

# Assessment of IgG Antibodies Against HSV1, HSV2, CMV and EBV in Patients with Pemphigus Vulgaris versus Healthy People

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## Abstract

**Objectives:** Regarding the implication of viruses particularly herpes in pemphigus vulgaris, we sought to assess and compare the level of immunoglobulin G (IgG) antibodies against herpes simplex virus types 1 and 2 (HSV1 and HSV2), cytomegalovirus (CMV) and Epstein-Barr virus (EBV) in patients with pemphigus vulgaris and healthy people.

**Materials and Methods:** In this cross-sectional study, 25 patients with pemphigus vulgaris and 27 healthy individuals comprised the experimental and control groups, respectively. Serum samples were taken from both groups; the levels of IgG antibodies against HSV1, HSV2, CMV and EBV were measured using ELISA.

**Results:** Immunoglobulin G titer was higher for all four viruses in the patient group in comparison to the control group. This difference was significant for anti-EBV (P= 0.005), anti-CMV (P=0.0001) and anti-HSV2 (P=0.001) but not significant for anti-HSV1 (P= 0.36).

**Conclusion:** Viruses including EBV, CMV, and HSV2 probably play a role in the pathogenesis of pemphigus in addition to the effects of genetics, toxins and other predisposing factors. In this study, no statistically significant relationship was observed between HSV1 and pemphigus vulgaris, which was probably due to the high titer of anti-HSV1 IgG in healthy individuals in the community. More studies must be done in this regard.

**Key words:** Cytomegalovirus; Epstein-Barr Virus Infections; Simplexvirus; Immunoglobulin G; Pemphigus vulgaris, familial

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## INTRODUCTION

Pemphigus disease is classified as an autoimmune disease in which auto-antibodies act against surface glycoproteins of epithelial

cells for some unknown reason. The most common type of this disease associated with the oral mucosal lesions is pemphigus vulgaris. Oral lesions in most cases are the first

symptoms, and treatment is extremely difficult. As a result, these lesions appear earlier and disappear later than all other types [1].

Nowadays, it is commonly believed that autoimmune diseases are the result of multiple factors. Among the factors, the role of endogenous (genetic) and exogenous factors in the prompting autoimmune diseases has been more significant [2].

Exogenous factors include viruses and previous infections that could be effective in setting off, triggering, and even causing progression of autoimmune diseases. There are several reports indicating the possible role of viruses, particularly that of the herpes family [2]. Herpes viruses are among the most important human pathogens.

The prominent feature of herpes viruses is their role in causing infections that have the ability to survive in the host for a lifetime and activate periodically [3].

Several studies have been conducted aiming at isolating viruses and/or triggering an immune response against a possible viral cause of pemphigus [4]. Some confirmed the role of viruses in this disease, but others rejected such a role [5,6].

In various studies, the role of viruses namely EBV, CMV, HSV1 and HSV2 in patients with pemphigus vulgaris has been confirmed. These studies have also proven the existence of the DNA of viruses in the blood smear and peripheral skin lesions of patients with pemphigus using polymerase chain reaction (PCR).

The presence of the antibody of these viruses in the blood has also been confirmed [7-10]. According to some studies, viral cultures can be as valuable in the diagnosis of herpes simplex as PCR; therefore, in patients with pemphigus resistant to treatment such viral cytological/serological examinations as well as PCR should be done [11].

In addition to the role of these viruses in the occurrence of the disease, the findings of various research studies indicated that

infections caused by these viruses, particularly by herpes simplex, can increase pemphigus mortality [12].

Viruses of the herpes group, especially HSV as well as CMV, can play an important role in development of oral lesions of pemphigus patients [13-21]. The onset of pemphigus after Cocksackievirus infection has also been reported [22].

The measurement of IgG can be a diagnostic tool. Clinically, measured IgG antibody levels are generally considered to be indicative of an individual's immune response to particular pathogens. A common example of this practice is titers drawn to demonstrate serological immunity to viruses. Specific IgG against viruses is produced one to two weeks after initial infection and increases to maximum titer in four to eight weeks and then decreases [23]. Considering the possible role of viruses, particularly herpes viruses in pemphigus and given that studying the relationship between the diseases and these viruses can play an important role in the diagnosis and treatment of these conditions, the goal of this study was to compare the level of IgG antibodies against HSV2, HSV1, CMV and EBV in patients with pemphigus vulgaris and healthy controls.

## MATERIALS AND METHODS

In this cross-sectional study, serum samples taken from 25 patients with pemphigus vulgaris and 27 healthy controls were evaluated. Participants of the patient group were selected from people referring to the Oral and Maxillofacial Department and Alzahra Hospital of Isfahan University of Medical sciences. Participants of the control group were selected from the personnel of these centers. This study was approved by the institution's Ethics Committee. All participants signed an informed consent form.

Initially, a detailed medical history was taken from the patients. Oral lesions were studied and a detailed history of the patient's lesions was assessed.

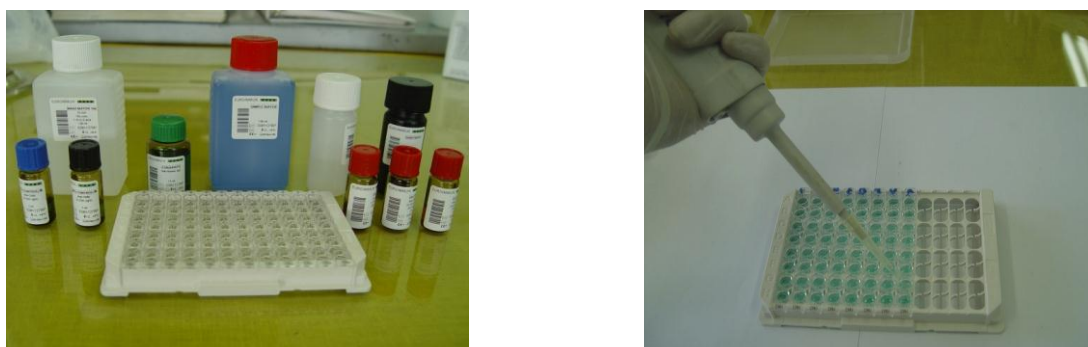


Fig. 1. Indirect ELISA

After biopsy, the skin lesions or mucosal samples were sent to a pathology lab and diagnosis of pemphigus vulgaris was confirmed based on histopathological pattern and direct immunofluorescence. Patients taking drugs such as captopril and D-penicillamine who fell into the category of drug-induced pemphigus and those diagnosed with cancer or leukemia, thymoma, and other malignancies that could cause paraneoplastic pemphigus were excluded from the study. The control group included healthy individuals who experienced no illnesses and were not taking any drugs. Blood samples were taken from patients and healthy individuals. After preparation, the samples were transferred to test tubes and centrifuged. Separated serums were isolated in separate tubes and were maintained at a temperature of  $-20^{\circ}\text{C}$ .

Indirect ELISA was employed. Measurement kits were used to assess anti-EBV (IgG), anti-CMV (IgG), anti-HSV2 (IgG), and anti-HSV1 (IgG) (Fig. 1). The data were presented as mean and standard deviation. SPSS software and independent t-test were used for statistical analysis and  $P < 0.05$  was considered statistically significant.

## RESULTS

In this study, 25 patients with pemphigus vulgaris, including 11 males and 14 females with a mean age of 36.6 years (range 25 to 48 years) were studied.

The control group of this study included 27 healthy individuals; 15 males and 12 females with a mean age of 36.6 years (range 25 to 45 years) who were healthy and were not taking any medications.

**Table 1.** Average anti-virus immunoglobulin G for Epstein-Barr virus, Cytomegalovirus, herpes simplex virus type 1 and herpes simplex virus type 2 separately for each study group

Immunoglobulin G anti-virus(UL/ML)		Descriptive Statistics					P value
		N	Minimum	Maximum	Mean	Std. Deviation	
Epstein-Barr virus	Patients	25	10.28	232.18	116.92	72.03	0.005
	Control group	27	9.74	203.04	77.07	71.25	
Cytomegalovirus	Patients	25	9.11	221.38	158.24	43.25	0.0001
	Control group	27	41.83	181.64	104.22	49.47	
Herpes simplex virus type 1	Patients	25	15.78	184.55	108	42.59	0.36
	Control group	27	15.59	273.69	95.11	57.40	
Herpes simplex virus type 2	Patients	25	22.55	132.12	73.8	36.57	0.001
	Control group	27	13.20	100.47	42.55	25.81	

The mean levels of anti-EBV, anti-CMV, anti-HSV1 and anti-HSV2 IgG in the study group are presented in Table 1. The difference in the mean levels of anti-EBV ( $P=0.005$ ), anti-CMV ( $P=0.0001$ ) and anti-HSV2 IgG was statistically significant ( $P=0.001$ ), but this difference was not significant for anti-HSV1 IgG ( $P=0.36$ ).

## DISCUSSION

Viral infections are the trigger factors of many autoimmune diseases such as type 1 diabetes mellitus, rheumatoid arthritis, lupus erythematosus, multiple sclerosis, Sjogren's syndrome, and Hashimoto's thyroiditis [11].

Several attempts have been made to isolate a virus from the blood, blister fluid and skin lesions in pemphigus patients, but no definitive results have been achieved from these studies [14]. The purpose of this study was to search for the presence or absence of a relationship between pemphigus vulgaris and viral infections, particularly herpes virus.

Comparison of the levels of anti-EBV IgG showed highly significant levels of antibodies in patients with pemphigus in comparison with the controls. The results of our study were consistent with those of Tufano et al, [6] in 1999, who observed the DNA of the EBV in peripheral blood smears and skin biopsies of 15% and 5% of patients, respectively.

Barzilai and colleagues [8] also found that anti-EBV IgG titer was higher in patients with pemphigus vulgaris compared with healthy controls, which conforms with the results of our study. These results support the hypothesis that EBV is truly associated with many autoimmune diseases including pemphigus vulgaris.

Regarding the level of anti-CMV IgG, the results of our study also showed that antibody titer increased significantly in patients with pemphigus vulgaris in comparison with the control group. These results are also consistent with those of Tufano et al, [6] where the anti-CMV IgM was found in 10% of pemphigus patients. The results of the study by Barzilai et

al. [8] showed anti-CMV antibody titer in 1565 sera from 23 different autoimmune diseases, including pemphigus vulgaris. Although higher antibody titer against CMV was observed in several diseases tested, including pemphigus, the increase was significant only in systemic lupus erythematosus patients. In a study by Kalra et al, [13] 16.6% of pemphigus vulgaris patients had anti-CMV IgM, which indicated acute infection and the probable role of CMV infection in the initiation and exacerbation of pemphigus in these patients. The results of our study also supported their findings. Therefore, it can be said that there is a strong relationship between CMV infection and the onset and exacerbation of pemphigus in genetically susceptible individuals.

The results of our study on HSV1 antibody showed an increase in the level of antibody titer in patients with pemphigus compared with the control group, but this difference was not significant. Likewise, numerous studies have reported the activation of pemphigus disease following herpes simplex infection [6,11,17,18]. In the study by Tufano et al, [6] the presence of the DNA of HSV1 and HSV2 was confirmed in 50% of peripheral blood smear cells and 71% of skin biopsies.

Also, in 95% of patients, anti-HSV IgG was higher than 1:8 titers. However, to determine whether the obtained results are really significant or not, it is better to study a larger number of participants so that the minor differences between the two groups can be interpreted correctly. The results of our study were also consistent with those of the study by Kalra et al, [13] in which patients who had more than 50% of mucosal involvement were positive for HSV antigen in oral scrapings and patients with less than 50% mucosal lesions were negative for HSV antigen. Kurata et al. [20] examined saliva for the presence of HSV DNA after the onset of pemphigus vulgaris initially localized to the oral lesions; they successfully detected high levels of HSV DNA in the saliva samples from six of 16 patients

with pemphigus vulgaris at the earliest stage. In addition, anti-HSV IgG titers in patients with pemphigus vulgaris were significantly higher than those in patients with virologically confirmed HSV-induced disorders.

This should also be mentioned that as far as the authors are concerned, all studies on HSV have been carried out without isolating HSV1 and HSV2; therefore, this study was the first to isolate HSV1 and HSV2 and their relationship with pemphigus vulgaris.

The results of our study showed a significant rise in HSV2 antibody titer in pemphigus vulgaris patients compared to the control group. Only one case has been reported by Kalajian and Callen [19] in 2007, where the disease manifested genital lesions and following diagnostic procedures, pemphigus vulgaris with herpes infection was diagnosed. This clarifies the fact that HSV2 infection may play a role in the onset and exacerbation of pemphigus. Results of the current study are also consistent with those obtained by Kalajian and Callen [19].

## CONCLUSION

Since herpes family of viruses poses a potential risk on patients with skin diseases such as pemphigus, it is necessary to use the best diagnostic techniques to discover the symptoms of such infections in these patients and employ the most effective anti-viral treatment to cure them.

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