BRIEF COMMUNICATION
Iran J Allergy Asthma Immunol
March 2009; 8(1): 53-56

Desmoglein ELISA in the Diagnosis of Pemphigus and Its Correlation with the Severity of Pemphigus Vulgaris

Hossein Mortazavi[1], Majid Shahdi[1], Ali Akbar Amirzargar[2], Zahra S.Naraghi[3], Mahin Valikhani[1], Maryam Daneshpazhooh[1], Amir Vasheghani-Farahani[1], Mojtaba Sedaghat[4], and Cheyda Chams-Davatchi[1]

[1] Department of Dermatology, Autoimmune Bullous Diseases Research Center, School of Medicine, Tehran University of Medical Sciences, Tehran Iran
[2] Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran Iran
[3] Department of Pathology, School of Medicine, Tehran University of Medical Sciences, Tehran Iran
[4] Department of Community Medicine, School of Medicine, Tehran University of Medical Sciences, Tehran Iran

Received: 2 October 2007; Received in revised form: 16 September 2007; Accepted: 12 February 2008

ABSTRACT

Anti-desmoglein 3 and 1 autoantibodies are involved in the pathogenesis of pemphigus diseases. Our objective was to assess the value of ELISA in the diagnosis of pemphigus and its correlation with the severity of pemphigus vulgaris.

Based on clinical presentation and histopathologic confirmation for the diagnosis of the pemphigus, 38 patients took part in the study. Sera of the patients were tested by desmoglein 1 and desmoglein 3 ELISA. Also, direct immunofluorescence was performed for all patients which revealed positive results in 36 patients (94.7%).

ELISA was positive in 37 of 38 pemphigus patients (Sensitivity: 97.3%). The relationship between desmoglein 1 index values and skin severity was statistically significant ($p<0.05$). Desmoglein 3 index values increased with oral severity although this was not statistically significant. Iranian patients similar to Indian patients had higher positive anti-desmoglein 1 autoantibodies.

Desmoglein-ELISA test is appropriate in the diagnosis of pemphigus. Desmoglein 1 index value is statistically correlated with the severity of pemphigus vulgaris.

Key words: ELISA; Desmoglein 1; Desmoglein 3; Pemphigus vulgaris

INTRODUCTION

Pemphigus Vulgaris (PV) and Pemphigus foliaceus (PF) are characterized by the presence of autoantibodies against desmosomal glycoproteins including desmoglein 3 (Dsg3) and desmoglein 1 (Dsg1).[1]

Corresponding Author: Maryam Daneshpazhooh, MD; Department of Dermatology, Razi Hospital, Vahdate Eslami Sq. Tehran, 11996, Iran.
Tel: (+98 21) 5561 8989, Fax: (+98 21) 5561 8989,
E-mail: maryamdanesh@yahoo.com or mortazavi_ho@yahoo.com

Circulating anti-Dsg1 and/or anti-Dsg3 IgG autoantibodies are present in patients with pemphigus.[2] Direct immunofluorescent (DIF) and indirect immunofluorescent (IIF) methods, as well as ELISA are used for the detection of pemphigus auto-antibodies. ELISA is a quantitative method for measuring antibody levels and a useful test for the diagnosis of pemphigus.[2,3] This method is more sensitive and specific than IIF. It is also superior to sophisticated immunoblotting techniques.[3,4] Furthermore, it has been shown that the sera of patients

in the active phase of pemphigus contain higher levels of anti-Dsg antibodies including IgG4 and IgG1 antibodies.[5] Moreover it has been reported that the severity of skin and mucosal lesions of pemphigus is related to anti-Dsg1 and anti-Dsg3 antibody levels.[6]

In PV, also the antibody profile correlates with the clinical phenotype of pemphigus, namely cutaneous (C), mucocutaneous (MC), and mucosal (M).[7]

In this study, the value of ELISA was determined in the diagnosis of pemphigus disease. Also, we tried to find relationships between the severity of PV and ELISA Dsg3 and Dsg1 index values (as a marker for anti-Dsg antibody levels). The influence of ethnicity on the results of ELISA[8,9] additionally highlights the importance of such a study among Iranian patients.

PATIENTS AND METHODS

Based on clinical presentation and histopathology, from July 2003 to July 2004, thirty eight pemphigus patients (35 PV, 3 PF) took part in this study. Patients included 25 women and 13 men. Mean age was 46.6 $\pm$16.5 years (17 to 75). Thirty out of 38 patients were new patients and eight patients were in relapse. The 30 new patients included 27 PV and 3 PF patients. At the time of admission, the mean duration of disease in new PV patients was 13.8±11.2 weeks. The eight relapsed patients showed severe flare-up of PV either under treatment of corticosteroid (mean: 23.125 mg/day in 4 patients) or after discontinuation of medications (in 4 patients). Dsg ELISA testing was performed on the sera of 38 patients. Treatment of PV patients was started with 2 mg/kg/day prednisolone plus 2 mg/kg/day azathioprine. Informed consent was obtained from all patients.

We classified the severity of the disease according to previous works.[6,7] The skin severity of pemphigus vulgaris patients was graded according to the involvement of body surface area (BSA) by vesicles, bullae, and erosions as follows: grade 0, no involvement; grade 1, mild disease (up to 10% BSA involvement); grade 2, moderate disease (11 to 30% BSA involvement); grade 3, severe disease (>30% BSA involvement). Also mucosal involvement was graded as follows: grade 0, no mucosal involvement; grade 1, mild disease (only one site with mild involvement); grade 2, moderate disease (only one site but severely involved); grade 3, severe disease (extensive oral erosions).

Anti-desmoglein autoantibodies were detected by ELISA method (kits from Medical and Biological Laboratories Co Ltd. Nagoya, Japan) and index values were calculated based upon manufacturer's instructions using 100-fold serum dilution. The index value of positive reactions was considered greater than 7 and 14 for Dsg3 and Dsg1, respectively. ELISA results of (Dsg3+, Dsg1±) and (Dsg3-, Dsg1+) are compatible with the diagnosis of PV and PF, respectively. Index values up to 150 have linear correlation with anti-Dsg antibody levels.[8] In case of negative ELISA or incompatible test with histopathology, the test was repeated. According to manufacturer, the sensitivity and specificity of ELISA kits were 96.1% and 86.3% for Dsg1 versus 100% and 70.6% for Dsg3, respectively. Direct immunofluorescence similar to procedure by Aithal et al.[10] was performed on skin specimens of all patients.

Kruskal-Wallis test was used to evaluate correlation of disease severity with desmoglein index values. New PV and recurrent patients as well as severity groups (0-4) were compared using Mann-Whitney test.

RESULTS

Dsg ELISA was positive in 37 patients (97.3%) and DIF in 36 patients (94.7%). In 32 out of 35 patients histologically diagnosed as PV, anti-Dsg antibody profile was compatible with PV. In two patients histologically diagnosed as PV, anti-Dsg antibody was in favor of PF. Only in a 38 year-old woman with 6 month history of oral involvement, ELISA was negative incompatible with histopathological and DIF findings in favor of PV. In the 3 patients diagnosed by histopathology as PF, anti-Dsg antibody profile was also in favor of PF. In 8 recurrent PV patients (one M, two C and five MC), concordant with histopathology, anti-Dsg1 and anti-Dsg3 were positive in all patients (100%). The phenotypic distribution of 27 new PV patients and ELISA results are shown in Table 1.

Table 1. ELISA results in different clinical phenotypes of 27 new PV patients

Clinical phenotype	No. of patients	No. (%) of patients with	
		Anti-Dsg1*	Anti-Dsg3
Mucosal	3	0 (0)	2 (66.66)
Cutaneous	5	4 (80)	4 (80)
Mucocutaneous	19	15 (78.94)	18 (94.73)
Total	27	19 (70.37)	24 (89)

* Dsg: desmoglein.

Skin and oral severity of 27 new PV patients and 8 recurrent patients according to Dsg1 and Dsg3 index values are shown in table 2. Dsg1 index values increased significantly in accordance with skin severity (P=0.038). There was no statistically significant relationship between Dsg3 index values and skin severity (P=0.211).

The mean index value of Dsg3 increased insignificantly with oral severity (P=0.241).

There was no statistically significant relationship between severity of disease and mean index values of Dsg1 and Dsg3 in eight recurrent patients.

Mean levels of Dsg 1 and Dsg 3 index values in new and recurrent PV patients were not statistically different (111.22±122.04 to 118.50±106.49 for Dsg1 and 174.30±26.82 to 259.75±37.67 for Dsg 3 in new and recurrent PV patients, respectively).

DISCUSSION

Histopathology is still used as the routine diagnostic test for pemphigus. Though DIF is essential in the diagnostic work-up of pemphigus diseases, its sensitivity is not 100% (10, 11). In patients with the disease duration of less than 3 months, it is shown that DIF provided sensitivity as low as 46.7%.[10]

In our study, the sensitivity of Dsg ELISA (97.3%) was comparable with previous studies[1,2] and higher than DIF (94.7%). Results for Dsg ELISA, similar to pathology, was in favor of PF in three PF patients.

On the other hand, in two patients with the clinicopathologic setting of PV, ELISA antibody profile was compatible with PF. Although transition of PV to PF has been reported,[12] clinical and histopathologic features were not suggestive for this assumption because of the incompatible results in the first patient with mucocutaneous phenotype. To describe the incompatibility in the second PV patient with cutaneous phenotype, we suggest that Anti-Dsg3 autoantibody level might be too low to cause mucosal lesions.[6,13] According to previous reports, some patients with the diagnosis of PV or PF may have anti-Dsg autoantibody levels below the cut-off value.[2] This fact could explain the only negative ELISA result in our study. Although the diagnosis of pemphigus is mainly based on histopathologic examinations and immunofluorescence tests (14), our study confirmed that the addition of Dsg ELISA to the above-mentioned tests increases the diagnostic yield.

Table 2. Skin and oral severity in 27 new and 8 recurrent PV patients according to Dsg1/3 index values

		Degree of severity	No. of patients	Mean of Dsg1 index value +SD*	P value	Mean of Dsg3 index value +SD	P Value
New Patients	Skin Severity	0	3	1.53+4.22	0.038	23.57+23.16	NS**
		1	4	93.95+117.68		173.50+133.48	
		2	11	81.16+68.78		220.00+153.53	
		3	9	192.22+154.96		169.04+124.32	
	Oral Severity	0	5	140.76+97.56	NS	104.00+112.51	NS
		2	8	117.68+154.59		146.39+179.40	
		3	14	97.00+115.82		215.36+115.96	
Recurrent Patients	Skin Severity	0	1	47.00+00.00	NS	310.00+00.00	NS
		1	1	28.00+00.00		227.00+00.00	
		2	2	113.50+119.50		183.00+24.04	
		3	4	161.50+121.60		293.75+140.14	
	Oral Severity	0	2	113.00+120.21	NS	213.50+19.09	NS
		1	1	29.00+00.00		166.00+00.00	
		2	1	71.00+00.00		108.00+00.00	
		3	4	155.50+127.98		344.25+69.48	

* Standard Deviation ** Statistically not significant

Eighty-nine percent of the new PV patients had anti-Dsg3 antibody. This finding is similar to previous studies.[9,15] On the other hand anti-Dsg1 autoantibody was positive in 70.3% of new PV patients. This finding showed that Iranian PV patients similar to Indian patients (75%) have higher frequency of positive anti-Dsg1 autoantibody compared with patients of north European origin.[7,9] Although all recurrent patients showed raised anti-Dsg1 and anti-Dsg3 autoantibodies, the number of patients was not enough to draw a conclusion.

Harman and his colleagues have reported a relationship between anti-Dsg1 and 3 autoantibody levels measured by Dsg ELISA and the severity score of cutaneous and oral involvement in pemphigus vulgaris, respectively.[6] Our results showed that index values of Dsg1, similar to findings by Sharma et al.[7] had statistically significant correlation with skin severity. There was an increasing trend of Dsg3 index values by the oral severity but in contrast to Sharma's findings, this correlation was not statistically significant. It may be due to the small sample size of our series.

Previously, Harman and colleagues showed that the presence of anti-Dsg1 autoantibodies could be suggestive of a more severe type of PV.[9] Thus, it appears that the anti-Dsg3 and specially anti-Dsg1 autoantibody levels could be good indicators of severity of PV and may be of great benefit in the management and monitoring of patients with PV and PF.[4,8,16]

In conclusion, the use of ELISA in combination with routine histopathology and immunofluorescence tests may be considered a helpful diagnostic tool in pemphigus. In addition to being helpful in differentiating pemphigus subgroups, it may increase the sensitivity of our diagnostic work-up.

REFERENCES

1. Harman KE, Gratian MJ, Seed PT, Bhogal BS, Challacombet SJ, Black MM. Diagnosis of pemphigus by ELISA: a critical evaluation of two ELISAs for the detection of antibodies to the major pemphigus antigens, desmoglein 1 and 3. Clin Exp Dermatol 2000; 25(3):236-40.
2. Amagai M, Komai A, Hashimoto T, Shirakata Y, Hashimoto K, Yamada T, et al. Usefulness of enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3 for serodiagnosis of pemphigus. Br J Dermatol 1999; 140(2):351-7.
3. Bhol K, Tyagi S, Natarajan K, Nagarawalla N, Ahmed AR. Use of recombinant pemphigus vulgaris antigen in development of ELISA and IB assays to detect pemphigus vulgaris autoantibodies. J Eur Acad Dermatol Venereol 1998; 10(1):28-35.
4. Ishii K, Amagai M, Hall RP, Hashimoto T, Takayanagi A, Gamou S, et al. Characterization of autoantibodies in pemphigus using antigen-specific enzyme-linked immunosorbent assays with baculovirus-expressed recombinant desmogleins. J Immunol 1997; 159(4):2010-7
5. Ayatollahi M, Joubeh S, Mortazavi H, Jefferis R, Ghaderi A. IgG4 as the predominant autoantibody in sera from patients with active state of pemphigus vulgaris. J Eur Acad Dermatol Venereol 2004; 18(2):241-2.
6. Harman KE, Seed PT, Gratian MJ, Bhogal BS, Challacombe SJ, Black MM. The severity of cutaneous and oral pemphigus is related to desmoglein 1 and 3 antibody levels. Br J Dermatol 2001; 144(4):775-80.
7. Sharma VK, Prasad HRY, Khandpur S, Kumar A. Evaluation of desmoglein enzyme-linked immunosorbent assay (ELISA) in Indian patients with pemphigus vulgaris. Int J Dermatol 2006; 45(5):518-22.
8. Cheng SW, Kobayashi M, Tanikawa A, Tanikawa A, Amagai M, Nishikawa T. Monitoring disease activity in pemphigus with enzyme-linked immunosorbent assay using desmoglein 1 and 3. Br J Dermatol 2002; 147(2):261-5.
9. Harman KE, Gratian MJ, Bhogal BS, Challacombe SJ, Black MM. A study of desmoglein 1 autoantibodies in pemphigus vulgaris: racial differences in frequency and the association with a more severe phenotype. Br J Dermatol 2000; 143(2):343-8.
10. Aithal V, Kini U, Jayaseelan E. Role of immunofluorescence on Tzank smears in pemphigus vulgaris. Diagnostic cytopathology 2007; 35(7):403-7.
11. Helander SD, Rogers RS. The sensitivity and specificity of direct immunofluorescence testing in disorders of mucous membranes. J Am Acad Dermatol 1994; 30(1):65-75.
12. Harman KE, Gratian MJ, Shirlaw PJ, Bhogal BS, Challacombe SJ, Black MM. The transition of pemphigus vulgaris into pemphigus foliaceus: a reflection of changing desmoglein 1 and 3 autoantibody levels in pemphigus vulgaris. Br J Dermatol 2002; 146(4):684-7.
13. Feliciani C, Motta A, Castellaneta M, Federica M, De Benedetto A, Toto P, et al. Coexisting pemphigus vulgaris and pemphigus foliaceus in the same patient. Int J Dermatol 2005; 44(2):139-41.
14. Mimouni D, Nousari CH, Cummins DL, Kouba DJ, David M, Anhalt GJ. Differences and similarities among expert opinions on the diagnosis and treatment of pemphigus vulgaris. J Am Acad Dermatol 2003; 49(6):1059-62.
15. Jamora MJ, Jiao D, Bystryn JC. Antibodies to desmoglein 1 and 3, and the clinical phenotype of pemphigus vulgaris. J Am Acad Dermatol 2003; 48(6):976-7.
16. Daneshpazhooh M, Chams-Davatchi C, Khamesipour A, Mansoori P, Taheri A, Firooz A, et al. Desmoglein 1 and 3 enzyme-linked immunosorbent assay in Iranian patients with pemphigus vulgaris: correlation with phenotype, severity, and disease activity. J Eur Acad Dermatol Venereol. 2007; 21(10):1319-24.