of HSV were isolated from colocalized lesions of pemphigus.

Demographic characteristics, pemphigus activity, and treatment characteristics among patients with and without HSV patients are presented in Table I.

Comparison of groups with and without HSV

EH (Fig 1, *B*) was diagnosed in 4 (17.39%) patients, and the rest (19 [82.6%]) were superinfected with HSV. None of the patients at baseline (n = 6) had evidence of HSV infection.

Patients included in the study presented with various clinical signs, the frequencies of which varied between patients with and without HSV (Table II). We recorded fever among patients both with (30.43%) and without HSV (27.03%). Of the various clinical presentations, fissures (P = .001), hemorrhagic crusts (P = .003), erosions with angulated margins (P = .024), linear erosions (P = .001), and raised ESR (P = .015) showed a statistically significant association with the presence of HSV infection. We did not adjust for multiple testing because we preferred to have false positive than false negative indication of HSV infection. We did not observe a significant association with disease flare or with the presence of refractory pemphigus lesions. We did not find a difference between the 2 groups concerning disease severity or duration of prior immunosuppressive therapy.

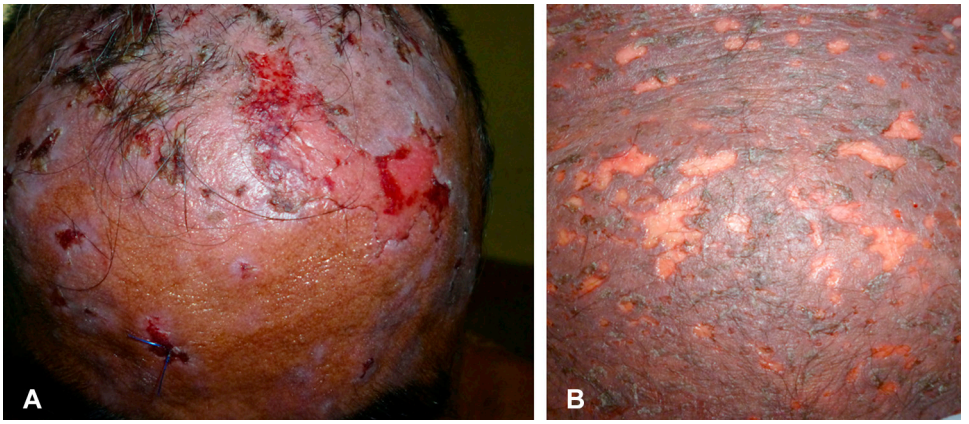

Fig 1. A, Pemphigus with herpes simplex virus superinfection: erosions with angulated margins on the scalp. **B**, Pemphigus with eczema herpeticum: multiple erosions with angulated margins on the abdomen.

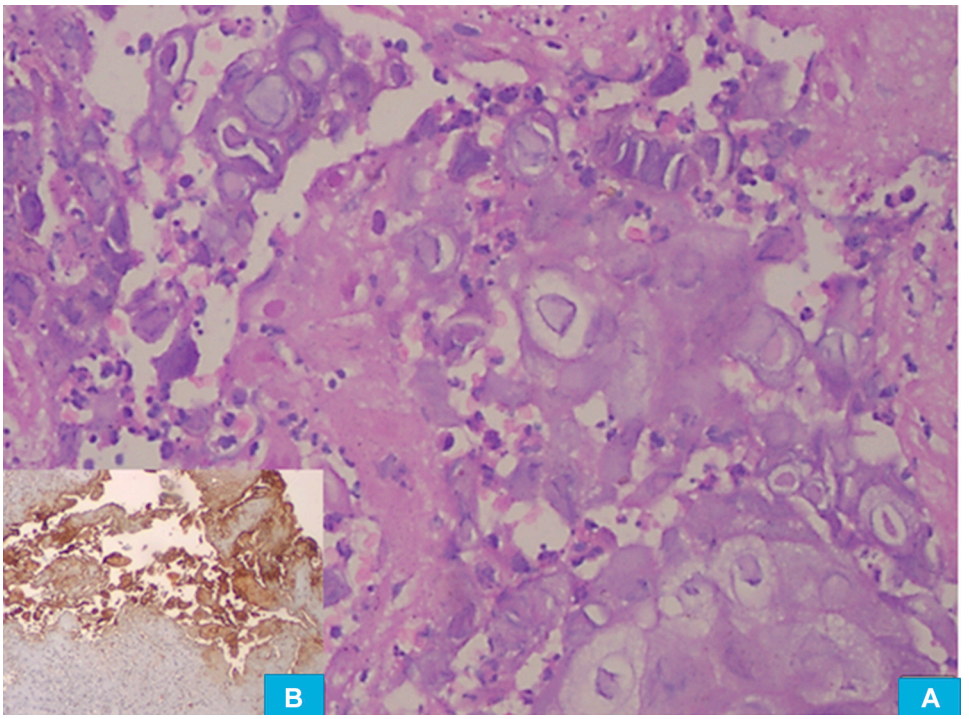

Fig 2. A, Pemphigus with herpetic infection. Sections show acantholytic cells in epidermis exhibiting intranuclear inclusions with clearing of chromatin, and some cells show prominent nucleoli. Hematoxylin-eosin stain; original magnification, ×400. **B**, Pemphigus with herpetic infection: sections show cytoplasmic and nuclear positivity for herpes simplex virus. Immunohistochemistry with Primary Antibody (BioGenex) and Secondary Antibody (Dako); diaminobenzidine stain; original magnification, ×100.

Comparison of EH with herpes superinfection

There was no significant difference with respect to fever (P = .34), PDAI score (P = .23), ESR (P = .08), and absolute neutrophil count (P = .84), although they were higher in the group with EH. Among the clinical markers, periorbital edema (P = .001) and fissures (P = .014) were significantly associated with EH.

Pemphigus severity and activity before and after acyclovir therapy

The mean PDAI of the 23 patients with HSV infection at the time of recruitment was 46.74 ± 21.92, which significantly decreased (P = .003) after 10 days of acyclovir therapy to 35.44 ± 17.95. To identify the significant independent predictors of HSV infection, a multivariate

Table I. Characteristics of patients with and without HSV infection

Characteristics	Group with HSV (n = 23)	Group without HSV (n = 37)	*P* value*
Demographic parameters			
Age in years, mean ± SD	44.7 ± 21.92	44.16 ± 11.6	.89
Sex			
Male, n (%)	14 (60.87)	12 (32.43)	
Female, n (%)	9 (39.13)	25 (67.57)	
Male:female	1.5:1	0.48:1	**.031**
Treatment: duration of immunosuppressive therapy in weeks, mean ± SD	17.52 ± 25.60	25.13 ± 31.18	.1
Mode of presentation, n (%)			
Recalcitrant lesions	3 (13.04)	9 (24.32)	.26
Flare	3 (13.04)	7 (18.91)	.35
Disease activity: PDAI, mean ± SD	46.74 ± 21.92	40.84 ± 26.28	.35
Laboratory value: ESR in mm Hg in first hour, mean ± SD	45.65 ± 15.43	36.11 ± 12.34	**.015**

ESR, Erythrocyte sedimentation rate; *PDAI*, Pemphigus Disease Activity Index; *SD*, standard deviation.
*Highlighted *P* values are significant.

Table II. Various clinical features of patients with and without HSV infection

Parameters, n (%)	Group with HSV (n = 23)	Group without HSV (n = 37)	*P* value*
Hemorrhagic crusts	12 (52.17)	6 (16.22)	**.003**
Pustules	10 (43.48)	13 (35.13)	.518
Erosions with angulated margins	8 (34.78)	4 (10.81)	**.024**
Linear erosions	8 (34.78)	1 (2.70)	**.001**
Periorbital edema	7 (30.43)	5 (13.51)	.111
Fissures in the skin	6 (26.09)	0 (0)	**.001**
Grouped vesicles	2 (8.70)	2 (5.40)	.461
Erosions with polycyclic margins	2 (8.70)	9 (24.32)	.13

**P* value was calculated by chi-squared or Fisher exact test.

logistic regression model was performed with HSV infection as the dependent variable and fissures, hemorrhagic crusts, erosions with angulated margins, linear erosions, and raised ESR as independent variables. Hemorrhagic crusts ($P = .015$) and linear erosions ($P = .008$) were found to be independent predictors of HSV infection after adjustment, including interaction (sex and ESR).

DISCUSSION

In our study, we confirmed HSV superinfection in 23 of 60 patients with pemphigus (38.33%). We also observed the following clinical markers: fissures ($P = .001$), hemorrhagic crusts ($P = .003$), erosions with angulated margins ($P = .024$), linear erosions ($P = .001$), and raised ESR ($P = .015$) to be significantly associated with HSV infection. Hemorrhagic crusts and linear erosions were found to be independent predictors of HSV infection.

The frequency of HSV superinfection among cases of pemphigus vulgaris has ranged from 0% to 60%.[2,6,8] The differences in the specimens used, sample site, sampling technique, techniques used for the detection of HSV, treatment status, and seroprevalence of HSV in the population might explain the discrepancy in the frequency of HSV infection between this study and other studies.

HSV in immunocompromised patients can be unrecognized because of small-sized lesions, atypical symptoms (eg, itching rather than tenderness), or atypical presentation including fissures or excoriations.[11] Kalajian and Callen[12] reported that, in the case of herpetic superinfection, multiple tiny grouped blisters are usually seen at the edge of the pemphigus lesions that rupture to form painful erosions with angulated margins. These erosions can coalesce to form typical polycyclic margins. In our study, we observed that the presence of fissures, hemorrhagic crusts, linear erosions, erosions with angulated margins, and raised ESR are strong clinical markers of HSV superinfection in patients with pemphigus. We observed that the severity of pemphigus in such patients decreased significantly after treatment with acyclovir. Thus, these features can aid in the early diagnosis of HSV infection, and early treatment with acyclovir can promote the healing of pemphigus lesions.

Dissemination of HSV in the lesions of underlying skin disease leads to the development of EH. EH is characterized by sudden onset of clustered, distinctly monomorphic, dome-shaped vesicles forming hemorrhagic crusts, which later fall off, leaving behind the characteristic multiple tiny, severely painful, punched-out or angulated erosions.[13] Fever, malaise, lymphadenopathy, and raised ESR usually accompany these cutaneous features. All 4 patients who with a diagnosis of EH in our study had the abovementioned clinical features, along with raised ESR. Periorbital edema and fissures were significantly associated with EH. However, the number of cases was too small to make a meaningful comparison with the group with HSV superinfection.

Multiple prior studies and reports[3,14,15] have described the association of HSV with recalcitrant lesions of pemphigus. In our study, HSV was detected from recalcitrant pemphigus lesions and exacerbating lesions of pemphigus. However, this was not found to be statistically significant.

Many prior studies[6,8] have established an association of HSV infection with corticosteroid therapy. In 6 of our patients who had not received prior treatment, HSV was not detected. However, we could not establish an association because serial swabbing of the lesions before and after immunosuppressive therapy was not performed in our study.

One of the major confounding issues with the study was the differentiation of occult colonization of bullous dermatoses from true infection.[7] This is primarily a problem with the oral mucosa, where intermittent shedding of HSV can occur. To avoid this issue, we performed biopsy and immunohistochemistry for HSV. Fourteen cases were confirmed with IHC. Of the 6 cases that were detected from the oral mucosa, 4 were confirmed by IHC to be a true infection. We did not perform polymerase chain reaction, which might have increased the detection of HSV DNA; however, this would not have addressed the issue of asymptomatic colonizers. We believe our approach with culture and IHC to be sufficient because we are able to address the issue of occult colonization of pemphigus lesions.

Another major issue is the generalization of our findings to all types of pemphigus on account of referral bias. Because our institution is a referral center for pemphigus in southern India, we treat the the most severe cases of pemphigus. Patients are usually admitted as inpatients and are receiving high doses of immunosuppressants, which may affect the magnitude of herpes superinfection.

To conclude, the physician may prescribe prophylactic acyclovir for a patient with severe pemphigus receiving long-term immunosuppressive therapy who has fissures, hemorrhagic crusts, linear erosions, erosions with angulated margins, and raised ESR.

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