CORRELATION OF PEMPHIGUS VULGARIS ANTIBODY TITERS BY INDIRECT IMMUNOFLUORESCENCE WITH ACTIVITY OF DISEASE BASED ON PEMPHIGUS AREA AND ACTIVITY SCORE (PAAS)

H. Mortazavi, K. Kiavash*, N. Esmaili, Ch. Chams-Davatchi

Department of Dermatology, Pemphigus Research Unit, Razi Hospital, Medical Sciences/ University of Tehran, Tehran, Iran

Abstract- Indirect immunofluorescence (IIF) has been used to identify and measure autoantibody levels in pemphigus vulgaris but data about relationship between clinical severity of disease and antibody titers by IIF have been conflicting. We conducted this cross-sectional study to correlate the severity of oral and/or cutaneous involvement in patients with pemphigus vulgaris based on Pemphigus Area and Activity Score with IIF titers. Sixty-one new pemphigus vulgaris patients were included in this study. Human prepuce was used as substrate for IIF and assessment of disease severity was based on Pemphigus Area and Activity Score. The mean±SD age was 44.04±30.46 years, with a range of 18 to 79 years. IIF was positive in 56 (91.8%) patients. There was a significant relationship between total disease score and IIF titers ((P<0.001). Also a significant relationship was found between skin score (P=0.04) and mucosal score (P=0.04) with IIF titers. Our results show that there is a significant relationship between disease activity based on Pemphigus Area and Activity Score and antibody titers by IIF. Further studies are recommended to determine the usefulness of this technique for monitoring disease.

Acta Medica Iranica, 46(3): 239-244; 2008

Key words: Pemphigus vulgaris, Indirect Immunofluorescence, Pemphigus Area and Activity Score

INTRODUCTION

The term "pemphigus", derived from the Greek word *pemphix* (blister), describes a group of chronic bullous diseases of the skin and mucosa. The two main subtypes are *pemphigus vulgaris*, with suprabasal acantholysis causing separation of basal cells from keratinocytes of the stratum spinosum, and *pemphigus foliaceus*, with acantholysis in the granular layers of the epidermis (1). Pemphigus vulgaris is the most common type of pemphigus which occurs with a worldwide distribution (2). Almost all patients have mucosal involvement and in two third of cases pemphigus vulgaris starts with the involvement of oral mucosa. Most patients also develop cutaneous lesions. In rare cases, only cutaneous lesions exist (cutaneous phenotype). Therefore, clinically, three phenotypes of mucocutaneous, mucosal, and cutaneous of pemphigus vulgaris occur. Pemphigus vulgaris has a devastating impact on the health of affected individual and it could be lethal in the absence of treatment (3). Therefore, close monitoring of the treatment and course of the disease is of major importance.

Received: , Revised: , Accepted:

Corresponding Author: Katrin Kiavash
Department of Dermatology, Pemphigus Research Unit, Razi Hospital, Medical Sciences/ University of Tehran, Tehran, Iran
Tel:+98 21 22344220
Fax:+98 21 22344220
Email: kiavashkatrin@yahoo.com

Pemphigus vulgaris is an autoimmune disease. Autoantibodies in the sera of pemphigus patients were discovered first in 1964 (4). It then became clear that autoantibodies are directed against cell-cell adhesion molecules in desmosomes, desmogleins (Dsg). In pemphigus vulgaris autoantibodies are directed against Dsg1 and/or Dsg3 (5).

Knowledge about the pathogenesis of disease has provided the possibility of developing laboratory methods for evaluation and monitoring of the disease. Indirect immunofluorescence (IIF) is a two-step procedure used to identify circulating autoantibodies to cutaneous or mucosal structures in patient's serum. These antibodies are most commonly of IgG or IgA classes. In the first step, serial dilutions of the patient's serum in phosphate-buffered saline (PBS) are incubated with frozen sections of the substrate. In the second step, the bound autoantibodies are labeled with fluorescein isothiocyanate (FITC)-conjugated anti-human immunoglobulins. IIF has been used since 1960s to identify and measure autoantibodies levels in pemphigus vulgaris. However, the data have been conflicting and while some studies have shown that IIF titers are useful for monitoring disease activity (6-8), other studies have shown otherwise (9-11). Use of different methods for measuring disease severity is an obvious drawback of previous studies (12). To create a universally acceptable specific system for scoring the clinical severity and progression of pemphigus vulgaris, Agarwal *et al.* proposed a new clinical scoring method along the lines of the Psoriasis Area and Severity Index (PASI) in 1998 (12). In this method, Pemphigus Area and Activity Score (PAAS), factors taken into consideration to evaluate the activity of disease are a) number of new lesions per day, b) peripheral extension of existing lesions, and c) the presence of the Nikolsky's sign perilesionally or at a distant site.

We conducted this cross-sectional study to correlate the severity of oral and/or cutaneous involvement in patients with pemphigus vulgaris based on PAAS score with IIF titers of pemphigus autoantibodies (with regard to PV phenotypes).

MATERIALS AND MEHTODS

Sixty-one new pemphigus vulgaris patients, based on clinical examination, histopathology, and direct immunofluorescence, were included in this study. Subjects were selected consecutively from patients admitted to Razi Hospital, Tehran, Iran, from October 2006 to October 2007. Patients with immune deficiency or malignancy-induced pemphigus vulgaris and pregnant women were excluded from study.

After obtaining written informed consent, serum samples were collected and stored at –70° C. Human prepuce was used as substrate. Sera were diluted in PBS over a range of nine doubling dilutions from 1: 10 to 1: 2560. Serum and sections were incubated for 30 minutes at 37°C and after washing with PBS, incubated with conjugated anti-immunoglobulins antibody (Dako, USA) for 30 minutes at 37°C.

Table 1. PAAS for cutaneous lesions

Clinical Marker	Clinical Score						
	0	**1**	**2**	**3**	**4**	**5**	**6**
A. Activity							
(a) number of new blisters/day	0	1-5	6-10	11-20	>20	-	-
(b) peripheral extension of blisters	nil	mild	mod	extensive	-	-	-
(c) Nikolsky's sign	negative	perilesional	distant	-	-	-	-
B. Area (%)	nil	0-15	16-30	31-50	51-70	71-90	>90

Table 2. PAAS for mucous membrane lesions alone

Markers	Clinical Scores			
	0	1	2	3
Area	Nil	1 site	2 sites	> 2 sites
Severity	Nil	Mild	Moderate	Severe

Assessment of disease severity was based on PAAS. In this scoring system the body is divided into four segments and a score given for each segment. Based on the data achieved according to Table 1, total skin score is calculated as sum of head score [(a+b+c) × score of area] × 0.1, trunk score [(a+b+c) × score of area] × 0.3, upper limbs score [(a+b+c) × score of area] × 0.2, and lower limbs score [(a+b+c) × score of area] × 0.4. Criteria for scoring mucous membrane involvement are shown in Table 2. Mucous membrane score is calculated as area score plus severity score. Total score is calculated as sum of cutaneous score and mucous membrane score.

The statistical software SPSS 13 for Windows (SPSS, Inc., Chicago, IL) was used for data analysis. To investigate correlations, we used Pearson correlation coefficient and ANOVA tests. $P < 0.05$ was considered significant.

RESULTS

Sixty-one Iranian new pemphigus vulgaris patients (32 men, 29 women) were enrolled. The mean ± SD age was 44.04 ± 3.9 years, with a range of 18 to 79 years.

The most frequent phenotype was mucocutaneous in 33 cases (54.1%); mucosal and cutaneous phenotypes were seen in 19 (31.1%) and 9 (14.8%) patients, respectively. Mucosal involvement was seen in 52 patients; the oral mucosa was affected in all of the patients with mucous involvement.

Skin and mucosal scores of patient with mucocutaneous phenotype are depicted in table 3. The scores for patients with mucosal and cutaneous phenotypes were also recorded. It is obvious that skin score of mucosal phenotype as well as mucosal score of cutaneous phenotype is zero. Mean skin and mucosal score of each phenotype as well as IIF results are depicted in table 4.

IIF was positive in 56 patients (Sensitivity: 91.8%). The mean IIF titer was 0.01 (SD: 0.02). There was no significant difference in mean IIF titer in 3 phenotypes. There was no significant relationship between sex/age with disease severity (total, skin, and mucosal score). Also there was no significant relationship between sex/age with IIF titers.

There was a significant relationship between total disease score and IIF titers (P < 0.001) (Fig. 1). Also a significant relationship was found between skin score (P = 0.04) and mucosal score (P = 0.04) with IIF titers. In addition, there was a significant relationship between total disease score and IIF titers in patients with mucocutaneous (P = 0.03) and mucosal (P = 0.03) phenotypes but not with cutaneous phenotype (P = 0.06).

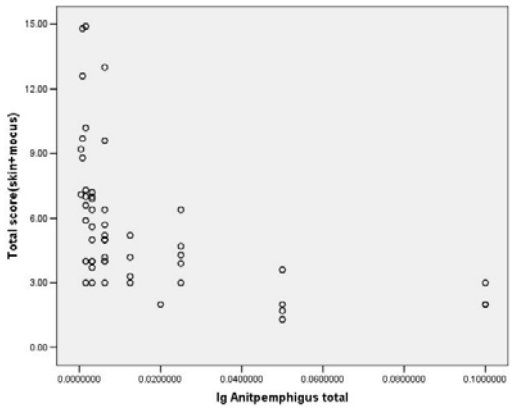

Fig. 1. Relationship between total disease score and IIF titers

Table 4. Mean skin/mucosal/total scores and frequency of positive IIF in three clinical phenotypes of patients with pemphigus vulgaris

Phenotype	Number	Mean Skin score (±SD)	Mean Mucosal score (±SD)	Mean Total score (±SD)	Positive IIF (%)
Cutaneous	9	2.63±1.74	0.00	2.63±1.74	5(55%)
Mucosal	19	0.00	3.37±0.96	3.37±0.96	19(100%)
Mucocutaneous	33	3.87±2.81	3.31±1.02	7.17±3.10	32(97%)
Total	61	2.48±2.73	2.84±1.48	5.32±3.13	56(91.8%)

DISCUSSION

In pemphigus vulgaris, autoantibodies are directed against Dsg1 and Dsg3, two antigens belonging to desmosomal cadherins (13). It seems that characteristic distribution of lesions is depended on relative expression of Dsg 1 and Dsg 3 (14). In patients with anti-desmoglein autoantibody profile of (Dsg3+, Dsg1-), only mucosal lesions or mucosal lesions with mild cutaneous involvement are noted; in patients with anti-desmoglein autoantibody profile of (Dsg3+, Dsg1+), mucocutaneous involvement is seen. In rare cutaneous pemphigus vulgaris, Dsg1 autoantibodies as well as anti-Dsg3 autoantibodies has been found (15). In our study, the frequencies of pemphigus phenotypes were similar to another study in our center (16). However, our results showed higher figures of cutaneous phenotype frequency.

Demographic features of our patients, including age and sex distribution, are very similar to results obtained in large study on Iranian patients (17).

For many years, IIF has been the standard test for detection of pemphigus autoantibodies. It is a more sensitive test for the detection of circulating pemphigus antibodies than immunoblotting, and is still standard assay to detect the intercellular antibodies associated with pemphigus (18). Although desmoglein-3 ELISA is a specific tool for the diagnosis of pemphigus, there is a positive correlation between IIF titers and ELISA values (19).

Furthermore, in most laboratories the diagnosis of pemphigus still depends on direct or indirect immunofluorescence (IIF) procedures (20).

In patients with pemphigus vulgaris, sensitivity of IIF has been reported 70% to 90%, depending in part to the substrate used (18). Harman, et al recommended the combination of substrates (normal human skin and monkey esophagus) to increase the sensitivity of IIF. By this modification IIF could still be used as an alternative test especially when ELISA is not available (20).

We used prepuce as substrate in our study. Foreskin has previously been proposed as an ideal substrate for indirect immunofluorescence (21).

Using human prepuce, we achieved a sensitivity of more than 90%, which seems an acceptable figure for routine use of this technique in laboratory assessment of patients with pemphigus vulgaris.

Use of different methods for assessment of severity of disease could be one of the reasons of conflicting results. In most studies, arbitrary scoring systems have been used to grade the severity of skin or mucosal involvement and number of lesions is the only factor considered (12, 22). To include other important variables, including frequency of new blister formation, in assessment of severity of disease, Agarwal et al. proposed a new scoring system along the lines of Psoriasis Area and Severity Index (PASI) named PAAS (12). In this scoring system, in addition to the extent of skin involvement, factors such as number of new blistering, peripheral extension of existing lesions, and the presence of Nikolsky's sign are being considered. Although there are some broad observer-based variation drawbacks (23), it seems this system can serve as a useful parameter for monitoring clinical activity of pemphigus vulgaris and for interphysician objective communication. Using

PAAS, we found a significant relationship between overall disease severity and antibody titers by IIF. There was also a significant relationship between skin score, mucosal score, and antibody titers. Relationship was stronger for mucosal than skin score.

This could be the result of using prepuce as substrate. In conclusion, our results show that there is a significant relationship between disease activity according to PAAS score and antibody titers by IIF. Further studies are recommended to determine usefulness of this technique for monitoring (grading PV) disease. We recommend using PAAS as an objective and reliable scoring system in the management of pemphigus vulgaris.

REFERENCES

1. Femiano F. Pemphigus vulgaris: recent advances in our understanding of its pathogenesis. Minerva Stomatol 2007;56:215-23.
2. Black M, Mignogna MD, Scully C. Number II. Pemphigus vulgaris. Oral Dis. 2005 May;11(3):119-30.
3. Ruocco E, Baroni A, Wolf R, Ruocco V. Life-threatening bullous dermatoses: Pemphigus vulgaris. Clin Dermatol. 2005 May-Jun;23(3):223-6.
4. Beutner EH, Jordon RE. Demonstration of skin antibodies in sera of pemphigus vulgaris patients by indirect immunofluorescent staining. Proc Soc Exp Biol Med 1964;117: 505–10.
5. Anhalt GJ, Díaz LA. Pemphigus vulgaris--a model for cutaneous autoimmunity. J Am Acad Dermatol. 2004 Jul;51(1 Suppl):S20-1.
6. Chorzelski TP, von Weiss JF, Lever WF. Clinical significance of autoantibodies in pemphigus. Arch Dermatol 1966; 93:570-6.
7. Beutner EH, Jordon RE, Chorzelski TP. The immunopathology of pemphigus and bullous pemphigoid. J Invest Dermatol 1968; 51:63-80.
8. Sams WM, Jordon RE. Correlation of pemphigoid and pemphigus antibody titres with activity of disease. Br J Dermatol 1971; 84:7-13.
9. Judd KP, Lever WF. Correlation of antibodies in skin and serum with disease severity in pemphigus. Arch Dermatol 1979; 115: 428-32.
10. Creswell SN, Black MM, Bhogal BS, Skeete MVH. Correlation of circulating intercellular antibody titers in pemphigus with disease activity. Clin Exp Dermatol 1981; 6: 477-83.
11. Judd KP, Mescon H. Comparison of different epithelial substrates useful for indirect immunofluorescence testing of sera from patients with active pemphigus. J Invest Dermatol 1979; 72: 314-16.
12. Agarwal M, Walia R, Kochhar AM, Chander R. Pemphigus area and activity score (PAAS)- a novel clinical scoring method for monitoring of pemphigus vulgaris patients. Int J Dermatol 1998; 37: 158-60.
13. Liu Z, Li N, Diaz LA. Immunopathological mechanisms of acantholysis in pemphigus vulgaris: an explanation by ultrastructural observations. J Invest Dermatol. 2004 May; 122(5):XIII-XIV.
14. Shirakata Y, Amagai M, Hanakawa Y, Nishikawa T, Hashimoto K. Lack of mucosal involvement in pemphigus foliaceus may be due to low expression of desmoglein 1. J Invest Dermatol 1998;110:76-8.
15. Yoshida K, Takae Y, Saito H, Oka H, Tanikawa A, Amagai M, Nishikawa T. Cutaneous type pemphigus vulgaris: A rare clinical phenotype of pemphigus. J Am Acad Dermatol 2005;52:839-45.
16. Esmaili N, Chams-Davatchi C, Valikhani M, Daneshpazhooh M, Balighi K, Hallaji Z, et al. Pemphigus vulgaris in Iran: a clinical study of 140 cases. Int J Dermatol. 2007 Nov; 46(11):1166-70
17. Chams-Davatchi C, Valikhani M, Daneshpazhooh M, Esmaili N, Balighi K, Hallaji Z, Barzegari M, Akhiani M, Ghodsi Z, Mortazavi H, Naraghi Z. Pemphigus: Analysis of 1209 cases. Intl J Dermatol. 2005, 44, 470 –476.
18. Jiao D, Bystryn J. Sensitivity of indirect immunofluorescence, substrate specificity, and immunoblotting in the diagnosis of pemphigus. J Am Acad Dermatol 1997;37:211-6.
19. Lenz P, Amagai M, Volc-Platzer B, Stingl G, Kirnbauer R. Desmoglein 3-ELISA: a pemphigus vulgaris-specific diagnostic tool. Arch Dermatol. 1999 Feb;135(2):143-8.
20. Harman KE, Gratian MJ, Bhogal BS, Challacombe SJ, Black MM. The use of two substrates to improve the sensitivity of indirect immunofluorescence in the diagnosis of pemphigus. Br J Dermatol 2000; 142: 1135-1139.

21. Friedman H, Campbell IT, Alvarez RR, Diaz LA, et al. [Indirect immunofluorescence in endemic pemphigus foliaceus. A contribution to its standardization] [Article in Portuguese]. Rev Inst Med Trop Sao Paulo. 1989 May-Jun;31(3):158-68.
22. Kumar B, Arora S, Kumaran MS, Jain R, Dogra S. Study of desmoglein 1 and 3 antibody levels in relation to severity in Indian patients with pemphigus. Indian J Dermatol Venereol Leprol 2006; 72 (3): 203-6.
23. Saraswat A, Kumar B. A new grading system for oral pemphigus. Int J dermatol 2003; 42:413-14.