Acta Dermatovenerol Croat 2018;26(2):100-104 CLINICAL ARTICLE

Evaluation of the Importance of Immunological Profile for Pemphigus Vulgaris in the Light of Necessity to Modify Compensation Theory

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Received: March 15, 2017
Accepted: May 15, 2018

ABSTRACT According to the "desmoglein compensation theory," anti-Dsg1 and anti-Dsg3 profiles are crucial for the clinical outcome of pemphigus vulgaris. However, recent studies have highlighted several cases with an incompatibility between the antibody profile and clinical manifestation. Data of 37 patients who had been diagnosed pemphigus vulgaris in our Department between January 2014-June 2016 were retrieved from our clinical database. Patients with ABSIS skin involvement scores, oral mucosa extent and severity scores, anti-Dsg1 and Dsg3 antibody profile were included in this retrospective study. Patients with discordance between clinical manifestations and immunological profile were considered as atypical clinical phenotype. Patients with missing data were excluded. In all 37 patients, Dsg1 and Dsg3 antibody titers at the baseline did not correlate with the concurrent ABSIS scores. At follow up, we detected statistically significant correlations between anti Dsg-1 profile and ABSIS skin involvement scores (p=0.006; r=0.588) and between anti-Dsg3 and ABSIS mucosal extent and severity scores (p=0.058; r=0.431). After treatment, the reduction of Dsg-1 antibody titers was statistically significant in remittent patients (p=0.027). We did not detect statistically significant reduction of Dsg-3 antibodies. Four subjects had incompatible antibody profile and clinical activity. Discordance between phenotype-antibody profile and clinical activity-Dsg titers support the idea that non-Dsg antigens may also be the target for pemphigus autoimmunity.

KEY WORDS: pemphigus vulgaris, compensation theory

INTRODUCTION

Pemphigus is a group of cutaneous autoimmune blistering diseases caused by IgG autoantibodies, which mainly target two desmosomal proteins, desmoglein 1 (Dsg1), and desmoglein 3 (Dsg3). Localization of the blister formation are explained by "desmoglein compensation theory". It is well-accepted that anti-Dsg1 and anti-Dsg3 profiles are crucial for the clinical outcome of the disease. However, recent studies have highlighted several cases with an incompatibility between the antibody profile and clinical manifestation (1-8). In addition, there is conflicting data about the utility of screening antibody titers during the follow-up period. In the literature, patients who are in remission yet still having positive anti-Dsg3 levels have been reported (2, 4, 9, 10).

The Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) is a validated scoring system to measure and capture changes in the disease activity (11). Since ABSIS is a reliable tool for evaluating the clinical activity of the disease, it has also been used in studies addressing the correlation between antibody profiles and clinical activity (9, 11). With respect to determining qualitative changes of mucosal disease in cases without any lesion size or number change, the combination of objective and subjective components may result in a comprehensive validation for oral mucosa involvement (11).

Acta Dermatovenerol Croat
2018;26(2):100-104

In this report, we aimed to improve our understanding of the link between clinical and immunological responses. We present the results of data analysis from 37 patients with the diagnosis of pemphigus vulgaris (PV) and describe a unique subset of patients with discordance between their antibody profile and clinical picture.

PATIENT AND METHODS

From the database of our Dermatology clinic we collected the data on 74 patients with the ICD code of pemphigus vulgaris in the period between January 2014 and June 2016. The patients who had a diagnosis other than PV, including pemphigus foliaceus, paraneoplastic pemphigus or other bullous disorders, during their follow-up and patients with the lack of clinical or immunological data were excluded. Thirty-seven patients were included in the final analysis. They were diagnosed according usual clinical, histopathological and immunological criteria. The clinical diagnosis was made on the basis of the existence of the depositions of IgG with or without C3 on the epithelial cell surface on direct immunofluorescence, flaccid blisters and/or erosions observed in the mucosa, skin, or both and intraepidermal blisters with acantholysis in histopathological examination (12).

We recorded the following data from the medical charts: age, gender, ABSIS skin and mucosa scores. ABSIS skin involvement scores, oral mucosa extent and severity scores were recorded separately. The titers of circulating antibodies were measured using the conventional enzyme-linked immunoassay (ELISA) kit. Anti-Dsg 1 and anti-Dsg3 profiles were measured at two time-points (baseline with/without follow-up) in 18 patients and only during the follow-up period in 19 patients. We compared these immunological profiles and concurrent ABSIS scores of the patients. The cases with antibody profiles that did not conform to compensation theory were discussed based on similar case reports, author's opinions and experimental studies. Also patients with immunological reactivity despite the sucessful control of the disease activity were also included in the study.

STATISTICAL ANALYSIS

Statistical analyses were performed using IBM SPSS for Windows Version 21.0 package program (IBM, USA). Numerical variables were tested for normality using visual (histogram, normality plots) and analytic (Kolmogorov-Smirnov test, variation coefficient and Kurtosis/Skewness index) tests. Categorical variables were presented as numbers and percentages and numerical variables were presented as mean ± standard deviation or median and interquartile range (IQR). Categorical variables were compared using chi-square test or Fisher's exact test; Mann Whitney U test was used for paired group comparison of numerical variables in case condition of normal distribution was not met. Correlation analysis was performed using Spearman's correlation test. The level of statistical significance was set at $p<0.05$.

RESULTS

Twenty-three female and 14 male patients were included to the study. The mean age was 50 years (ranging 27-74). The median mucosal involvement extent and severity scores were 2.19 and 18.6, respectively. The mean skin involvement score was 20.72 at initial presentation. Oral mucosa ABSIS scores were similar in both genders but skin ABSIS scores were higher in female than male patients ($p=0.046$) At baseline, Dsg1 and Dsg3 titers did not correlate with the ABSIS oral mucosa severity scores. We detected statistically significant correlations at follow-up between anti Dsg-1 profile and ABSIS skin involvement scores ($p=0.006$; $r=0.588$) and between anti-Dsg3 and ABSIS mucosal extent and severity scores ($p=0.058$; $r=0.431$). After the treatment period in remittent patients, the reduction of Dsg-1 antibody titers was statistically significant ($p=0.027$). On the other hand, we did not detect statistically significant reduction of Dsg-3 antibodies in the post-treatment period.

We observed one case of cutaneous pemphigus without Dsg1 positivity, one mucosal pemphigus without Dsg-1 and Dsg-3 positivity and two cutaneous pemphigus with Dsg3 positivity (Table 1). These patients were considered to have an atypical clinical phenotype. Anti-Dsg3 titers of five patients with successfully controlled of disease activity remained at higher levels during the follow-up period (Table 2). The average length of time to achieving the control of disease activity in these five patients was 10.4 months (range 6-12 months).

DISCUSSION

The compensation theory suggests that antibodies against Dsg1 and/or 3 are adequate to disrupt epidermal integrity (13). However, in recent years it has been proposed that the compensation theory based on this monopathogenetic justification is insufficient to explain pemphigus pathogenesis (14, 15).

Studies regarding the correlation between ELISA testing for anti-Dsg1 and anti-Dsg3 with disease activity and clinical phenotype have conflicting results. Some studies report that serum testing for antibodies against desmoglein 1 and 3 correlates well with the severity of disease (8) and is a good tool for moni-

toring disease activity (5-7). Contrary to these results, some authors think desmoglein ELISA testing is not practical for the follow-up (4). In addition, anti-Dsg1 reactivity is found more predictive for disease activity (2, 3). In our study, significant reduction of the Dsg 3 autoantibodies was not observed in patients with remission. In the light of the scientific literature this may be explained by the presence of non-Ca^{+2} dependent epitopes or predominance of non-pathogenic IgG1 versus IgG4 (16-18).

The monopathogenetic hypothesis cannot explain the observed negativity of both anti-Dsg1 and anti-Dsg3 antibodies seen in 5-33% of patients with pemphigus vulgaris (1, 19-24). Similarly, in our series, one of our cases (5.5%) had negative ELISA results for both anti-Dsg1 and anti-Dsg3. There are reports in literature on patients with anti-Dsg3 positivity in the absence of oral mucosal involvement (25-27), patients with oral mucosal involvement despite anti-Dsg3 negativity (28, 29), cutaneous pemphigus vulgaris cases lacking Dsg1 autoantibodies (30) and the discordance between clinical activity and Dsg titers (2, 4).

According to the multipathogenetic explanation, as opposed to the monopathogenetic hypothesis, other coexisting pathogenic antibodies rather than a monotypic pathogenic antibody lead to the simultaneous influence of multiple biological functions of *keratinocytes, and the activity of* anti-Dsg antibodies is augmented by non-Dsg antibodies (14). In a study which investigated the complementary activities of non-Dsg and anti-Dsg antibodies, it was established that acantholysis due to anti-*mitochondrial antibodies (AMA) is reversible, contrary to the irreversible acantholysis effect of AMA coupled with anti-Dsg antibodies* (31). It was demonstrated that high doses of monoclonal anti-Dsg antibodies can lead to acantholysis, but this effect was absent at low antibody dilutions (31). Interestingly, acantholysis occurred when AMA was added to a non-acantholytic concentration of anti-Dsg antibodies (31). According to these findings, either anti-Dsg or non-Dsg autoantibodies are believed to be responsible for the development of the acantholysis in pemphigus (14). In relation to this finding, Sajda *et al*. reported non-desmoglein reactivities toward muscarinic acetylcholine receptor subtypes 3,4,5 and thyroid peroxidase in PV patients (32).

In the report of Grando *et al.* (15), the existence of over 50 proteins that interact with the pemphigus IgG type antibody, generation of desmosomes by Dsg1-3 deficient keratinocytes due to gene silencing (33), and suprabasal acantholysis observed in Dsg3 neonates after the transfer of antibodies from patients with pemphigus vulgaris (34), provide supporting evidence for the inadequacy of the compensation theory.

Lower pathogenicity of anti-Dsg3 antibodies is another explanation for atypical clinical presentation (35). According to this hypotheses, low levels of pathogenic antibodies are sufficient to block skin Dsg3 accompanied by anti-Dsg1 antibodies, but not of the oral mucosal Dsg3, where Dsg3 is dominant (25). Although it is not possible to make strong conclusions based on the retrospective design of our study and lack of long-term follow-up, we believe that our findings and other relevant data call for further review of compensation theory and monopathogenic hypothesis.

Table 1. Baseline Dsg antibody levels of 18 of patients

Age	Gender	DSG 1	DSG3	Oral involvement extent (0-11)	Oral involvement severity(0-45)	Skin involvement (0-150)
69	M	NEGATIVE	1:1000	5	39	35
66	M	1:100	1:320	5	39	10
59	F	NEGATIVE	1:1000	0	0	18*
53	F	NEGATIVE	1:320	3	30	45
52	F	NEGATIVE	1:1000	5	42	15
45	M	NEGATIVE	1:1000	3	30	45
35	M	1:200	1:200	4	30	45
36	M	NEGATIVE	1:320	3	30	45
51	F	NEGATIVE	NEGATIVE	4	30	0*
33	F	NEGATIVE	1:320	3	30	0
55	M	NEGATIVE	1:320	6	42	0
73	F	1:320	1:1000	3	30	45
55	F	NEGATIVE	1:100	5	39	0
27	M	1:320	1:320	0	0	50*
69	F	NEGATIVE	1:320	5	39	0
56	F	1:320	1:1000	6	42	60
43	F	1:200	1:320	0	0	60*
50	M	1:100	1:1000	10	42	5

* Patients with an incompatibility between the antibody profile and clinical manifestation at initial presentation

Table 2. Dsg 1 and Dsg 3 titers of patients in clinical remission (CR)

Patient age/gender (n=8)	Duratin of CR (months)	Treatment at the time of ELISA test	Dsg1 ELISA	Dsg3 ELISA
48/F	10	16 mg/day Pred., 50 mg/day AZA	NEGATIVE	1/1000
34/F	12	7,5/day Metilpred., 100mg/day AZA	NEGATIVE	NEGATIVE
38/F	12	100 mg/day AZA, IVIg	NEGATIVE	1/320
70/M	12	5 mg/day Fluocort. 50 mg/day AZA	NEGATIVE	1/200
54/M	12	100 mg/day AZA	NEGATIVE	1/320
42/M	9	IVIg	NEGATIVE	NEGATIVE
53/M	12	5 mg/day metilpred., 50 mg/day AZA	NEGATIVE	NEGATIVE
53/M	6	32 mg/day pred., 100 mg/day AZA	NEGATIVE	1/320

Pred.: prednisolone, Metilpred.: methylprednisolone, AZA: azathioprine, Fluocort.: fluocortolone

CONCLUSION

The discordance between phenotype-antibody profile and between clinical activity-Dsg titers supports the idea of non-Dsg antigens having a key role in pemphigus autoimmunity.

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