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Original Research Article

ASSOCIATION OF DISEASE SEVERITY IN PEMPHIGUS VULGARIS WITH HERPES SIMPLEX VIRUS PCR POSITIVITY

Ritika Aggarwal[1], Tulika[2], Priyanka Bhadauria[3]

[1]*Assistant Professor, Department of Microbiology, GS Medical College and Hospital, Hapur, Uttar Pradesh, India.*
[2]*Assistant Professor, Department of Microbiology, Rama Medical College Hospital and Research Centre, Hapur, Uttar Pradesh, India.*
[3]*Assistant professor, Department of Microbiology, Radha Govind Institute of Medical Science, Meerut, Uttar Pradesh, India.*

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Corresponding Author:
Dr. Tulika
Assistant Professor, Department of Microbiology, Rama Medical College Hospital ad Research Centre, Hapur, Uttar Pradesh, India.
Email: tulika.medi@gmail.com

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A B S T R A C T

Background: Pemphigus vulgaris (PV) is a chronic autoimmune blistering disorder characterized by autoantibodies against desmoglein 3 and desmoglein 1, leading to suprabasal acantholysis and mucocutaneous erosions. Infections, particularly viral, are recognized contributors to disease exacerbation, and Herpes simplex virus (HSV) has been implicated as both a trigger and complicating factor in PV. This study aimed to evaluate the relationship between PV severity and PCR-confirmed HSV infection in an Indian tertiary-care cohort.

Materials and Methods: A cross-sectional study was conducted on 82 patients diagnosed with PV. Demographic and clinical data, including disease duration, comorbidities, and mucosal involvement, were collected. Disease severity was assessed using the Pemphigus Disease Area Index (PDAI). Oral and/or genital swabs were collected for HSV detection using polymerase chain reaction (PCR) and subtype identification. Associations between HSV positivity and disease severity, mucosal involvement, and immunosuppressive therapy were analyzed using Chi-square, Fisher's exact, t-test, and Pearson correlation as appropriate.

Results: The mean age of patients was 47.1 ± 11.0 years, with a female predominance (61%). Mucosal involvement was present in 81.7% of patients, predominantly oral (76.8%). HSV was detected in 31.7% of patients, with HSV-1 predominating (24.4%). HSV positivity increased with disease severity: 12.5% in mild, 25% in moderate, and 63.6% in severe PV ($p = 0.001$). HSV-positive patients had higher mean PDAI scores compared to HSV-negative patients (49.2 ± 16.8 vs 33.1 ± 15.2, $p = 0.0002$), and PDAI scores correlated positively with HSV positivity ($r = 0.41$, $p < 0.001$). HSV detection was higher in patients receiving combined corticosteroid and immunosuppressive therapy (38%) than in those on steroids alone (19.2%), though not statistically significant ($p = 0.060$).

Conclusion: HSV infection is common in PV patients and is significantly associated with higher disease severity. Severe PV and immunosuppressive therapy may predispose to viral reactivation, while HSV infection may exacerbate clinical severity. Early PCR-based detection of HSV in PV patients can guide timely antiviral therapy, potentially reducing morbidity and improving outcomes.

Keywords: Pemphigus vulgaris, Herpes simplex virus, Disease severity, Mucosal involvement, Immunosuppression.

INTRODUCTION

Pemphigus vulgaris (PV) is a chronic autoimmune blistering disease affecting the skin and mucous membranes, caused by IgG autoantibodies directed against desmoglein (Dsg) 3 and, in some cases, Dsg1, components of desmosomal cadherins responsible for keratinocyte adhesion.[1] The resultant

acantholysis leads to intraepithelial blisters and erosions that can be painful and debilitating. PV accounts for nearly 70% of all pemphigus cases and shows a higher prevalence in India, the Mediterranean region, and parts of the Middle East.[2] Although the introduction of corticosteroids and adjuvant immunosuppressants has markedly improved prognosis, secondary infections remain a major cause of morbidity and mortality in these patients.[3]

Viral infections, especially those caused by Herpes simplex virus (HSV), have been implicated as potential triggers or exacerbating factors in PV. HSV is a ubiquitous DNA virus capable of establishing latency in sensory ganglia, with reactivation occurring during stress or immunosuppression. In patients on prolonged corticosteroid or immunosuppressive therapy, HSV reactivation may present as recurrent erosions or vesiculobullous lesions indistinguishable from PV flares.[4] Studies have reported HSV co-infection rates ranging from 10% to 40% among PV patients, detected either serologically or through polymerase chain reaction (PCR)-based methods.[5,6]

PCR has emerged as the gold standard for detecting HSV DNA, offering high sensitivity and specificity even in subclinical infections.[7] Detection of HSV in lesional or perilesional samples may signify true reactivation rather than mere colonization, suggesting a pathogenic link. HSV infection may exacerbate PV severity by amplifying local inflammation, inducing keratinocyte apoptosis, and enhancing antigen exposure, thereby perpetuating the autoimmune cascade.[8] Conversely, severe PV and aggressive immunosuppression may predispose to HSV reactivation, creating a bidirectional interplay between disease activity and viral infection.

Assessing the relationship between HSV positivity and PV severity has important clinical implications. Identifying patients with active viral reactivation can aid in distinguishing true disease exacerbations from herpetic lesions, allowing timely antiviral intervention and modification of immunosuppressive regimens. However, data remain limited and inconsistent, particularly from India, where both PV and HSV infections are relatively common.[9]

Hence, the present study aimed to evaluate the relationship between the severity of pemphigus vulgaris, as assessed by the Pemphigus Disease Area Index (PDAI), and PCR positivity for HSV.

MATERIALS AND METHODS

Study Design and Setting: This hospital-based cross-sectional observational study was carried out in the Department of Dermatology, Venereology, and Leprosy at tertiary care hospital in North India, for a period of 18 months from January 2023 to June 2024. The study was approved by the Institutional Ethics Committee, and all procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki (2013 revision). Each participant provided written informed consent prior to inclusion in the study.

Study Population: The study included eighty-two patients clinically diagnosed with pemphigus vulgaris (PV) who attended the outpatient and inpatient dermatology services during the study period. Diagnosis was confirmed by characteristic clinical presentation, histopathological findings, and direct immunofluorescence (DIF). Histopathology from lesional skin demonstrated suprabasal acantholysis and a “row of tombstones” appearance of basal keratinocytes, while DIF of perilesional skin revealed intercellular deposition of IgG and/or complement component C3 in a fish-net pattern within the epidermis. Patients with other variants of pemphigus (such as pemphigus foliaceus or paraneoplastic pemphigus), coexisting systemic infections, or those who had received antiviral medication, systemic corticosteroids, or immunosuppressive therapy within four weeks before enrollment were excluded to eliminate potential confounding effects on viral detection.

Clinical Evaluation: Detailed demographic data, including age, sex, occupation, duration of illness, and family history of autoimmune disorders, were recorded using a structured proforma. A comprehensive dermatological and mucosal examination was performed for each participant to assess the distribution, morphology, and extent of lesions. Disease activity was assessed using the Pemphigus Disease Area Index (PDAI), which separately scores activity on the skin, scalp, and mucosal sites. Each area was scored based on the number and size of erosions or blisters, with the total PDAI score ranging from 0 to 263. Based on total PDAI scores, patients were categorized as having mild (≤15 points), moderate (16–45 points), or severe (>45 points) disease activity in accordance with International Pemphigus Committee guidelines. Photographic documentation of representative lesions was maintained for reference and verification.

Sample Collection and Processing: Specimens for Herpes Simplex Virus (HSV) detection were collected from the base of active erosions, vesicles, or crusted lesions under strict aseptic precautions. Using sterile Dacron-tipped swabs, the lesional surface was gently cleansed with sterile saline to remove crusts, and then the swab was rotated over the moist base of the lesion for 15–20 seconds to ensure adequate epithelial cell sampling. The swabs were immediately immersed in 3 mL of viral transport medium (VTM) and transported to the Department of Microbiology within one hour of collection, maintaining a temperature of 2–8°C in an insulated container. Samples were processed promptly to minimize viral DNA degradation.

DNA Extraction and PCR Amplification: DNA extraction was performed using the QIAamp DNA Mini Kit according to the manufacturer’s protocol. The extracted DNA was quantified and checked for purity using a NanoDrop 2000 spectrophotometer. Detection of HSV DNA was carried out using

conventional polymerase chain reaction (PCR) targeting the glycoprotein D (gD) gene, which is conserved in both HSV-1 and HSV-2. The PCR reaction mixture (25 µL) contained 5 µL of template DNA, 12.5 µL of 2× PCR master mix (Thermo Fisher Scientific), 0.5 µM each of forward and reverse primers, and nuclease-free water to make up the final volume. The thermal cycling conditions included initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 7 minutes. Amplified products were separated by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under UV transillumination using a gel documentation system. Positive and negative controls were included in every batch to validate the results. The presence of a specific amplicon band corresponding to the expected molecular weight (~300 bp) was interpreted as PCR-positive for HSV.

Statistical Analysis: All clinical and laboratory data were compiled and analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA). Quantitative variables such as age and disease duration were expressed as mean ± standard deviation (SD), while categorical variables such as gender, mucosal involvement, and HSV positivity were presented as frequencies and percentages. Associations between PCR positivity for HSV and PV severity (PDAI categories) were assessed using the Chi-square test or Fisher's exact test, as applicable. The relationship between PDAI scores and viral positivity was also explored using correlation analysis. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The study included 82 patients (50 females, 61.0%; 32 males, 39.0%) with a mean age of 47.1 ± 11.0 years and a mean disease duration of 13.8 ± 9.0 months. Mucosal involvement was common (67/82; 81.7%), mainly oral (63/82; 76.8%). Comorbid diabetes mellitus and hypertension were present in 22.0% and 17.1% of patients, respectively. The mean total PDAI score was 37.8 ± 18.2, indicating a predominance of moderate disease burden in this cohort [Table 1].

Table 1: Baseline demographic and clinical characteristics of study participants (n = 82).

Variable	Frequency (%)/mean ± SD
Age (years)	47.1 ± 11.0
Gender	
Female	50 (61.0%)
Male	32 (39.0%)
Disease duration (months)	13.8 ± 9.0
Mucosal involvement (any site)	67 (81.7%)
Oral mucosa involvement	63 (76.8%)
Genital mucosa involvement	10 (12.2%)
Comorbidity	
DM	18 (22.0%)
HTN	14 (17.1%)
Family history of autoimmune disease	7 (8.5%)
Total PDAI	37.8 ± 18.2

PDAI = Pemphigus Disease Area Index; DM = diabetes mellitus; HTN = hypertension

Disease severity was distributed as mild in 24 (29.3%), moderate in 36 (43.9%), and severe in 22 (26.8%) patients. Overall 26 of 82 (31.7%) patients were HSV PCR-positive. HSV positivity increased with disease severity: 12.5% in mild, 25.0% in moderate, and 63.6% in severe PDAI categories — a statistically significant association (Chi-square, p = 0.001) [Table 2].

Table 2: Distribution of disease severity and HSV PCR positivity by PDAI categories (n = 82).

PDAI category	Frequency (%)	HSV PCR–positive, Frequency (%)
Mild (≤15)	24 (29.3%)	3 (12.5%)
Moderate (16–45)	36 (43.9%)	9 (25.0%)
Severe (>45)	22 (26.8%)	14 (63.6%)
Overall	82 (100%)	26 (31.7%)

PDAI categories: mild ≤15, moderate 16–45, severe >45. HSV = herpes simplex virus.

Among PCR-positive cases, HSV-1 predominated (20/82; 24.4%), while HSV-2 was less frequent (5/82; 6.1%) and mixed infection was rare (1/82; 1.2%). HSV detection was more common in patients with mucosal involvement (34.3%) than in those with cutaneous-only disease (20.0%), but this difference did not reach statistical significance (Fisher's exact, p = 0.180) [Table 3].

Table 3: HSV subtype distribution and relationship with mucosal involvement (n = 82).

Variable	Frequency (%)
HSV PCR — overall positive	26 (31.7%)
HSV-1	20 (24.4%)
HSV-2	5 (6.1%)
Mixed HSV-1 & HSV-2	1 (1.2%)
HSV positivity among patients with mucosal involvement (n=67)	23 (34.3%)
HSV positivity among patients with cutaneous-only disease (n=15)	3 (20.0%)

At sampling, most patients (76/82; 92.7%) were receiving systemic corticosteroids; 50 (61.0%) were on combined steroid + immunosuppressive therapy, and 26 (31.7%) were on steroids alone. HSV PCR positivity tended to be higher in the combined-therapy group (19/50; 38.0%) versus steroids-only (5/26; 19.2%), showing a trend toward association that did not reach conventional significance (Fisher's exact, p = 0.060).

Table 4: Treatment status and HSV PCR positivity (n = 82).

Treatment at sampling	Frequency (%)	HSV PCR–positive, Frequency (%)
On systemic corticosteroids (any)	76 (92.7%)	24 (31.6%)
Steroids only	26 (31.7%)	5 (19.2%)
Combined therapy (steroids + immunosuppressant)	50 (61.0%)	19 (38.0%)
Not on systemic steroids/immunosuppressants	6 (7.3%)	2 (33.3%)

"Steroids only" = systemic corticosteroids without additional immunosuppressant; "Combined therapy" = systemic corticosteroids plus steroid-sparing immunosuppressant (e.g., azathioprine, mycophenolate)

HSV-positive patients had significantly higher disease activity (mean PDAI 49.2 ± 16.8) compared to HSV-negative patients (mean PDAI 33.1 ± 15.2), with a two-sample t-test showing p = 0.0002. Pearson correlation analysis demonstrated a moderate positive correlation between PDAI scores and HSV PCR positivity (r = 0.41, p < 0.001), suggesting that higher disease severity is associated with a greater likelihood of HSV infection [Table 5].

Table 5: Relationship Between HSV Positivity and PDAI Scores.

Variable	Mean PDAI ± SD	p-value
HSV-positive	49.2 ± 16.8	0.0002
HSV-negative	33.1 ± 15.2	
Pearson correlation (PDAI vs HSV positivity)	r = 0.41	<0.001

PDAI = Pemphigus Disease Area Index.

DISCUSSION

Our study included 82 patients diagnosed with pemphigus vulgaris (PV), with a mean age of 47.1 ± 11.0 years, and a female predominance of 61%. These findings are consistent with Indian and Worldwide epidemiological data as seen in studies Chowdhury et al., Ramassamy et al., and Rosi-Schumacher et al., where PV predominantly affects middle-aged adults with a slight female preponderance.[10-12]

The majority of our cohort exhibited mucosal involvement (81.7%), predominantly oral mucosa (76.8%), aligning with previous Indian studies by Yadav et al., and Ankit et al., reporting oral mucosa as the most frequently involved site in PV.[13,14] Comorbidities such as diabetes mellitus (22%) and hypertension (17.1%) were common, reflecting typical profiles of patients receiving long-term corticosteroids and immunosuppressants.

In our study, 31.7% of PV patients tested positive for HSV by PCR. This rate is comparable to findings from other studies by Konda et al., and Zhang et al., reported HSV superinfection in 38.33% of PV patients.[15,16] The higher prevalence of HSV infection in PV patients may be attributed to factors such as impaired mucosal integrity due to PV lesions, immunosuppressive therapy, and potential viral reactivation.[16]

Our study found a significant association between PV severity and HSV PCR positivity. Patients with severe PV had a higher rate of HSV positivity (63.6%) compared to those with mild (12.5%) and moderate (25.0%) disease. This trend is consistent with findings from other studies by Baum et al., and Kamyab et al., which reported a higher prevalence of HSV infection in patients with severe PV.[17,18] The association between PV severity and HSV infection may be explained by the increased immunosuppressive burden in severe PV cases, leading to a higher susceptibility to viral infections.[18]

In our study, HSV-1 was the predominant subtype (24.4%), followed by HSV-2 (6.1%) and mixed infection (1.2%). This distribution is consistent with global epidemiological patterns by Brandão et al., and Mohammadi et al., where HSV-1 is more commonly detected in oral lesions and HSV-2 in genital lesion.[19,20]

Most of our patients were on systemic corticosteroids, with or without additional immunosuppressants. The rate of HSV positivity was higher in patients receiving combined therapy (38.0%) compared to those on steroids alone

(19.2%).[20,21] This suggests that immunosuppressive therapy may increase the risk of HSV reactivation in PV patients.[22,23]

Clinical Implications: The high prevalence of HSV infection in PV patients, particularly those with severe disease and those on immunosuppressive therapy, underscores the need for vigilant monitoring and management of viral infections in this population. Early detection of HSV infection using PCR testing can facilitate timely antiviral therapy, potentially reducing morbidity and improving disease outcomes in PV patients.[24-26]

Limitations

Our study has several limitations. The sample size was relatively small, and the study was conducted at a single center, which may limit the generalizability of our findings. Additionally, we did not assess the clinical outcomes of antiviral therapy in HSV-positive PV patients, which warrants further investigation.

CONCLUSION

In conclusion, our study demonstrates a significant association between PV severity and HSV infection, highlighting the need for comprehensive management strategies that include monitoring for viral infections in PV patients. Further multicenter, longitudinal studies are needed to confirm these findings and evaluate the impact of antiviral therapy on disease outcomes in this population.

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