

The potential roles of herpesvirus and cytomegalovirus in the exacerbation of pemphigus vulgaris

Fariba Mohammadi¹, Zahra Khalili¹, Sayed Mahdi Marashi², Amirhoushang Ehsani¹, Maryam Daneshpazhooh¹, Majid Teymoori-Rad², Kamran Balighi¹, Ahmad Nejati², Shohreh Shahmahmoodi², Shima Izadidakhrabadi², Hamidreza Mahmoudi¹, Pedarm Noormohammadpour¹

¹ Autoimmune Bullous Disease Research Center, Department of Dermatology, Razi Hospital, Tehran University of Medical Sciences, Tehran, Iran

² Virology Department, School of Public Health (SPH), Tehran University of Medical Sciences (TUMS), Tehran, Iran

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Corresponding author: Pedarm Noormohammadpour, MD, Autoimmune Bullous Disease Research Center, Razi Hospital, Vahdate-Eslami Square, 11996, Tehran, Iran. Email: normohamad@razi.tums.ac.ir

ABSTRACT **Background:** Among exogenous etiologies, the critical role of microbial agents such as herpesviruses (HSV1/2) and cytomegalovirus (CMV) in triggering and flaring autoimmune conditions such as pemphigus vulgaris (PV) has been recently discovered.

Objectives: The present study aimed to investigate the plausible role of these viruses in the exacerbation of PV using serological and molecular methods.

Patients/Methods: Sixty patients with PV (30 with relapse type and 30 with remission type) were recruited for the purpose of this case-control study. Skin, mucosal, and throat specimens were obtained and examined for viruses by reverse transcriptase polymerase chain reaction. To determine the immunoglobulin G (IgG) titer, enzyme-linked immunosorbent assay was used.

Results: Desmoglein1-specific IgG was positive in 56.7% of patients with the relapse form and in 20.0% of those with the remission form indicating a significant difference across the 2 groups ($P = 0.003$), but the rate of positivity for desmoglein3-specific IgG in the relapse and remission types was 76.7% and 63.3%, respectively, with no significant difference ($P = 0.260$). There was no difference in the mean levels of HSV-IgG and CMV-IgG in the relapse and remission groups. HSV and

ABSTRACT CMV positivity in PV patients was independent of the site of the samples. Using the multivariable linear regression model, the level of CMV-IgG in PV patients was directly affected by female sex and advanced ages.

Conclusions: Our study could not demonstrate the role of HSV1/2 and CMV as triggering factors for PV exacerbation. Further studies are needed to evaluate the potential role of these viruses in PV exacerbation especially considering demographic variables.

Introduction

Pemphigus disease is an autoimmune disease that is characterized by secretion of autoantibodies that act against surface glycoproteins of epithelial cells [1]. The immunological basis for pemphigus disease includes autoantibodies targeted against keratinocyte surface antigens desmoglein1 and desmoglein3 (DSG1, DSG3) [2]. These proteins are primarily involved in intercellular cell-to-cell adhesion structures. Pemphigus vulgaris (PV) is a common type of pemphigus disease with painful blistering on the skin and mucous membranes [3]. Overall, the close link between autoimmune diseases and both endogenous (genetic) and exogenous (environmental) factors has been clearly understood. Exogenous factors include thiol drugs, physical trauma such as burn, ultraviolet exposure, X-ray, neoplasm, hormones and pregnancy, nutritional factors, and emotional stress [4-7].

Among exogenous etiologies, the critical role of microbial agents such as viruses in triggering and flaring autoimmune conditions has been recently discovered. In this regard, the causative role of herpesviruses (HSVs) as the most important human pathogens in the pathogenesis of PV has been recently suggested [8]. Thus, efforts have been made to clarify viral etiologies for PV and in this way the role of HSVs and cytomegalovirus (CMV) is taken into consideration [9,10]. The main common features of both pointed viruses include their ability to survive in the host body for a long time as well as activating periodically [11]. The human HSVs including herpesviruses 1 and 2 (HSV1/2) and CMV infect between 60% and 90% of the adult population worldwide [12,13]. After primary infection, the virus establishes lifelong latency, with periodic reactivations that are effectively controlled by a robust immune response in most infected individuals despite the virus-producing proteins that interfere with adaptive and innate immunity. The activation of T cells is the important aspect of adaptive immunity. Tissue-resident memory (Trm) T cells are a subtype of memory lymphocytes that enter nonlymphoid tissues such as skin and become permanently established without recirculating. Local immune control of viruses can be mediated by Trm T cells through direct killing of infected cells and recruiting circulating memory CD8+ to the skin. Also, Trm T cells maintain HSV1 latency by secreting granzyme B, which degrades the early protein ICP4 that is important in viral replication [14,15].

Atypical HSV infections have been described in immunosuppressed patients [16]. Several studies have suggested the activation or exacerbation of pemphigus after HSV or CMV infections [17]. More recently, Japanese researchers have detected high levels of HSV in the saliva samples of PV patients [18]. In this regard, several studies have shown the benefits of adding antiviral therapies to an immunosuppressive therapy in recalcitrant disease [19,20]. Several studies also failed to detect herpesviruses in pemphigus patient specimens [21,22], suggesting that viruses may have only a transient role for exacerbation of pemphigus disease, although yet to be further defined.

The number of patients with PV in Iran appears to be increasing, and little information is available with regard to the role of HSV1/2 and CMV in triggering PV among the Iranian population. The present study aimed to investigate the plausible role of these viruses (HSV1/2 and CMV) in the exacerbation of PV using serological and molecular methods.

Materials and Methods

Disease Definitions in PV Patients

PV patients were categorized as follows: relapse (development of more than 3 new lesions/month that do not heal within a week without treatment, or development of established lesions in a patient whose disease was controlled) and complete remission (absence of new or established lesions in a patient whose all-systemic therapy is discontinued for ≥ 2 months, or while the patient is receiving minimal therapy) [23]. Scoring of disease was recorded based on the pemphigus disease area index.

Study Population

Sixty patients with PV (27 male, 33 female; mean age 46.3 ± 12.7 years) were recruited for the purpose of this case-control study. Patients were selected among inpatients and outpatients who were referred to the pemphigus clinic of Razi Hospital (Tehran, Iran). The diagnosis of pemphigus had been confirmed by histology and direct immunofluorescence. Among patient subjects, 30 were in complete remission as in the control group, whereas the rest ($n = 30$) were in relapse as in the case group. None of the patients had overt labial herpetic lesions at the time of sampling. All study participants

gave informed consent to provide samples for the study, which was approved by the Local Research Ethics Committee of Tehran University of Medical Sciences (approval number: IR.TUMS.MEDICINE.REC.1395.1017 and IR.TUMS.MEDICINE.REC.1395.2472).

Sample Preparation

For viral detection and determination of specific antibodies, we used blood, skin biopsy, and throat swab samples from patients. After patient consent forms were obtained, one skin specimen or mucosal specimens were obtained by punch biopsy (3 mm) and blood samples were taken from all patients. In addition, a sample from the throat was collected by swabbing. All samples were transferred in cold chain conditions to the virology laboratory. As soon as samples were received, preparation and storage were carried out. Sera (for serology) and buffy coat (for virus detection) were isolated from whole blood and stored at -20°C . DNA extraction from buffy coat, skin biopsy, and throat swab were performed using DNA Isolation Kit for Cells and Tissues according to the manufacturers' instructions (Roche, Berlin, Germany). After DNA extraction, it was eluted in 50 μL of buffer and then adjusted to a definitive concentration of 500 $\text{ng}/\mu\text{L}$.

Virus-Specific Antibody Responses

HSV1/2- and CMV-immunoglobulin G (IgG) antibodies were determined using enzyme-linked immunosorbent assay (ELISA). Quantification of IgG antibodies was determined by standard curve as RU/mL according to the manufacturer (values greater than 22 RU/mL were defined as seropositive).

DSG-Specific IgG

The levels of autoantibody to DSG1 and DSG3 were measured by ELISA. The cutoff values were 20 U/mL for both.

Determination of Viral Load

The absolute quantification of HSV1/2 and CMV-specific DNA was performed by real-time polymerase chain reaction (PCR) using the RealStar HSV PCR Kit 1.0 and RealStar CMV PCR Kit 1.0, respectively (Altona Diagnostics; Hamburg, Germany). All stages of the amplification of the viruses' specific target sequences for the detection and quantification of the amplified DNA were carried out according to the manufacturer's instructions. Ten microliters of the DNA extracted from samples in distilled water, and the 4 controls were included in each experiment as a template and a negative control and quantification standards, respectively. Moreover, we performed internal control to identify possible PCR's inhibitor and to confirm the reliability of all reagents of the kit used for each sample. Finally, the concentration of the samples was calculated in copies per microliter and copies per milliliter.

Statistical Analysis

The results were presented as mean \pm standard deviation for quantitative variables and were summarized by absolute frequencies and percentages for categorical variables. Normality of data was analyzed using the Kolmogorov-Smirnoff test. Categorical variables were compared using chi-squared test or Fisher's exact test when more than 20% of cells with an expected count of less than 5 were observed. Quantitative variables were also compared with t test or Mann-Whitney U test. The association between quantitative variables was assessed using the Pearson correlation test. To determine the main correlates of IgG antibodies against viruses, multivariable linear regression analysis was used. For the statistical analysis, the statistical software SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL) was used. P values of 0.05 or less were considered statistically significant. We used GraphPad Prism software (GraphPad Software, La Jolla, CA) for plotting graphs.

Results

In total, 30 patients with the relapse type of PV and 30 patients with the remission type were assessed. The demographic and clinical features of patients are presented in Table 1. Comparison of the baseline characteristics of the 2 groups (Table 2) showed no difference in gender, mean age, subtypes of disease (mucous or mucocutaneous), or disease duration and oral medications. Regarding medical history, hypertension and hyperlipidemia were more prevalent in those with relapse PV than in the remission group, while no difference was revealed in other underlying comorbidities including diabetes mellitus, history of smoking, coronary artery disease, or thyroid abnormalities.

With regard to DSG-specific IgG positivity in the relapse and remission forms of PV, DSG1-specific IgG was positive in 56.7% of patients with the relapse form and in 20.0% of those with the remission form, indicating a significant difference across the 2 groups ($P = 0.003$), but the rate of positivity for DSG3-specific IgG in the relapse and remission types was 76.7% and 63.3%, respectively, with no significant difference ($P = 0.260$). The mean HSV-IgG in the relapse and remission groups was 154.47 ± 66.80 and 151.17 ± 62.73 , respectively, with no significant difference ($P = 0.844$). Moreover, the mean CMV-IgG in the subgroups was 130.30 ± 47.88 and 127.93 ± 53.56 , respectively, with no meaningful difference ($P = 0.857$). There was no correlation between prednisolone dose and HSV-IgG ($P = 0.373$) or CMV-IgG ($P = 0.647$). As shown in Table 3, HSV and CMV positivity in PV patients was independent of the source of the samples.

TABLE 1. Demographic and clinical characteristics of pemphigus vulgaris patients

ID	Sex	Age (Years)	Type	Duration (Years)	Activation	PDN Dose (mg/d)	Drug History	DSG1	DSG3	PDAI
P1	F	72	MCPV	2	Relapse	15	Azathioprine	Positive	Positive	94
P2	F	41	MPV	2.5	Remission	5	Azathioprine	Negative	Positive	0
P3	F	47	MCPV	4	Remission	5	Mycophenolic acid, Azathioprine	Negative	Positive	0
P4	F	48	MCPV	1.5	Remission	5	Mycophenolic acid	Negative	Positive	0
P5	F	31	MCPV	2	Remission	5	Azathioprine, rituximab	Positive	Positive	0
P6	F	28	MCPV	5	Relapse	10	Mycophenolic acid, Azathioprine	Positive	Positive	12
P7	M	61	MCPV	4	Remission	5	Azathioprine, Mycophenolic acid, rituximab, Mtx	Negative	Positive	0
P8	M	67	MCPV	10	Relapse	30	Cyclophosphamide, Mycophenolic acid, rituximab	Negative	Positive	34
P9	F	63	MCPV	3.5	Remission	0	Mycophenolic acid monotherapy	Negative	Negative	0
P10	M	41	MCPV	3	Remission	0	Mycophenolic acid	Positive	Negative	0
P11	M	60	MCPV	2.5	Remission	5	Azathioprine	Negative	Negative	0
P12	M	45	MCPV	7	Remission	5	Mycophenolic acid, Azathioprine	Negative	Negative	0
P13	M	32	MCPV	10	Remission	7.5	Azathioprine	Negative	Positive	0
P14	M	41	MCPV	1.5	Remission	5	Azathioprine, rituximab	Negative	Negative	0
P15	M	38	MCPV	4	Remission	10	Azathioprine, mycophenolic acid, rituximab	Positive	Negative	0
P16	M	55	MPV	2	Remission	7.5	Azathioprine	Negative	Positive	0
P17	M	65	MCPV	2	Relapse	5	Azathioprine	Negative	Positive	14
P18	F	45	MCPV	2	Relapse	10	Azathioprine	Positive	Positive	12
P19	F	58	MCPV	2	Remission	7.5	Azathioprine	Negative	Positive	0
P20	M	39	MPV	2	Remission	1.25	Azathioprine	Negative	Negative	0
P21	F	45	MPV	2	Relapse	7.5	Mycophenolic acid	Negative	Positive	5
P22	M	21	MCPV	1	Relapse	15	Azathioprine	Positive	Positive	34
P23	F	65	MCPV	12	Remission	5	None	Negative	Positive	0
P24	F	45	MCPV	10	Remission	5	Azathioprine	Negative	Positive	0
P25	F	33	MCPV	5	Relapse	0	Azathioprine			15
P26	M	44	MPV	19	Remission	7.5	Mycophenolic acid, Azathioprine	Negative	Positive	0
P27	F	54	MCPV	2.5	Remission	5	Mycophenolic acid, rituximab	Positive	Positive	0
P28	F	62	MCPV	3	Remission	2.5	Azathioprine	Negative	Negative	0
P29	F	55	MCPV	1.5	Remission	2.5	Mycophenolic acid, Mtx	Negative	Negative	0
P30	M	38	MCPV	1.5	Remission	7.5	Rituximab	Positive	Positive	0
P31	F	67	MCPV	11	Remission	5	Azathioprine, mycophenolic acid, rituximab, Mtx	Negative	Positive	0
P32	F	46	MCPV	0.5	Remission	7.5	Rituximab	Negative	Negative	0

(Continued next page)

TABLE 1. Demographic and clinical characteristics of pemphigus vulgaris patients (continued)

ID	Sex	Age (Years)	Type	Duration (Years)	Activation	PDN Dose (mg/d)	Drug History	DSG1	DSG3	PDAI
P33	F	40	MCPV	12	Remission	10	Azathioprine	Negative	Positive	0
P34	M	40	MCPV	3	Relapse	25	Azathioprine, rituximab	Negative	Positive	5
P35	M	55	MCPV	6	Remission	7.5	Azathioprine	Negative	Negative	0
P36	M	35	MCPV	14	Remission	7.5	Mycophenolic acid, Azathioprine, Mtx, rituximab, IVIG	Negative	Positive	0
P37	M	65	MCPV	5	Relapse	0	Mycophenolic acid, Azathioprine	Positive	Negative	23
P38	F	74	MCPV	2	Relapse	7.5	Azathioprine	Positive	Positive	24
P39	F	32	MCPV	2	Relapse	10	Rituximab	Negative	Positive	14
P40	F	39	MCPV	8	Relapse	2.5	Mycophenolic acid, rituximab	Positive	Positive	3
P41	F	36	MCPV	5	Relapse	5	Mycophenolic acid, Azathioprine	Positive	Positive	34
P42	F	54	MCPV	2	Relapse	5	Mycophenolic acid, Azathioprine, Mtx	Positive	Positive	12
P43	F	54	MCPV	6	Relapse	25	Rituximab, mycophenolic acid	Negative	Positive	11
P44	M	20	MCPV	1	Relapse	15	Rituximab	Negative	Negative	5
P45	F	42	MPV	1	Remission	2.5	Rituximab	Negative	Positive	0
P46	M	38	MPV	3	Relapse	15	None	Negative	Positive	27
P47	F	46	MCPV	15	Relapse	20	Mycophenolic acid, Azathioprine, Mtx, rituximab, IVIG, cyclophosphamide	Positive	Positive	0
P48	M	27	MCPV	1.5	Relapse	30	Mycophenolic acid, Azathioprine	Positive	Positive	0
P49	M	42	MCPV	0.5	Relapse	35	None	Negative	Negative	17
P50	F	34	MPV	1	Relapse	10	Mycophenolic acid, Azathioprine, Mtx, rituximab, IVIG, cyclophosphamide			10
P51	F	34	MCPV	1	Relapse	6.25	Rituximab	Positive	Negative	38
P52	F	37	MCPV	2	Relapse	25	Azathioprine, rituximab	Positive	Positive	19
P53	M	57	MCPV	3	Relapse	15	Azathioprine	Positive	Positive	84
P54	M	54	MCPV	5.5	Relapse	2.5	Azathioprine, mycophenolic acid, rituximab, Mtx	Positive	Negative	12
P55	F	54	MCPV	2	Relapse	12.5	Rituximab	Negative	Positive	8
P56	F	48	MCPV	1	Relapse	10	Azathioprine	Positive	Positive	23
P57	F	60	MCPV	10	Remission	10	Azathioprine	Negative	Positive	0
P58	M	29	MCPV	3	Relapse	5	Mycophenolic acid, rituximab	Positive	Positive	14
P59	M	45	MCPV	1	Relapse	15	Azathioprine	Positive	Positive	21
P60	F	39	MCPV	1.5	Relapse	35	Mycophenolic acid, Azathioprine	Negative	Positive	15

Abbreviations: Mtx, methotrexate; DSG, desmoglein; MCPV, mucocutaneous PV; MPV, mucous PV; PDN dose, oral prednisolone dose at the time of the study; PDAI, pemphigus disease area index

TABLE 2. Comparing baseline characteristics between the patients with relapse and those in remission

	Relapse Type (n = 30)	Remission Type (n = 30)	P Value
Male	12 (40.0)	14 (46.7)	0.602
Mean Age, Years (\pm SD)	44.90 \pm 14.30	47.83 \pm 11.04	0.377
Disease Duration, Years (\pm SD)	3.42 \pm 3.14	5.23 \pm 4.75	0.086
Medical History			
History of hypertension	9 (30.0)	2 (6.7)	0.020
History of hyperlipidemia	5 (16.7)	0 (0.0)	0.050
History of diabetes mellitus	5 (16.7)	2 (6.7)	0.424
History of smoking	1 (3.3)	1 (3.3)	1.000
History of coronary disease	2 (6.7)	1 (3.3)	0.998
History of AVN	2 (6.7)	0 (0.0)	0.492
History of hypothyroidism	2 (6.7)	0 (0.0)	0.492
Form of Disease			0.706
Mucous	3 (10.0)	5 (16.7)	
Mucocutaneous	27 (90.0)	25 (83.3)	
Medication			
Azathioprine	19 (63.3)	20 (66.7)	0.787
Mycophenolic acid	13 (43.3)	13 (43.3)	1.000
Rituximab	12 (40.0)	10 (33.3)	0.592
Methotrexate	4 (13.3)	4 (13.3)	1.000
IVIG	2 (6.7)	1 (3.3)	0.999
Cyclophosphamide	3 (10.0)	0 (0.0)	0.237

Unless noted otherwise, values represent number (percent).

TABLE 3. Comparison of HSV and CMV detection based on reverse transcriptase polymerase chain reaction techniques according to the source of samples between the patients with relapse and those in remission

	Relapse Type (n = 30)	Remission Type (n = 30)	P Value
HSV			
Skin	1 (3.3)	0 (0.0)	0.998
PBMC	1 (3.3)	2 (6.7)	0.898
Throat	0 (0.0)	0 (0.0)	1.000
CMV			
Skin	3 (10.0)	0 (0.0)	0.237
PBMC	5 (16.7)	0 (0.0)	0.052
Throat	3 (10.0)	0 (0.0)	0.237

Values represent number (percent).

Abbreviation: PBMC, peripheral blood mononuclear cell.

As indicated in Table 4 and Figure 1 and using the multi-variable linear regression model, the level of HSV-IgG was not associated with the baseline characteristics including demographics, underlying comorbidities, disease duration, and form of PV. However, as shown in Table 5 and Figure 2, the level of CMV-IgG in PV patients was affected by the gender and age factors. The mean CMV-IgG was significantly higher in female than in male patients suffering from PV (142.21 \pm

46.60 vs 112.00 \pm 50.86, $P = 0.020$). Also, a direct correlation was revealed between patients' age and the level of CMV-IgG (r coefficient = 0.404, $P = 0.001$).

Discussion

Several studies have investigated the relationship between viral infections and the initiation or exacerbation of autoimmune

TABLE 4. Multivariable linear regression model to assess the main correlates of HSV-IgG antibody in patients with pemphigus vulgaris

	Unstandardized Coefficients		Standardized Coefficients	t	P Value	
	(Constant)	B	Std. Error			Beta
Sex		-35.727	164.984		-0.217	0.829
Age		-3.982	18.674		-0.213	0.832
Type		0.492	0.809		0.608	0.546
Duration		-10.558	26.124		-0.404	0.688
Form		0.825	2.345		0.352	0.726
Hypertension		-15.421	19.720		-0.782	0.438
Hyperlipidemia		0.185	34.852		0.005	0.996
Diabetes		58.505	46.066		1.270	0.210
Coronary Disease		-14.900	33.394		-0.446	0.657
Smoking		-2.827	45.347		-0.062	0.951
(Constant)		67.297	54.454		1.236	0.222

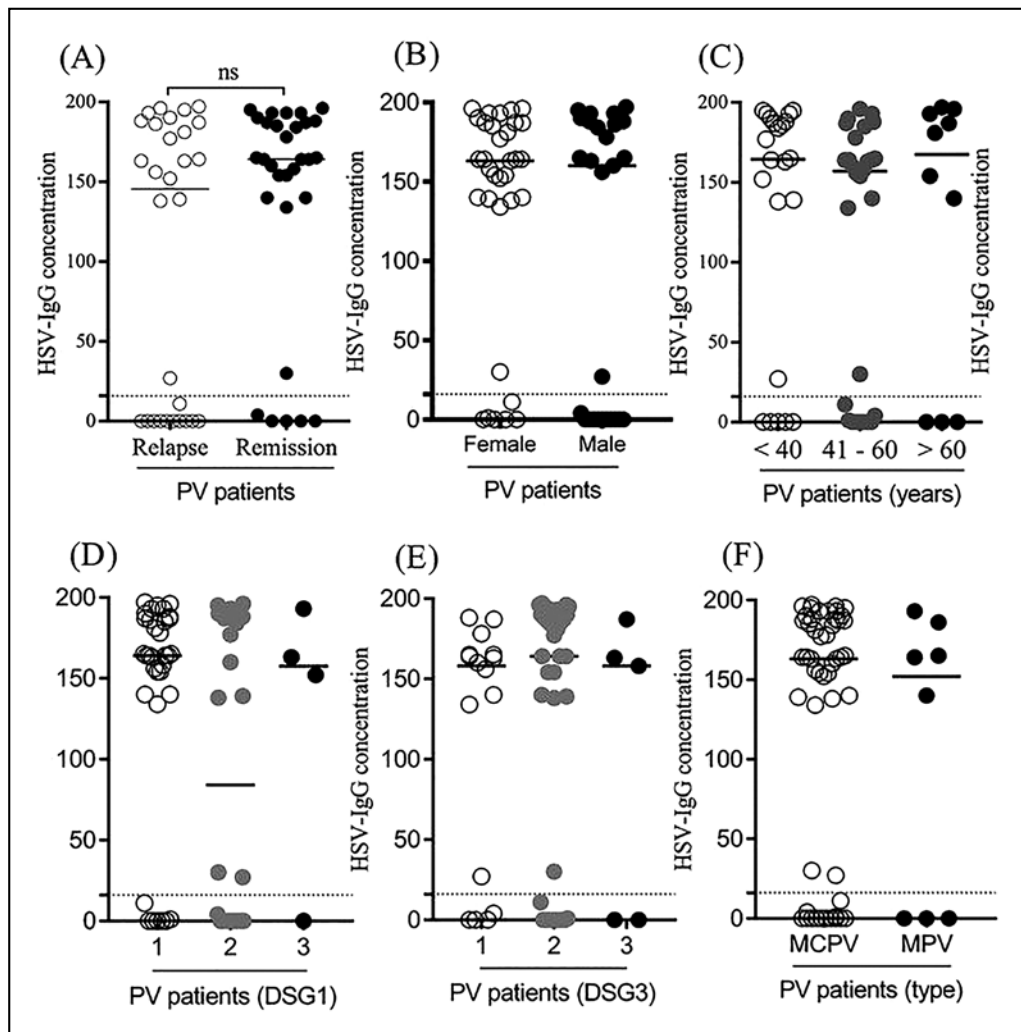


Figure 1. Level of HSV-IgG antibody (cut-off value: 22 RU/mL) according to baseline variables.

TABLE 5. Multivariable linear regression model to assess the main correlates of CMV-IgG antibody in patients with pemphigus vulgaris

	Unstandardized Coefficients		Standardized Coefficients	t	P Value	
	(Constant)	B	Std. Error			Beta
Sex		-62.825	114.201		-0.550	0.585
Age		28.334	12.926	0.281	2.192	0.033
Type		1.196	0.560	0.303	2.135	0.038
Duration		24.026	18.083	0.163	1.329	0.190
Form		-0.515	1.623	-0.042	-0.317	0.752
Hypertension		-2.244	13.650	-0.022	-0.164	0.870
hyperlipidemia		-14.238	24.124	-0.110	-0.590	0.558
Diabetes		12.675	31.886	0.070	0.397	0.693
Coronary dis.		35.510	23.115	0.228	1.536	0.131
Smoking		-46.372	31.389	-0.202	-1.477	0.146
(Constant)		39.650	37.693	0.142	1.052	0.298

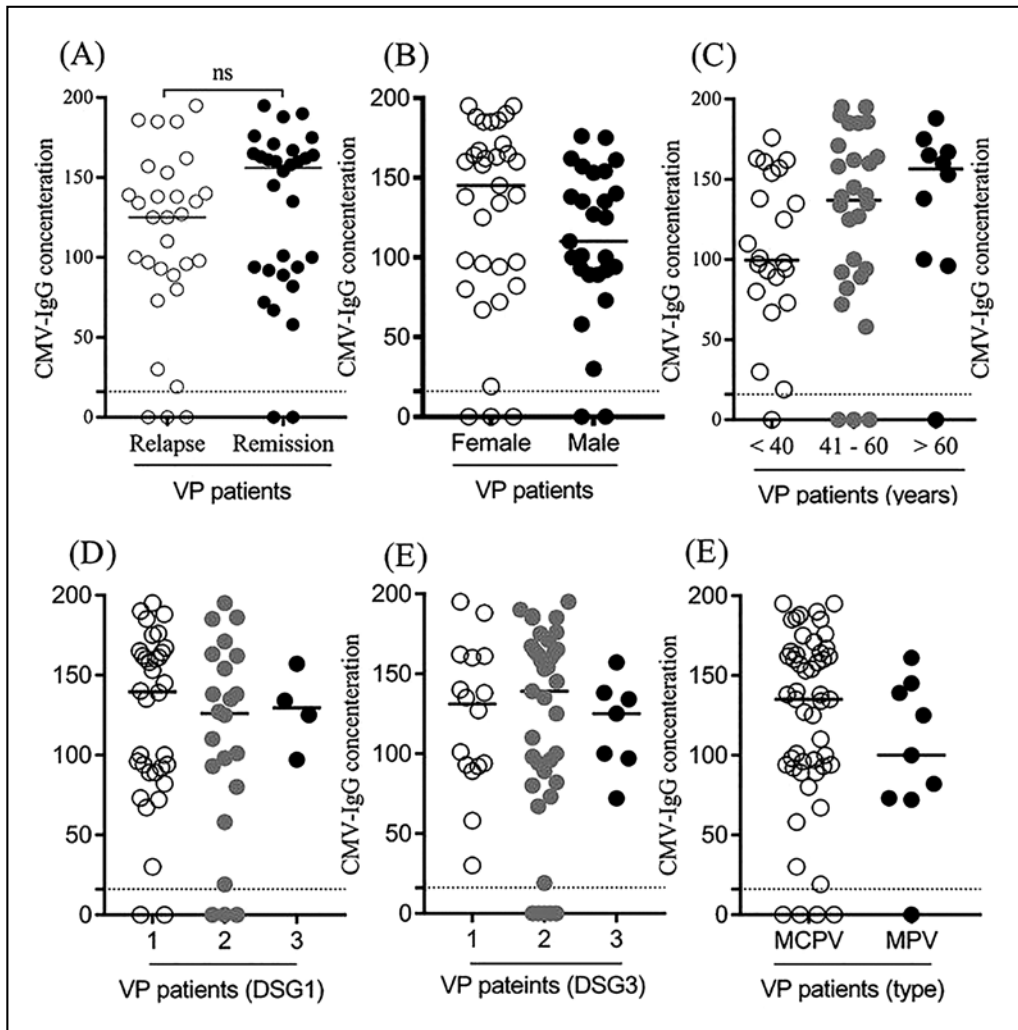


Figure 2. Level of CMV-IgG antibody (cut-off value: 22 RU/mL) according to baseline variables.

diseases such as systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and diabetes mellitus [24]. In this regard, efforts have been made to detect viral pathogens in blood samples and skin specimens of patients with pemphigus by techniques such as immunochemistry and PCR for DNA particles. The association between onset or reactivation of pemphigus disease and viral infections may be causal, or as a result of drug-induced immunosuppression which can increase the likelihood of viral infections, or established on the pathogenic connection between viral infection and immune dysregulation leading to autoimmunity. The mechanism of viral induction of autoimmunity can be explained in several ways, including molecular mimicry (due to cross-reactions between pathogen-derived epitopes and self-derived epitopes); bystander activation (due to activation of preexisting autoreactive T cells as a result of the inflammatory environment); epitope spreading (when antigen-presenting cells recognize self-antigens which were not initially directed by the immune system); polyclonal activation (due to infection of B cells by lymphocytotropic viruses which lead to B cell proliferation and increased antibody production); and viral superantigens (encoded by some viruses, are able to activate polyclonal T cells) [25]. Molecular mimicry is possibly the most acceptable explanation for the association of viral infections and pemphigus. Further explanation is that viruses upregulate production of interferon and interleukins. High levels of interferon gamma lead to increased expression of HLA2 on keratinocytes, making the pemphigus antigen structural site active. Also, overproduction of IL4 and IL10 causes a shift of TH1 to TH2 response which potentiates antibody response [26,27].

The role of viruses in the pathogenesis of pemphigus has been assessed in some previous studies. The importance of viral infections as an exacerbating factor of PV is neglected by some clinicians. Concerning this, in our current case-control study, we evaluated the probable role of HSV1/2 and CMV in the exacerbation of PV. In our study, a significantly higher number of PV patients (regardless of disease activity) were positive for HSV- and CMV-IgG antibodies, similar to the studies by Tufano et al and Sagi et al [28,29]. This may show a history of HSV and CMV exposure in most patients and may indicate more frequent recrudescence of latent viruses [30]. A significant increase in IgG antibody levels, discovered by comparing relapse and remission samples, indicates an active infection. According to a general consensus, relatively higher IgG levels are associated with a higher probability of having an active viral infection and relatively lower IgG levels represent a previous viral infection rather than an active viral infection. In this regard, Senger et al showed higher levels of HSV1 antibody in patients in the active phase of disease than in remission patients [31]. In contrast to our study, there was no significant difference in antibody levels between relapse and remission patients. In the studies of Marzano et al and Rah-

mati-Roodsari et al, similar to our study, only 1 patient had a positive HSV PCR result for skin samples [32,33]. These values are less than the findings of the study by Tufano et al, reporting positive HSV PCR in 5 of 7 skin specimens [29]. Other PCR results show evidence of HSV1 infection in 10% of all blood samples and CMV infection in 16.6% of all blood samples, 10% of all skin samples, and 10% of all throat samples. On the other hand, several studies failed to detect herpes DNA viruses in pemphigus patient specimens [26,27]. Senger et al showed the role of HSV1 in exacerbation of pemphigus by using the ELISA technique [31]. However, we found no correlation between HSV and CMV PCR results or IgG antibody levels and disease activity (relapse or remission). These findings cannot rule out the role of these viruses in exacerbation of the disease; improvement in some patients, especially those with the mucosal form, after receiving antiviral therapy as well as exacerbation of pemphigus after HSV infection are clinical evidence of the possible role of viruses in disease exacerbation [22]. Therefore, the role of viruses in exacerbation of pemphigus remains a hypothesis and requires further study.

Our study is unique for several reasons. First, we used both ELISA and PCR techniques to find evidence of viruses, whereas most previous studies used only one technique. Second, the samples were sourced from several sites including skin, throat, and blood. Third, we evaluated virus status in relapse and remission PV patients to determine the role of viruses in the exacerbation of the disease, whereas most previous studies examined the status of viruses in newly diagnosed patients and healthy controls to determine the etiological role of viruses. However, this study had some limitations. We could not evaluate other types of pemphigus disease due to their lower prevalence. Because of high costs of the techniques, we were able to investigate only the role of HSV1/2 and CMV in the exacerbation of disease.

Conclusions

In conclusion, our study could not demonstrate the role of HSV1/2 and CMV as triggering factors for PV exacerbation. Further studies are needed to evaluate the potential role of these viruses in PV exacerbation.

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