HLA-G in patients with pemphigus vulgaris: does it correlate with disease severity?

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Background: Pemphigus vulgaris (PV) is an autoimmune disease with worldwide distribution. Human leukocyte antigen G (HLA-G) is postulated to be associated with this inflammatory and autoimmune condition. However, its role has not been well established in the literature. The study aimed to evaluate the plasma level of HLA-G in PV patients and assess its correlation with disease severity and compare it with normal subjects.

Methods: Thirty PV patients were enrolled in this cross-sectional study. A blood sample was taken from each participant; samples were analyzed for the soluble HLA-G (sHLA-G) plasma level by applying an ELISA kit (sHLA-G ELISA kit; Exbio, Czech Republic). Patients' clinical and demographic data were recorded and analyzed.

Results: Higher levels of sHLA-G were seen in PV patients compared to the control group ($P < 0.05$). There was a negative linear relationship between plasma HLA-G level and PV based on all ABSIS indices except for oral involvement ($-1 < R < 0$); however, these correlations were not statistically significant ($P \geq 0.05$).

Conclusion: Our data showed higher plasma sHLA-G levels in PV patients, which did not correlate with disease severity.

Keywords: pemphigus vulgaris, HLA-G Antigens, plasma

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INTRODUCTION

Pemphigus vulgaris (PV) is a group of threatening bullous autoimmune diseases that involve skin and mucosa membranes [1]. Histologically, there are intra-epidermal blisters and decreased cell-cell adhesions of keratinocytes [2]. PV has a worldwide distribution, and its incidence ranges between 0.5 to 3.2 per 100,000 persons. Its incidence rate has been estimated at 1 per 100,000 persons per year in Iran [3].

PV is a dangerous disease, and lack of treatment greatly increases morbidity and mortality risks [4]. Diagnosis of this disease is based on clinical

manifestations, pathology, and immunopathology findings. Determining IgG antibodies on keratinocytes' cell surface is characteristic for PV diagnosis [5].

Although PV has genetic underpinnings, it has not been recognized as an inheritable disease. The most significant sources of information supporting this theory are studies conducted on the association between HLA and PV. Some studies indicate a meaningful relationship between PV and HLA-A10 [6,7], while the disease has been associated with HLA-DRW4 in Jewish patients [8].

Human leukocyte antigen G (HLA-G) is one of the main MHC class 1 antigens coded by genes in the 6P21 location. HLA-G has limited tissue distribution and polymorphisms. Due to these characteristics, it is different from other class 1 HLA molecules [9]. It has been proven that HLA-G is expressed under physiologic conditions in a few tissue types such as the cornea, thymus, erythroid precursor cells, and endothelial cells [10,11].

It seems that the main source of this molecule's production is activated monocytes that have the CD14 marker [12]. Both mRNA and HLA-G are found in decreased or inappropriate amounts in some pathological conditions such as eclampsia or recurrent spontaneous abortions [13,14]. Therefore, there is significant evidence that HLA-G has a major role in several pathological conditions. In fact, HLA-G can induce two different effects in pathological conditions: it can be protective in inflammatory and autoimmune conditions or dangerous in infections and tumors [15].

HLA-G expression is associated with malignant transformation, advanced clinical stages, and high histological grades of malignancies. It is used as a prognostic indicator; elevated amounts of HLA-G in biopsy specimens and its soluble form in plasma (sHLA-G) are significantly associated with poor prognosis in several forms of malignancies [16].

Few studies have been conducted on the role of HLA-G in pemphigus disease, and most of them were done on skin biopsy specimens. Genetic diversities in different races can affect results in studies. Researchers of the current study believe that if the plasma HLA-G level is significantly correlated with disease variables in PV patients, it could be used for diagnostic or therapeutic purposes or even as a prognostic factor.

MATERIALS AND METHODS

A total of 60 subjects were enrolled in this study. The first group included 30 newly diagnosed patients with PV referred to outpatient dermatology clinics or inpatient wards of the Emam Reza and Ghaem hospitals (Mashhad, Iran) between June 2013 and March 2015. The second group included 30 healthy people as controls.

Two attending dermatologists examined the patients independently and documented their medical history via structured interviews. PV diagnosis was based on clinical manifestations, as well as histopathological and direct immunofluorescence results. Eligible subjects were enrolled after providing informed consent. The inclusion criteria encompassed newly diagnosed PV patients from the outpatient clinic or dermatology wards of Emam Reza and Ghaem hospitals (Mashhad, Iran). Patients with congenital heart diseases, severe malnutrition, chronic renal failure, diabetes mellitus, and evidence of systemic infection were excluded.

PV patients were assessed based on the Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) for determining the extent and severity of the disease.

None of the enrolled patients received treatment until after blood samples were collected. The control group was matched with the patient group based on gender and age, and there was no family or personal history of autoimmune disease.

On admission, blood samples were collected from patients' venous blood. An ELISA kit (sHLA-G ELISA kit; Exbio, Prague, Czech Republic) was used to measure the plasma sHLA-G level according to the manufacturer's instructions to avoid technical errors and obtain accurate results. All specimens were tested twice in the laboratory.

Statistical analysis

Data analysis was done using the SPSS software (version 16, US). A descriptive analysis was used to compare the patient and control group averages. The Kolmogorov-Smirnov test was applied to verify the normal distribution of data in both groups. Due to the abnormal distribution of data in both groups, coefficients for HLA-G concentrations and other possible correlated factors were determined

using the Mann-Whitney and Kruskal-Wallis tests where appropriate. Differences were considered significant when $P < 0.05$.

RESULTS

There were 15 male and 15 female participants in each group. The mean age of participants was 50.5 ± 14.84 years in the patient group and 38.97 ± 10.8 years in the control group; the age of the subjects in the patient group was significantly higher ($P = 0.001$).

There was a direct correlation between plasma sHLA-G and age, but this relationship was not significant ($P = 0.47$, r = 0.096). Our findings also revealed no significant correlation between plasma sHLA-G level and gender ($P = 0.09$).

The median HLA-G level was 13.30 (10.74 - 44.20) for those who had pemphigus vulgaris, and this value was 11.03 (0.8-13.56) for the control group. The plasma sHLA-G level was significantly higher in the patient group compared to the control group ($P = 0.022$).

The correlation between sHLA-G level and severity of oral and skin involvement (each separately), the severity of skin involvement multiplied by its extent, and the total disease severity based on the ABSIS score are shown in Table 1. As it represents, no significant statistical relationship was found between these variables and the sHLA-G level ($P > 0.05$)

DISCUSSION

Human leukocyte antigen G (HLA-G) is not limited to protecting a fetus from the mother's immune system and is also expressed during viral infections and cancers. The presence of sHLA-G has been identified in various diseases. Plasma HLA-G is a unique and advanced biomarker used to confirm the diagnosis of some infectious diseases and cancers. Despite the ability to suppress the immune system, HLA-G is a great target for treating diseases such as multiple sclerosis [17]. Ectopic expression of HLA-G molecules is observed in non-physiological conditions such as viral infections, cancers, organ transplantation, and conditions of inflammation and autoimmune diseases [18-22].

Table 1. Correlation between plasma sHLA-G level and severity of pemphigus vulgaris

	R	*P*-value
Extent of oral involvement	0.148	0.435
Severity of oral involvement	-0.009	0.963
Extent of skin involvement	-0.254	0.176
Severity of skin involvement	-0.249	0.184
Severity of skin involvement multiplied by the extent	-0.193	0.306
Severity score of disease	-0.091	0.633

In a study by Yari *et al.*, HLA-G proteins were compared in sections prepared from healthy skin biopsies and individuals with pemphigus vulgaris (PV). In the skin of normal people, the expression of HLA-G was only at the transcriptional level. However, in skin samples taken from patients with PV, this expression was high and measurable at both transcriptional and translation levels. In addition, the RT-PCR multiplication pattern showed a decrease in HLA-G2 transcripts and an increase in HLA-G1 transcripts in PV patients relative to controls [23]. A review study by Gasit *et al.* aimed to clarify the association between HLA molecules and PV, demonstrating a relationship between PV and HLA-G in Jewish patients [24]. Our study also showed that HLA-G has a significant correlation with PV ($P = 0.022$), which is consistent with the results of other studies.

Our findings indicated a direct correlation between the plasma level of HLA-G and age in the pemphigus group ($P = 0.47$ and r = 0.09), although this was not statistically significant. Also, gender was matched between patient groups and their control groups, revealing no significant relationship between sex and plasma HLA-G levels in PV patients ($P = 0.09$).

Our study showed a reverse relationship between the severity of pemphigus in terms of the ABSIS index and the level of plasma HLA-G in PV patients ($P = 0.63$ and r = - 0.091).

The association between plasma HLA-G level and gender, age, and disease severity was not studied in any previously published research except for a study conducted by Cardili *et al.* in 2010; they assessed the association between the level of HLA-G expression and severity of psoriasis disease. The results were similar to our study, without a correlation between HLA-G expression and other variables [25].

In the present study, only the soluble form of HLA-G was evaluated and its level on the cell surface was not measured, limiting our study

results. Nevertheless, several studies have shown that the level of this molecule, both soluble and on the cell surface, increases in patients with PV. Also, the level of different HLA-G isoforms was not measured in this study. Meanwhile, it has been shown that in comparison with the control group, the rate of HLA-G1 transcription is increased and HLA-G2 is decreased in patients with PV [23].

Regarding the reverse relationship between PV severity and sHLA-G level, we note that our sample size was small. We think future studies with more samples can accurately prove this hypothesis.

CONCLUSIONS

Our study suggests that the plasma HLA-G level is significantly higher in pemphigus vulgaris patients compared to controls. However, this increase did not correlate with the disease severity. Therefore, our hypothesis of an association between the HLA-G level and the disease severity was disproven, warranting further studies in this area.

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Conflict of Interest: None declared.

Abbreviations

ABSIS, Autoimmune Bullous Skin Disorder Intensity Score; PV, Pemphigus Vulgaris; HLA-G, Human Leukocyte Antigen G; sHLA-G, Soluble Human Leukocyte Antigen G

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